SUBACUTE ORAL TOXICITY OF PROFENOFOS AND HEPATOPROTECTIVE EFFECTS OF *Tephrosia purpurea* IN GRAMAPRIYA BIRDS

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PATHOLOGY

BY

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Enrolment No: - V/13/087

College of Veterinary and Animal Sciences, Parbhani

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(INDIA)

2021

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled, "**SUBACUTE ORAL TOXICITY OF PROFENOFOS AND HEPATOPROTECTIVE EFFECTS OF *Tephrosia purpurea* IN GRAMAPRIYA BIRDS**" or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

Place : Parbhani
Date: / / 2021

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CERTIFICATE

This is to certify that the thesis entitled, **"SUBACUTE ORAL TOXICITY OF PROFENOFOS AND HEPATOPROTECTIVE EFFECTS OF *Tephrosia purpurea* IN GRAMAPRIYA BIRDS"** submitted by Shri. **SHETE HARSHAL JAYPAL** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Associate Dean
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Affectionately

Dedicated

To My Beloved

Parents

Mr. Jaypal

and

Mrs. Sarojani Shete

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"Great things happen to those who don't stop believing, trying, learning, and being grateful."

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Place: Parbhani

Date: / /2021 **(Shete Harshal Jaypal)**

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ABBREVIATIONS

%	:	Per cent
&	:	And
@	:	At the rate of
°F	:	Degree Fahrenheit
µg	:	Microgram
AchE	:	Acetyl choline Esterase
Ad lib.	:	*Ad-libitum* (sufficient)
ALP	:	Alkaline phosphatse
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
ATP	:	Adenine triphosphate
BUN	:	Blood urea nitrogen
BWG	:	Body weight gain
CCL4	:	Carbon tetrachloride
CD	:	Critical difference
CM	:	Centimeter
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
COVAS	:	College of Veterinary and Animal Sciences, Parbhani
CRD	:	Controlled randomised design
CT	:	Clotting time
Cumm	:	Cubic millimetre
DLC	:	Differential leucocyte count
DNA	:	Deoxyribo Nucleic Acid
EDTA	:	Ethylene Diamine Tetraacetate

et al.	:	*et alia* (and others)
etc.	:	et cetera
FCR	:	Feed conversion ratio
FCT	:	Fibrous connective tissue
Fig.	:	Figure
gm/dl	:	Gram/decilitre
gm/kg	:	Gram per kilogram
Gr. I	:	Normal feeding & watering (Healthy control)
Gr. II	:	Profenofos @ 1.6 mg/kg Bwt through oral gavage daily
Gr. III	:	*Tephrosia purpurea* leaves powder @ 0.1 % of feed daily
Gr. IV	:	Profenofos @ 1.6 mg/kg Bwt. through oral gavage daily + *Tephrosia purpurea* leaves powder @ 0.1 % of feed daily
gm or g	:	Gram
H & E	:	Haematoxylin and Eosin
Hb	:	Haemoglobin
i.e.	:	That is
IAEC	:	Institutional Animals Ethics Committee
ISP	:	Isoprotrenol
IU/L	:	International units per litre
Kg	:	Kilogram
Ltd	:	Limited
MCHC	:	Mean corpuscles haemoglobin concentration
MCV	:	Mean corpuscles value
MAFSU	:	Maharashtra Animal and Fishery Sciences University
mg	:	Milligram
mg/dl	:	milligram per decilitre

ml	:	Millilitre
MNC	:	Mononuclear cell
Na	:	Sodium
NADH	:	Nicotinamide adenine dinucleotide
NS	:	Non significant
OECD	:	Organisation for economic cooperation and development
P.O	:	Per oral
PCT	:	Proximal convoluted tubule
PCV	:	packed Cell volume
RBC	:	Red blood cells
S.E	:	Standard error
Sec	:	Seconds
SGOT	:	Serum glutamic oxaloacetic transaminase
SGPT	:	Serum glutamate pyruvate transaminase
STP	:	Serum total protein
SUN	:	Serum urea nitrogen
SUA	:	Serum uric acid
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count
WASP	:	Web Based Agricultural Statistics Software
WBC	:	White blood cells

Introduction

CHAPTER: -1

INTRODUCTION

India became the sixth largest economy by sustaining growth rates higher than China, thereby earning the epaulette of being the fastest growing major economy in the world with an output grew at 3.6 per cent in 2014 and again in 2018. India's livestock sector (containing poultry) is one of the largest in the world with a holding of 11.6% of world livestock population. Contribution of livestock sectors to the national economy in terms of Gross Domestic Product (GDP) is 4.1% (Islam *et al.,* 2016). In India Livestock and poultry sectors play a multi-faceted role in socio-economic development of rural households, as these assets are more equitably distributed than land. Landless and small scale farmers rear an average flock size of 8 to 12 birds through their backyard poultry practices in India. Which showed that over 50 % of small scale and marginal farmers depend on poultry and small ruminant rearing (Jha and Chakrabarti, 2017). Further, that livestock and poultry can be used as an effective tool for reducing rural poverty (Ali., 2007).

From last few decade, poultry industry has made considerable growth in both commercial and backyard poultry sector in India. Which was not only in terms of size but also in productivity, sophistication and quality. The total Poultry population in the country is 851.81 Million in 2019, which was increased by 16.8% over previous Census. However, the total Backyard Poultry population in the country is 317.07 Million in 2019, which showed the increment of 45.8% over previous Census (20th Livestock Census, 2019).

In India for upliftment of small scale farmers we should look for the best alternative such as backyard poultry farming, which helps in improving their subsidiary income. Backyard poultry products have more price values than that of commercial poultry products, which is the best alternative for the rural farmers and provides them the socio-economic improvement also (Singh *et al.,* 2017). Also, the rural backyard poultry farming has the pivotal role in elevating the food and nutrition securities of the poorest households and farmers, which helps them

to improve livelihood standards. It also is the source of the nutritional supplement in the form of animal protein for poor households in India. As this way the rural backyard poultry farming can improve the income generation problem, poverty issues and nutritional problems in rural India (Pica-ciamarra *et al.,* 2010). Other benefits of backyard poultry farming includes the improvement of the soil fertility in backyard areas and provide egg and meat through very less investment. Also, considering recent human health issues, backyard poultry is capable to provide egg and poultry meat containing low cholesterol concentration as compared to intensive poultry system (Rath *et al.,* 2015).

In rural India, until now most of the chicken breeds reared were generally Desi type (Non-descript), which had low egg and meat production capacity. In last decades their contribution to the total egg production was stagnant due to their low production capacity (Rath *et al.,* 2015). Considering this points many research institutes have found out many improved and upgraded chicken varieties, which can be successfully reared in backyard, semi intensive and intensive system throughout the various parts of India (Rath *et al.,* 2015). Some of these improved varieties are specifically used for egg or meat purpose production, whereas others are available with their ability of dual purpose production. Some examples of the improved varieties of the chickens are Gramapriya, CARI-nirbhic, CARI-Shyama, Vanaraja, Gramalaxmi and Nicobari (Rath *et al.,* 2015).

Gramapriya is dual purpose crossbred chicken developed through a Hyderabad-based project under an All India Co-ordinated Research Project (AICRP). Promising features of the Gramapriya are their attractive multicolor feather pattern, high general immune competence, better performance with poor quality feed and also grows faster and produce brown colored eggs. These characteristics of Gramapriya bird made it more useful in rural and tribal areas of India than any other desi bird (Pathak and Nath, 2013). In India, Gramapriya birds showed better performance in case of age at first egg laying, annual egg production and body weight under backyard system of rearing. Considering these points, Gramapriya birds can improve the livelihood and nutritional requirements of small scale farmers in rural and tribal parts of India. Also, rearing of

Gramapriya birds could help in fulfillment of protein requirements of pregnant women, feeding mothers and children in rural and tribal areas of India. So Gramapriya birds are more preferred among farmers of India for backyard poultry farming (Sree *et al.,* 2017).

Considering above mentioned points the Indian poultry industry is growing very fast comparing other agricultural industries. In spite of these development and growth in poultry industry, it also facing many problems such as diseases and toxin residues in poultry products resulting in poor growth and immune suppression in the birds. These problems have significant international implications and economic losses to farmers as well as to the Indian economy. Among such diseases, toxicity of organo-phosphoros/ organo-chlorine compounds through the feed and water and their residues in poultry products is commonly seen in India (Aulakh *et al.,* 2006). These organo-phosphoros/ organo-chlorine compounds are generally ingredients of pesticides used in agricultural sector.

Majority of the population in India (about 56.7%) is engaged in agriculture sector. In order to achieve maximum production the crop protectants are being used indiscriminately. India is the largest producer of pesticides among the Asian countries, where India is at 12^{th} rank in the world for the use of pesticides in agricultural practices. India is producing about 90,000 tons of pesticides annually (Chitra *et al.,* 2006). Synthetic insecticides (Organophosphates) were commonly used for the control of the insects and pest. This uncontrolled use of pesticides consequences many serious problems including toxic residues in crops and cereals, which are further used as the source of feed to mammals and birds (Rhayf *et al.,* 2012). Low concentration of pesticide causes toxic effects in birds as it is used on crops and around the poultry premises.

Among these OP compounds, Profenofos is one of them extensively used as a crop protectant in the field, especially in and around Parbhani, as per the market survey made. Profenofos was readily used by farmers on the maize and soybean crops, which are further used as principle feed constituents in poultry feed. In recent years, profenofos was more preferred as it is a broad spectrum pesticide

and it can also easily decompose through environment. Like other organophosphates profenofos also causes many toxic effects on poultry. As this way profenofos was directly related to the residual effects in poultry and the poultry feed, therefore, it was selected for this study.

In India it is observed that there is increase in use of herbal medicines for various disease conditions. There were large number of plants and herbal formulations have hepatoprotective activity. About 87 plants are used in 33 patented and proprietary polyherbal formulations for their hepatoprotective property (Khatri *et al.,* 2009). Among them *Tephrosia purpurea* was readily used as the hepatoprotective plant. In India *Tephrosia purpurea* is a copiously branched herbaceous perennial plant distributed throughout the tropics and commonly known as 'sarponkha' and 'saraphunkha'. It has been used as traditional herbal ingredients in different herbal formulation due to its traditional claim in Ayurveda. The whole plant of the *Tephrosia purpurea* are reported to be useful in various liver disorders (Mathews *et al.,* 2012). Considering this trend and hepatotoxic activity of profenofos compound, it was thought to use the hepatoprotective plant to treat toxicity of profenofos.

Finally, considering the current facts of increasing use of profenofos, losses being caused in poultry and a trend of using herbal medication; the present trial has been planned with the following objectives,

OBJECTIVES:

1) To study the effects of profenofos compound toxicity on Growth performance in Gramapriya birds
2) To record the effect of profenofos compound toxicity on hemato-biochemical alterations and clotting time in Gramapriya birds
3) To note the gross and histopathological changes in profenofos toxicated Gramapriya birds
4) To evaluate the hepatoprotective effects of *Tephrosia purpurea* in induced subacute oral toxicity of profenofos in Gramapriya birds

Review of Literature

CHAPTER: - 2

REVIEW OF LITERATURE

2.1 Evaluation of LD_{50} values of profenofos in laboratory animal and poultry:

FAO and WHO, (2007) described two case studies described in a toxicological evaluation report of pesticide residues in food. First case study was carried out with White Leghorn chickens, which were given profenofos at a dose rate of 60 mg/kg bwt. This study resulted in death of White Leghorn chickens after administration of first dose. However 2nd study was also carried out on profenofos toxicity in White Leghorn chickens, which showed that LD_{50} of profenofos in White Leghorn chickens was 45.7 mg/kg bw.

El- bendary *et al.,* (2014) examined pathological alterations in male albino mice which was toxicated by profenofos (Dose @ $1/10^{th}$ of LD_{50}). The study was carried out at Cairo University, Egypt during the period of June 2012 to January 2013. The experimental study showed that the LD_{50} value of the profenofos in mice was 350 mg/kg/b.wt.

Kafle *et al.,* (2018^{b}) studied pathological changes in broiler birds following Profenofos administration. The experiment was conducted at Assam Agricultural University, Guwahati. During this experiment, pilot study was carried out to obtain the LD_{50} of profenofos in broiler birds and the same was found to be 16 mg/kg/b.wt.

2.2 Effects of OP compound toxicity on Growth performance in poultry:

Garg *et al.,* (2004) examined the chronic toxicity of organophosphate pesticides in broiler chicks. They were stated that the body weights of toxin treated groups, recorded at the end of experiment did not vary considerably as compared to the healthy control group.

Naraharisetti *et al.,* (2009) in a 28 day study, inspected the effect of Malathion (@500 ppm through feed) in broiler chickens. The study noted that

weight gain of toxicated birds were significantly decreased as compare to healthy control group.

Al-Baggou., (2014) studied subacute toxicity of chlorpyrifos (100 ppm) in day-old Ross chicks via drinking water. The study observed that chlorpyriphos did not markedly alter the growth rate and body weights of the chicks as compared to the healthy control group.

Ghaffar *et al.,* (2014) recorded significantly reduced feed intake in Triazophos toxicated Japanese quail than the control group. Simultaneously, the study was also showed the significant decreased body weights in Triazophos toxicated Japanese quail than the control group.

Ahmad *et al.,* (2015) studied Impact of chlorpyrifos on health of broiler chicks, which was carried out with different dose rates of chlorpyrifos such as 5 mg/kg bw, 10 mg/kg bw and 20 mg/kg bw etc. The all treatment groups showed significantly decreased feed intake and body weights on the 2^{nd} & 3^{rd} week of the experiment compared to control group. They found the same results in body weights of treatment groups up to 5^{th} week of the experiment. Whereas, they observed non-significant results of feed intake in the last three weeks of experiment.

Begum *et al.,* (2015) studied the chronic chlorpyrifos (@ 0.36 mg/kg b.w.) intoxication in indigenous chicken and examined the changes in growth parameter. Feed intake and body weight gain were markedly reduced throughout the experimental trial of 12 weeks.

Wani *et al.,* (2017) observed the chlorpyrifos (3.2, 1.6 and 0.64 mg/kg) induced oxidative stress in broiler chicken. The study revealed that the feed consumption, body weight, body weight gain were markedly decreased in chlorpyrifos treated groups as compared to healthy control group. Also there is significant increase in FCR of toxin treated groups were observed in experimental trial.

EL-Nahhal and Lubbad, (2018) observed toxicity effects of chlorpyrifos when fed @ 0.1 mg/kg/day, diuron (@ 0.1 mg/kg/day), and their combination (@ 0.05 mg/kg/day each compound) on chicken. These treatments were given to

chickens daily for two weeks of period. The study showed the changed feeding behaviour of chickens in all treatment groups, which were consequences to the significant decreased feed intake in the treatment groups than control group. However, all treatment groups showed significantly lowered body weights of chickens than the control group. The study revealed that the OP compounds had a significant effects on body weights and feed intake of chickens.

Hussain *et al*., (2019) studied pathological alterations in trichlorfon toxicated adult cockerels. Observations showed that the feed intake and weight gain were significantly decreased in trichlorfon treated groups than the control groups.

2.3 Haematological and Blood clotting time alterations in subacute oral toxicity of OP compounds:

Moregaonkar (1990) reported Butacarboxime; a carbamate insecticide toxicity in poultry. He noted that the chicks received 1500 ppm of butocarboxime showed significantly elevated BCT (Blood clotting time) at 60th day and 75th day intervals as compared to healthy control group.

Garg *et al*., (2004) were studied the chronic toxicity of Monocrotophos (OP Pesticide) and its pathophysiological effects in Broiler Chicks. The study mentioned that mean total erythrocyte count, packed cell volume and haemoglobin remained unaltered and showed non-significant change in treatment groups throughout the experiment. Whereas, the mean total leucocyte count showed significant decrease in Monocrotophos treated group. Also, further differential leucocyte count reveals significant increase in heterophil percentage in treatment group and significant decrease in lymphocyte percentage in treatment group. However, the percent eosinophil and monocyte showed non-significant change.

Kammon *et al*, (2011) conducted experiment to study chronic toxicity of chlorpyrifos in broilers. He administrated chlorpyrifos compound at the dose rate of 0.8 mg/kg body weight PO, which was $1/50^{th}$ of LD_{50}. They revealed that the

administration of chlorpyrifos at the dose rate of 0.8 mg/kg bwt did not produce any significant changes in the concentration of haemoglobin, TLC and DLC in broiler chickens on day 24 and day 45. The findings of their study suggested that chronic exposure of broilers to chlorpyrifos at 0.8 mg/kg bwt has no significant toxic effects on haemopoietic system.

Rhayf *et al*., (2012) observed the dimethoate induced hematological alterations in local layer chickens. The study stated that the mean values of haemoglobin concentration, total erythrocyte count and total leucocyte count were significantly decreased in the toxin treated group.

Ghaffar *et al.*, (2014) examined clinico-hematological alterations in Triazophos toxicated Japanese quail. They revealed that the significant decrease in the haemoglobin (%) and Total Erythrocyte Counts were noticed throughout the experiment in Triazophos treated groups. However, comparing the control group, the Packed Cell Volume (PCV) values were significantly increased throughout the experiment in Triazophos treated groups. In case of the erythrocytic indices the mean corpuscular hemoglobin concentration values were decreased in Triazophos treated groups. However, the mean value of corpuscular volume were significantly increased in Triazophos treated group. DLC examination revealed that the leukocyte and monocyte counts were decreased, while heterophil and lymphocyte values were increased significantly in Triazophos treated groups.

Ahmad *et al.,* (2015) studied Impact of chlorpyrifos at the dose rate of 20 mg/kg bwt on broiler chicks. They found that haemoglobin concentration was significantly decreased in chlorpyrifos (CPF) treated groups as compared to the control group. Haematocrit (PCV) significantly altered among all CPF treated groups compared to control. Significantly lower TLC values were observed in the CPF treated group as compared to other groups.

Begum *et al*., (2015) studied haematological changes in chronic chlorpyrifos intoxicated indigenous chickens. They stated that the Heamoglobin (%) and TEC showed significant increase in chlorpyrifos treated groups. The further DLC examination revealed that the heterophil percent was significantly

increased, however the lymphocyte percent decreased levels in the chlorpyrifos treatment group. Monocyte, eosinophil, and basophil percent remained unaltered in both groups.

Kafle *et al*., (2018) observed the subchronic profenofos toxicity in broiler birds. The study noted that the mean values of the haemoglobin, Total erythrocyte count and total leucocyte count of treatment group were significantly increased from 3rd week of experiment. Differential leucocyte count reveals that mean percentage of the lymphocyte was decreased significantly, whereas the mean percentage of the heterophil was significantly increased throughout the experiment.

Kulthe *et al.,* (2018) examined chlorpyrifos induced subacute toxicity in Japanese quails. The study noted that there was significant alterations in haematological parameters like mean Hb, PCV, TEC and mean values of TLC in toxin treated group. However, the absolute heterophil and lymphocyte counts marked decline as compare to healthy control group.

Shingumare (2018) studied on ameliorative effect of *Swertia chirata* in o, s- dimethyl acetyl phosphoramidothioate induced toxicity in male Wistar rats. The study observed significantly elevated BCT (Blood Clotting Time) at 14th and 28th day of experiment in toxicated rats as compared to rats in healthy control group.

Londhe (2018) studied subacute toxicity of o,s dimethyl acetyl phosphoramidothioate in female Wistar rats. In which she observed significantly increased BCT (Blood clotting time) at 28th day of experiment in rats of toxicated group as compared to BCT levels in rats of healthy control group.

Hussain *et al*., (2019) examined the serum biochemical alterations due to effect of trichlorfon in adult cockerels. The study observed that the mean values of total erythrocyte count, haemoglobin concentration and haematocrit values were significantly decreased in treatment groups, throughout the experimental period. Whereas, the total leucocyte count were significantly elevated in toxin treated groups as compared to control group.

2.4 Serum biochemical alterations in subacute oral toxicity of OP compounds in poultry:

Garg *et al.,* (2004) studied the chronic toxicity of Monocrotophos (OP Pesticide) and its pathophysiological effects in Broiler Chicks. The study revealed elevated levels of serum alkaline phosphatase in Monocrotophos treated birds. However, the Monocrotophos treated birds were showed significant decrease in the serum total protein levels throughout the experiment.

Kammon *et al.,* (2010) examined the biochemical parameters with related to hepatotoxicity and nephrotoxicity in chlorpyrifos (CPF) toxicated layer chickens. The biochemical parameters of chlorpyrifos toxicated chickens showed significant elevation in serum glucose, AST, AKP, ALT and uric acid levels, as compared to control group. Whereas, the plasma levels of the total protein and albumin were unaffected in treatment group. The study also revealed the significant inhibition of the cholinesterase enzyme in the CPF treated group.

Kammon *et al*, (2011) conducted an experiment to study the chronic toxicity of chlorpyrifos in broilers. They administrated chlorpyrifos compound at the rate of 0.8 mg/kg body weight orally, which was $1/50^{th}$ of LD_{50}. Evaluation of the biochemical parameters was done on two intervals i.e. at 24^{th} day and 45^{th} day of experiment. They found that there was no significant change in activity of serum AChE in broiler chickens administered with chlorpyrifos. There was non-significant increase in levels of serum AST and ALT in chlorpyrifos-treated group. Also, chlorpyrifos did not significantly influence the activity of serum AKP. However, serum AKP was found higher on 24^{th} day than on 45^{th} day due to increased bone development. Whereas, the uric acid and creatinine kinase showed non-significantly elevated values in chlorpyrifos treated group. Chlorpyrifos did not produce significant changes in serum levels of glucose, cholesterol, total protein and albumin.

Ahmad *et al.,* (2015) studied Impact of chlorpyrifos at the dose rate of 20mg/kg bwt on broiler chicks. They found that the total protein values did not alter significantly in different groups. However, serum albumin values were

altered significantly in the chlorpyrifos treated group as compared to control. Whereas, the serum globulin was altered non-significantly throughout the experiment. Serum ALT values were recorded higher in the chlorpyrifos treated group at day 14^{th} of the experiment. Chlorpyrifos treated group showed significantly decreased values of AChE as compared to control group at experimental days 7^{th} and 14^{th}. Also, they stated that 5 mg/kg BW dose of chlorpyrifos might decreases AChE values in serum and plasma on experimental day 14^{th}.

Begum *et al.,* (2015) studied the chronic chlorpyrifos intoxication in indigenous chicken and examined the hematobiochemical changes in these. The chlorpyrifos treated group showed significantly changed mean values of ALP, AST, and ALT as compared to control group. Also, there was significant inhibition of cholinesterase enzyme in treatment group. The serum uric acid levels of treatment group showed markedly elevated values. Conversely, the mean values of total protein left unchanged as compared to control group.

Singh *et al.,* (2016) recorded the pathological effects of sub-chronic chlorpyrifos toxicity in broilers. The chlorpyrifos toxicated group was showed significant increase in mean values of AST and ALT. Furthermore, there were significant elevation in glucose, urea and uric acid concentrations of treatment group. However, the mean values of serum total protein, albumin and globulin were significantly decreased in treatment groups as compared to the control.

Wani *et al.,* (2017) performed the chlorpyrifos induced oxidative stress in broilers. The biochemical examination revealed the hypoproteinemia, hypoalbuminemia with decreased albumin:globulin ratio in chlorpyrifos treated group. Though there were increase in serum ALT and serum AST values of toxin treated group.

Khudair *et al.,* (2017) examined the biochemical changes of chlorpyrifos poisoning in local layer hens, which showed that the serum AST and ALT levels were significantly increased in chlorpyrifos treated group. However, there was no significant change in the serum ALP values in chlorpyrifos treated groups. Serum uric acid and creatinine values were significantly increased in chlorpyrifos treated

group. Whereas, serum triglyceride and total Protein levels showed significant decrease in intoxicated group when comparing with control group.

EL-Nahhal and Lubbad, (2018) observed effects of low concentrations of chlorpyrifos (0.1 mg/kg/day), diuron (0.1 mg/kg/day), and their combination (0.05 mg/kg/day each compound) on chicken, which was introduced daily for two weeks of period. There was non-significantly increase in serum ALT, AST, and ALP values in treatment groups. However, group treated with combination of chlorpyrifos and diuron showed highest serum ALP and ALT levels. Chlorpyrifos treated group showed the significant effects on uric acid and total protein levels. Diuron showed the lowest effect on uric acid levels and the potent effect on urea levels. Statistical analysis revealed a significant difference between the control group and treatment groups (chlorpyrifos and diuron treatment group) in the activity of serum ACHE.

Kulthe *et al.,* (2018) examined chlorpyrifos induced subacute toxicity in Japanese quails. The study stated that there was non-significant alterations in mean serum total protein, albumin, globulin and A:G ratio in toxin control group as compared to control group. Whereas the serum creatinine, ALT, AST and GGT were statistically elevated in toxin treated group.

2.5 Clinical signs and symptoms in oral toxicity of OP compounds in poultry:

Mohammad *et al*., (2008) conducted acute oral toxicity of chlorpyrifos, diazinon, and dichlorvos in broiler chicks. Toxicity signs were observed within two hours after dosing of chlorpyrifos, diazinon, and dichlorvos. Marked salivation, lacrimation and gasping were observed in toxicated chicks. Frequent defecation and drooping of wings were also noticed in treatment groups. Tremors, convulsions and recumbency were consequenced to death of broiler chicks in treatment group.

Kammon *et al.,* (2010) studied Pathological alterations in chlorpyrifos and imidacloprid toxicated layer chickens. Layer chickens showed signs of toxicity within 2 hours of administration of chlorpyrifos (Dose of 55 mg/kg orally). The clorpyrifos treated birds showed signs like sluggishness, watery diarrhoea and marked salivation leading to change in stance and drooping of wings. The

chlorpyrifos treated chickens were unable to stand. Convulsions were significantly seen before death of the chlorpyrifos treated chickens.

Ghaffar *et al.,* (2014) observed the triazophos (OP Compound) in Japanese quail. The study revealed clinical signs like ruffled feathers, tremors, watery droppings, salivation, torticollis and less foam production. However, decreased frequency of crowing and mating with their pen mates were also observed at high doses of triazophos as compared to the control group.

Ahmad *et al.,* (2015) observed the effects of chlorpyrifos toxicity on health biomarkers of broiler chicks. The study was mentioned the clinical signs like salivation, lacrimation, gasping, frequent defecation, tremors and convulsions, which were observed in treatment groups as compared to control group.

Begum *et al*., (2015) recorded the chronic signs observed in chlorpyrifos toxicated indigenous chickens. The chlorpyrifos treated chickens were seems to be active and alert. Signs observed after 2 months of treatment were staggering gait, leg weakness, pale mucous membrane and curled toes appearance. However, tremors and diarrhoea were also noticed in chlorpyrifos treated group.

Singh *et al*., (2016) performed sub-chronic chlorpyrifos toxicity in broilers. The study revealed clinical signs like diarrhoea, reduced appetite gasping, dullness, depression, listlessness, difficult breathing, inability to stand, stiffness, incoordination in movement, muscle twitching, enlarged joints, scaly skin, reduced growth and ruffled feathers. These signs were observed mainly in toxin treated groups as compared to control group.

Wani *et al*., (2017) examined the chlorpyrifos induced oxidative stress in broiler chickens. The study noted clinical signs like weakness, inappetence, depression, dullness, lethargy, ruffled feathers and change in behaviour as compared to the control group. They study also found that the mentioned signs were observed after four weeks of experiment.

Kafle *et al.,* (2018) studied pathological alterations caused due to profenofos toxicity in broiler birds. The signs observed within 3 hr after administration of profenofos and they found the clinical signs like depression,

ruffled feather and hurdling. However, some birds also showed hypersalivation, drooling of wings and diarrhoea. Incoordination and convulsion in treatment group might proceed to finally death.

Hussain *et al*., (2019) examined the pathological alterations due to effect of trichlorfon in adult cockerels. The study observed the clinical signs included watery droppings, tremors, less frequency of crowing, depression and salivation in toxin treated groups as compared to control healthy group.

2.6 Alterations in Absolute organ weight in subacute oral toxicity of OP compounds:

Garg *et al.,* (2004) examined the chronic toxicity of organophosphate pesticides in broiler chicks. They were observed the significant decrease in relative weight of the lymphoid organs (spleen and bursa of fabricius) in monocrotophos (OP compound) treated group.

Naraharisetti *et al.,* (2009) in a 28 day study, inspected the effect of Malathion (OP Compound) in broiler chickens. They observed the significant decrease in the absolute weights of the liver, kidney and heart were significantly decreased in Malathion treated group. However, there was no significant difference in absolute weight of brain in Malathion treated group.

Shahzad *et al*., (2013) observed immuno-pathologic effects of oral administration of chlorpyrifos (OP Compound) in broiler chicks for the experimental period of 45 days. Study showed that there was significant reduction in the relative weights of spleen and bursa of chlorpyrifos treated birds as compared to control group.

Al-Baggou, (2014) examined effects of subacute chlorpyrifos toxicity through drinking water in day-old Ross chicks. The study revealed that the chlorpyrifos treated group was indicated significant increase in mean brain weights. Whereas on 14^{th} day of experiment, mean liver weights were significantly decreased in chlorpyrifos treated group.

EL-Nahhal and Lubbad, (2018) observed the chlorpyrifos toxicity in chicken. They were found that the chlorpyrifos toxicity was resulted into the significant increase in weight of the heart. Further they were clarified that the increased weight of heart was due to the hypertrophy. Additionally, they were observed the decreased weights of liver in chlorpyrifos treated groups.

Hatipoglu *et al.,* (2008) observed the subacute oral toxicity of endosulfan in male New Zealand white rabbits. The study were noted that the significant increase in absolute weights of liver in endosulfan treated rabbits group. Whereas, the absolute weights of kidney, lungs, heart and testis were statistically at par with healthy control group.

2.7 Gross and histopathological changes in subacute oral toxicity of OP compounds in poultry:

Kammon *et al.,* (2010) studied on the Patho-biochemical alterations against chlorpyrifos toxicity in layer chickens. They introduced chlorpyrifos (CPF) toxicity at the dose of 55 mg/kg b.wt. orally. Liver and kidneys were with generalized ecchymotic hemorrhages and pale areas on outer surfaces. Microscopically, liver tissues showed degeneration, coagulative necrosis and hemorrhages. Ecchymotic hemorrhages and vacuolar degeneration of tubular epithelial cells were also observed in kidneys.

Kammon *et al*, (2011) conducted an experiment to observe the toxicity of chlorpyrifos in broilers. They administrated orally chlorpyrifos compound at the dose rate of 0.8 mg/kg (b.wt.), which was $1/50^{th}$ part of LD_{50}. The gross lesions observed in chickens administered chlorpyrifos were pale and flaccid consistency of liver with significant hepatomegaly. Histopathologically, mild necrosis of glandular cells and accumulation of exfoliated cells in the lumen of proventricular glands. Intestines revealed mild degenerative changes, sloughing of epithelial cells were significantly observed in intestine, which was mainly due to the mild necrotic changes in intestinal wall. Liver sections of Chlorpyrifos treated group Showed significant vacuolar degenerative changes. Degenerative Changes like fatty changes and coagulative necrosis were also significantly seen in

histopathological sections of liver. Kidneys were with significant changes such as mild proliferative glomerulitis and moderate necrosis of glomeruli. Mild degenerative changes were observed in pancreas.

Khudair., (2017) examined the histopathological changes of chlorpyrifos poisoning in local layer hens, which showed that are characterized by the presence of necrotic areas and deposition of hemosiderin in the spleen. Severe congestion and haemorrhages were observed in the mucosal layer of intestines. Lungs were with significant edematous and haemorrhagic changes. Degenerative changes were seen in columnar epithelial layer of proventriculus with infiltration of inflammatory cells.

Kafle *et al.,* (2018) observed histopathological changes against profenofos compound toxicity in broiler birds. Histopathologically, liver was with significant congestion, haemorrhagic changes. Also, vacuolar degeneration of hepatocytes and focal mononuclear cells infiltration were observed in liver sections. Focal coagulative necrosis was observed in kidney. Tubular epithelium of kidney were seen with degenerative changes such as cellular swelling and mild vacuolar degeneration. Lungs were with focal areas of pneumonia and alveolar capillary congestion.

Rhayf *et al*., (2012) observed the dimethoate induced histopathological alterations in local layer chickens. The study noted the severe histopathological changes associated with liver, kidney and brain in treatment group. Histopathology of liver included the swelling of hepatocyte including cytoplasm vaculation furthermore lymphocytes infiltrations were observed surrounding portal vein with focal necrosis. Kidney was marked haemorrhage and congestion and severe degenerative changes of tubular epithelial layer indicated by sloughing of tubular epithelial. However, brain showed vacuolated spaces in glial cells, gliosis and finely branching small blood vessels in toxin treated group.

Sodhi et al., (2008) recorded hepatopathy induced by Malathion in chicks. The study observed the liver for histopathological alterations showed moderate to severe necrotic and degenerative changes in Malathion toxicated group. Whereas, bile duct proliferation and congestion of hepatic sinusoids with

lymphomononuclear cells infiltration were also significantly seen in the toxin treated group.

Begum *et al.,* (2015) studied the chronic chlorpyrifos (CPF) intoxication in indigenous chicken and examined the histopathological changes. The histopathological examinations of liver revealed mild congestion with hemorrhages, mononuclear cell infiltration and degenerative changes along with focal areas of hepatocellular necrosis in CPF treated group as compared to control group. However, the mild renal histopathlogical changes were noted in CPF treated group up to 3rd week of the experiment. However, from 4th weeks ahead cellular swelling, necrosis, tubular degeneration and focal to diffuse haemorrhages were observed as marked renal changes.

Hussain *et al*., (2019) examined the pathological alterations due to effect of trichlorfon in adult cockerels. From 45th experimental day onwards histopathology of testis showed markedly congestion with arrest of process of the spermatogenesis and presence of necrotic spermatids in the lumen of the seminiferous tubules. Whereas, the liver was noted the microscopic lesions included pyknosis, fragmentation and disintegration of nuclei of the hepatocytes were seen in the toxin treated groups. The study also noted the microscopic lesions of the kidney such as necrosis of tubular epithelium with presence of necrotic epithelial cells in lumen of the renal tubules, while congestion was noted in all organs (testis, liver and kidney) microscopically in treatment groups.

Shahzad et al., (2013) observed pathologic effects of oral administration of chlorpyrifos (CPF) in broiler chicks. Chicks in Treatment group with higher dose of CPF (20 mg/kg BW) were showed mild congestion, increased inter-follicular connective tissue proliferation, and moderate cytoplasmic vacuolation; however Demarcation between cortex and medulla was lost with oedema fluid accumulation in bursa on microscopic evaluation. Whereas, the microscopic examination of spleen reveals cytoplasmic vacuolation and congestion degenerative changes as well as increased hyperplasia among reticular cells in CPF treated groups as compared to the control group.

2.8 About *Tephrosia purpurea*:

2.8.1 Origin:

Tephrosia purpurea is a species of flowering plant in the pea family (Fabaceae) that has a pantropical distribution. It is a common wasteland weed. In many parts it is under cultivation as green manure crop. It is found throughout India and Sri Lanka in poor soils. *Tephrosia purpurea* is a self-generating erect or spreading perennial herb found throughout India. It can be found as an ingredient in traditional herbal formulations. Phytochemical investigation of *Tephrosia purpurea* revealed the presence of rotenoids, isoflavones, flavonoids, glycosides, flavanones, chalcones, flavanols and sterols *(*Gora *et al*., 2014). Due to its rich content of flavonoid and polyphenol*, Tephrosia purpurea* can be used for the treatment of various clinical conditions such as jaundice, diarrhoea, rheumatism, asthma and urinary disorders (Mathews *et al.,* 2012*).*

2.8.2 Taxonomy and botanical classification:

Sr. No.	Taxonomical classification	
1.	**Kingdom:**	Plantae
2.	**Subkingdom:**	Angiosperms
3.	**Class:**	Eudicots
4.	**Subclass:**	Rosids
5.	**Order:**	Fabales
6.	**Family:**	Fabaceae
7.	**Tribe:**	Millettieae
8.	**Genus:**	*Tephrosia*
9.	**Species:**	*T.* ***purpurea***
10.	**Binomial name:**	*Tephrosia* ***purpurea***

2.8.3 Common names of *Tephrosia purpurea* are:

Fish poison, Wild indigo (English); **Sarphonk, Sharpunkha** (Hindi); **Sharpankha, Unhali** (Marathi);**Masa** (Rajasthani); **Kolinchi, Kollukkai Velai, kaaivelai** (Tamil); **Vempali , Pampara chettu** (Telugu); **Jangli neel** (Bengali);

Kozhinnila (Malayalam); **Kaggi** (Kannada); **Sarapunkha** (Sanskrit); **Sarphoka, Sarphooka, Sarphuka** (Urdu); **Ghodakan** (Gujarati) are the common names of *T. purpurea* plant in different parts of India.

2.8.4 Morphological characters:

The plant is a polymorphic, greenish Grey coloured, much branched subtract, perennial herb of 30-60 cm height. Leaves are imparipinnate, 5-15 cm long leaflets, 9-21 narrow, oblanceolate, green and glabrous above and obscurely silky beneath. Flowers are red or purple in leaf -opposed racemes (Gopalakrishnan *et al*., 2009). Flowers are about 7 mm long with stalks are 3-4 mm long; bracts about 2 mm long. Calyx is 3-4 mm long, velvet-hairy; sepals tapering to a point. Pods are 2.5-4 cm long, 3-4 mm broad, linear-oblong, 5-7-seeded. Seeds were ellipsoid and dark brown in colour.

2.8.5 Medicinal properties of *Tephrosia purpurea*:

Sr. No.	Useful part	Effect observed	Work done by
1.	**Roots**	Anti-ulcer activity	Deshpande *et al*., (2003)
		CNS depressant and analgesic	Valli *et al*., (2011)
		Cytotoxic	Sandhya, (2011)
		Anti-pyretic, anti-inflammatory	Valli *et al*., (2011)
2.	**Leaves**	Antihyperlipidemic	Akhthar and Ahmad (2011)
		Hepatoprotective	Jain *et al*., (2006)
		Nephroprotective and curative	Jain *et al.,* (2009)
		Attenuates pain and inflammation	Gulecha and Sivakumar (2011)
3.	**Aerial parts**	Hepatoprotective	Murthy *et al.,* (1993)

(Mathews *et al.*, 2012)

2.8.6 *Tephrosia purpurea* phytochemical properties:

Jain *et al.,* (2006) observed the phytochemical properties of the *Tephrosia purpurea.* They found that the phytochemical investigation of *Tephrisia purpurea* shown presence of the coumarins, flavonoids and rotenoids, flavanones and isoflavanones and quercetin.

Verma *et al.,* (2017) performed the phytochemical examination of the methanol extract of *Tephrosia purpurea* stem. They revealed that the methanol extract of *Tephrosia Purpurea* stem contains flavonoids, phytosterols, alkaloids

and proteins. Further, they stated that the glycosides and terpenoids were absent in *Tephrosia purpurea* stem.

Kumar *et al.,* (2019) analysed the phytochemicals in aqueous extract of *Tephrosia purpurea* whole plant. The study showed that aqueous extract of *Tephrosia purpurea* contains glycosides, terpenoids, tannins and flavonoids. Also, they were noted the presence of phenols and saponins in the aqueous extract of the *Tephrosia purpurea.*

Mathews *et al.,* (2012) conducted a basic research on the herb *Tephrosia purpurea*. In which they stated that alkaloids, saponins, glycosides, tannins and flavonoids were the ingredients of *Tephrosia purpurea*. Further, the study reveals that the constituents in *Tephrosia purpurea* reduces the toxicity or stimulate the action via synergistic activity.

Khatri *et al.,* (2009) performed the phytochemical analysis of *Tephrosia purpurea* they discovered the presence of the glycosides (rutin, quercetin and osyritin); retenoids (deguelin, elliptone, rotenone and tephrosin); flavonoids (lanceolatin, purpurin, purpurenone and purpuritenin); sterols for example β-sitosterol. Also, the study explained other constituents of the *Tephrosia purpurea*, which includes isoflavone, 7, 4 -dihydroxy-3, 5 - dimethoxyisoflavone, and a chalcone and tephropurpurin.

Gopalakrishnan *et al* (2009) studied the phytochemical and pharmacognostical screening of the *Tephrosia purpurea*. They noted that the presence of reducing sugars, phrnolic compounds, saponins, tannins and flavonoids in aqueous extract of the aerial part of *Tephrosia purpurea*. Further, the study revealed about the occurrences of alkaloids, phenolic compounds, saponins and flavonoids in ethanol extract of the *Tephrosia purpurea* aerial part.

2.8.7 Hepatoprotective properties of *Tephrosia purpurea*:

Deshpande *et al*., (2003) antiulcer activity of *Tephrosia purpurea* in indomethacin induced gastric ulceration in rats. They stated that the aqueous extract of *T. purpurea* had significant anti-ulcer effect but not ant secretory effect on gastric mucosa of rat.

Jain *et al., (*2006) studied the hepatoprotective activity of ethanol extract of *Tephrosia purpurea* leaves. They studied the hepatoprotective activity of the extract in CCl4 induced hepatotoxicity rats. They used *T. purpurea* leaves extract at the dose rate of 100mg/kg bwt.daily and found that the *Tephrosia purpurea* leaves extract showed significant hepatoprotective activity against the CCl4 induced toxicity rat.

Pavana *et al*., (2007) studied antihyperglycemic and antilipidperoxidative effects of *T. purpurea* seed extract in streptozotocin induced diabetic rats. The study observed antidiebetic and anti-hyperglycemic activity of *T. purpurea* in which they noted the significantly improved blood glucose levels in *T. purpurea* fed rats as compared to levels in diabetic control rat group.

Khatri *et al., (*2009) evaluated the hepatoprotective activity of aerial parts of *Tephrosia purpurea* on Wistar albino rats. They prepared Aqueous- alcoholic extract of *Tephrosia purpurea* and introduced in thioacetamide-induced hepatotoxicity rats. They administered this extract at the dose rate of 500 mg/kg b.wt.daily. Finally, they concluded that *Tephrosia purpurea* acts as a potential antioxidant, which showed significant hepatoprotective activity. They were mentioned the LD_{50} value of the ethanolic extract of aerial part of the *Tephrosia Purpurea,* which was 5.12 gm/kg.

Jain *et al.,* (2013) studied the nephroprotective effect of *T. purpurea* leaves on gentamicin induced toxicated rats. The study noted the significant ameliorative effect of *T. purpurea* in renal histo-architectural protection and also observed the improved BUN and serum creatinine levels in rats from *T. purpurea* fed group as compared to levels in rats from toxicated group.

Dalwadi *et al*., (2014) reviewed the phytochemistry and pharmacological properties of *Tephrosia purpurea* plant in which they noted the hepatoprotective, anti-cancerous, anti-hyperglycemic, anti-pyretic, anti-oxidant and anti-inflammatory properties of the *T. purpurea* leaves.

Gora *et al*., (2014) observed hepatoprotective activity of *Tephrosia purpurea* against arsenic induced toxicity in wistar albino rats. They used aerial parts of *Tephrosia purpurea* extract in hydro- alcoholic solution at the dose rate

of 500 mg/kg/b.wt. They stated that *Tephrosia purpurea* had the free radical scavenging activity and hepatoprotective activity due to presence of poly-phenolic compounds and flavonoids in leaves. They observed that *Tephrosia purpurea* decreases oxidative stress and protects the tissues from oxidative damages. This were observed by the significant increase in body weight of *Tephrosia purpurea* extract (TPE) treated group. Authers concluded that the *Tephrosia purpurea* plant extract could significantly ameliorate the hepatotoxicity by reducing oxidative stress.

Verma *et al.,* (2017) evaluated hepatoprotective activity of *tephrosia purpurea* stem. They performed CCl_4 toxicity in Wistar rat. They prepared Methanol extract of *T. purpurea* stem. They found that the stem of *T. purpurea* contains flavanoids, phytosterols and alkaloids compounds. They observed that 150 mg/kg b.wt. PO dose of the extract showed significant hepatoprotective effects. However, they found that *T. purpurea* stem extract was safe upto 2000 mg/kg b.wt. PO in wistar rats.

Lipinski *et al*., (2019) studied the effect of Superliv herbal feed additive (Ayurvet; Delhi, India) on the growth performance, carcass characteristics and meat quality of Ross 308 broiler chickens. *Tephrosia purpurea* is the major content of the Superliv herbal feed additive. The herbal feed additive were supplemented @ dose rate of 500 gm/ tonne of feed (0.05 % of the feed). The treatment group supplemented with herbal additives showed significant increase in the growth performance.

Materials and Methods

CHAPTER: - 3

MATERIAL AND METHOD

PLAN OF RESEARCH WORK

The present experiment was planned to study the sub-acute oral toxicity of profonofos in Gramapriya birds through oral gavage for 28 experimental days. Also, hepatoprotective effects of *Tephrosia purpurea* were assessed in toxicated birds for 28 experimental days. Birds were acclimatized to experimental room for 7 days of period. At different time intervals (0^{th}, 14^{th} and 28^{th} day of experiment) the blood was collected and processed for hematological and biochemical studies. Also, the Gross & histopathological observations in the selected vital organs were recorded at the time of sacrification of birds.

3.1 Shed preparation:

After selection of proper poultry shed for experimental trial, dry cleaning was carried out by scrubbing and sweeping the floors and walls of shed. Which was essential to remove out the dirt and reduce the residual infection. The wet cleaning and washing were carried out by detergent and plain water, whereas the further disinfection was performed with phenyl. It included the cleaning and disinfection of the poultry shed, Instruments and equipment (feeders, chick guards, waterers, chick plates and water storage). After these cleanings flame gun was used to burn the floor, side walls, wire nets and metal divider.

Four separate pens (25 sq.ft. area) were made with the help of metal divider for each a group was made. Rice husk was used as a litter material. For brooding of chicks, proper arrangement of sufficient light and heat were provided by using electric bulbs in each pen. Before arrival of birds the formalin fumigation was carried out for sanitation of poultry shed and equipment. Arrangement for ad libitum fresh, clean and cool drinking water was carried out five hours before the arrival of chicks. All the precautionary measures against all diseases was taken

throughout the experimental period of 35 days (including acclimatization period - 7 days).

3.2 Experimental birds:

A total of 100 healthy day old 'Gramapriya chicks were procured from Government Central Hatchery; Padegaon, Aurangabad and sheltered in animal house of Department of Veterinary Pathology of College of Veterinary and Animal Sciences, Parbhani. All the chicks were vaccinated with Marek's disease vaccine on the day of hatching at hatchery and maintained till the end of trial. Birds were indiscriminately allotted to the control and treatment groups and were kept in four separate pens. Considering the age groups, birds were given *ad libitum* nutritionally balanced Gavaran feed i.e. starter feed and finisher feed. Also, offered ample quantity of fresh drinking water. In accordance with poultry scientific vaccination schedule, birds were vaccinated against different poultry diseases. On the way to vaccination the minimal stress were maintained in flock with the help of proper scientific management and medicines, which were helped to decrease the chances of vaccination failure (Plate 4).

3.3 Poultry feed:

Nutritionally balanced feed as per its specification for each age group was purchased from reputed commercial feed producer. Approximately 4 hours before scheduled arrival of the chicks, waterers were placed and grinded maize was spread over the newspaper. Grinded maize was continued for first three days afterwards the standard formalised feed was used as feed for the birds throughout the experiment. The starter and finisher gavaran poultry feed were analysed for the proximate principles from AFAQAL laboratory, Nammakkal. Where we found the following values for proximate principles and gross energy of feed used during the experimental trial.

Table 3.1 Proximate Analysis and Total Gross Energy of Starter Gavaran poultry feed

Sr. No.	Feed Analysis Name	Results
1	Moisture	9.75 %
2	Crude Protein	21.86 %
3	Crude Fibre	4.48 %
4	Ether Extract	2.44 %
5	Total Ash	5.70 %
6	Gross Energy	3876 Kcal/kg

Table 3.2 Proximate Analysis and Total Gross Energy of Finisher Gavaran poultry feed

Sr. No.	Feed Analysis Name	Results
1	Moisture	8.04 %
2	Crude Protein	18.07 %
3	Crude Fibre	7.97 %
4	Ether Extract	2.96 %
5	Total Ash	9.66 %
6	Gross Energy	3751 Kcal/kg

3.4 Biochemical kits:

Standard biochemical kits required for estimation of biochemical parameters were procured.

3.5 Procurement of Profenofos Pesticide:

Profenofos was procured from the local market Parbhani

3.6 Physicochemical Properties of Profenofos:

1)	**Chemical Name:-**	O-(4-bromo-2chlorophenyl) O-ethyl S-propyl ester
2)	**Chemical Class:-**	Organophosphate insecticide
3)	**Molecular Formula:-**	$C_{11}H_{15}BrClO_3PS$
4)	**Molecular Weight:-**	373.63 $g \cdot mol^{-1}$
5)	**Odour:-**	Garlic like odour
6)	**Specific Density:-**	1.455 at 20°C
7)	**Physical State:-**	Oily liquid
8)	**Solubility in Water:-**	28 mg/L at 25°C
9)	**Solubility in other solvents:-**	Readily miscible with most organic solvents.
10)	**Colour:-**	Pale yellow to amber colour

3.7 *Tephrosia purpurea:*

Tephrosia purpurea is a species of flowering plant in the pea family that has a pantropical distribution. It is a common wasteland weed and perennial herb found throughout India. The *Tephrosia purpurea* plant was acquired from the local areas of Parbhani and villages surrounding the Parbhani (Plate 1).

3.7.1 <u>Preparation of plant leaves powder:</u>

Tephrosia purpurea plant was obtained from the surroundings of Parbhani (MS) and thoroughly washed with clean water to remove all dust and dirt. Subsequently, the plant leaves were dried in room under controlled environment. Afterwards it will be grinded by using electric grinder. The coarse powder will be prepared from the dried leaves of *Tephrosia purpurea*. This powdered form of *Tephrosia purpurea* plant will be used throughout the experiment (Plate 2).

3.8 Dose and route of administration of profenofos:

Profenofos was given to the treatment group chicks through oral gavage (with water as a vehicle) @ 1.6 mg/kg Bwt. starting from 1st day (after acclimatization period for a week) until end of experiment (Plate 3).

Plate 1: *Tephrosia purpurea* Plant

Plate 2: *Tephrosia purpurea* plant powder

Plate 3 Dosing of a bird (Through oral gavage) with Profenofos (@ 1.6 mg /kg bwt.)

Plate 4: An arrangement made for housing the experimental birds

3.9 Duration of experiment:

The experimental trial was conducted for 35 days in animal house, College of Veterinary and Animal Sciences, Parbhani.

3.10 Acclimatization and grouping:

Acclimatization period for all birds were 7 days. Thus 8^{th} day of chick age was considered as 1^{st} day of experimental trial. Similarly, 21^{st} and 35^{th} day of age were considered as 14^{th} and 28^{th} day of experimental trial, respectively. The birds were distributed equally in four groups i.e. Group I to Group IV.

3.11 Plan of experiment:

A biological experiment (*in vivo* trial) was conducted to study the hepatoprotective effect of *Tephrosia purpurea on* experimentally induced sub-acute toxicity of profenofos in Gramapriya birds.

3.12 Experimental design:

The study was carried out for a period of 28 experimental days in 100 Gramapriya birds. Which were uniformly distributed into four groups of 25 birds in each, as represented in Table 1. The group I was served as healthy control and was given standard feed and water ad libitum for 28 days. The birds of groups II were intoxicated daily with a solution of Profenofos @ 1.6 mg/kg body weight through oral gavage. The birds of group III were treated on plant control and fed with a *Tephrosia purpurea* leaves powder @ 0.1 % of feed daily for 28 days. The group IV was treated with Profenofos @ 1.6 mg/kg body weight daily through oral gavage + *Tephrosia purpurea* leaves powder @ 0.1 % of feed daily. Duration of experiment was 28 days (Table 3.3).

Table 3.3: Details of experimental groups of birds

Sr. No.	Group	Treatments & Dose	No. of Birds
1.	**Group I**	Normal feeding & watering (Healthy control)	25
2.	**Group II**	Profenofos @ 1.6 mg/kg Bwt through oral gavage daily	25
3.	**Group III**	*Tephrosia purpurea* leaves powder @ 0.1 % of feed daily	25
4.	**Group IV**	Profenofos @ 1.6 mg/kg Bwt. through oral gavage daily + *Tephrosia purpurea* leaves powder @ 0.1 % of feed daily	25
		Total	**100**

The birds were reared according to standard managemental practices and also the experimental protocol was get approved from Institutional Animal Ethics Committee (IAEC) before the start of experiment. All the birds in different groups were kept under closed observation during whole experimental period.

3.13 Collection of material

3.13.1 Collection of blood:

Blood collection was carried out from all the experimental birds on 0, 14th and 28th day of experiment from wing/ jugular vein and dispersed in an EDTA vials for hematological and non heparinized vials for separation serum, for biochemical estimations.

3.13.2 Collection of tissues:

Tissues were collected from various organs of the sacrificed birds at the 14th & 28th day of experimental trial and was preserved in 10 per cent neutral buffered formalin. The pieces of suitable thickness of brain, heart, lungs, liver, kidneys, spleen, bursa and intestines were collected for histopathological examination.

3.14 Parameters studied

Various parameters were observed and analysed during study on hepatoprotective effect of *Tephrosia purpurea* against Profenofos induced toxicity in Gramapriya birds. The parameters studied during experiment were body weights, feed consumption, haematological, biochemical estimations, organ weights and gross as well as histopathological alterations in tissues collected.

3.15 Growth performance

3.15.1 Average Weekly Feed consumption (gm/bird):

Measured quantity of feed was fed every 24 hrs and the feed in balance was recorded after 24 hrs. The difference between the feed offered and balanced was calculated to know the actual feed consumed by each group. The feed consumption was expressed as gm/day/group. The calculated feed intake and the mean individual feed intake of each bird in gm/chicks/week were calculated. From this data, daily and weekly feed consumption were recorded in all experimental groups.

3.15.2 Average Weekly Body weights (gm/bird):

Chicks from each group were weighed on day 0 and on weekly intervals by using digital weighing machine. The average body weight gain (gm/bird) was computed at weekly intervals.

3.15.3 Average Weekly Feed Consumption Ratio (FCR):

On the basis of weekly weight gain and weekly feed consumption, the FCR of each group was calculated at weekly intervals as per the following standard formula-

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Average weekly feed consumption per bird (gm)}}{\text{Average weekly weight gain per bird (g)}}$$

3.16 Haematological parameters:

Haematological investigations such as Hb, PCV, TEC, TLC, DLC and clotting time were carried out for all groups at 0, 14th and 28th day of study. The blood samples were collected from the birds in clean, dry and heparinized vials. The details of haematological investigations along with methods adopted during investigations are enlisted in (Table 3.4).

Table 3.4: Haematological parameters studied in birds

Sr No.	Parameters	Method of estimation	Described by
I	Haemoglobin (gm/dl)	Acid hematin method	Jain (1986)
II	Total Erythrocyte Counts (10^6/ mm^3)	Neubaures chamber	Sastry (1989)
III	Packed Cell Volume (%)	Microhaematocrit method	Jain (1986)
IV	Total Leukocytes Counts (10^3/ mm^3)	Neubaures chamber	Sastry (1989)
V	Differential leucocyte counts (%)	Giemsa stain	Weiss and Wardrop (2010)
VI	Clotting Time (Sec.)	Capillary method	Benjamin (1978)

3.17 Biochemical Studies:

On 0, 14th and 28th day of experiment, the blood samples were collected from the birds into clean, dry and sterilized test tubes without anticoagulant and used for separation of serum samples. The same was kept in refrigerator at -20ºc temperature, until analysed.

Biochemical parameters such as Serum Total Protein, Serum Albumin, Serum glucose, Serum AST, Serum ALT, Serum Alkaline phosphatase, Serum urea nitrogen, Serum uric acid and Serum AchE were analysed on 0, 14th and 28th

day of study by using ready to use kits and through semi-automatic biochemical analyser. The methods employed for biochemical analysis are mentioned in Table 3.5

Table 3.5: Biochemical studies conducted during and at the end of experimental trial

Sr. No	Parameters	Method of estimation	Described by
I	Serum Total Protein (gm/dl)	Biuret method	Varley (2005)
II	Serum albumin (gm/dl)	Biuret method	Varley (2005)
III	Serum glucose (mg/dl)	GOD POD method	Tinder (1969)
IV	Serum AST (IU/L)	UK Kinetic method	Teitz (1976)
V	Serum ALT (IU/L)	UK Kinetic method	Teitz (1976)
VI	Serum Alkaline Phosphatase (IU/L)	Kinetic rate method	Robison1 (1923)
VII	Serum urea nitrogen (mg/dl)	Berthelot method	Chaney and Marbach (1962)
VIII	Serum uric acid (mg/dl)	Uricase/POD method	Fossati et *al*.(1980)
IX	Serum AchE (U/L)	Butyrylthicholine method	Henry Hallett Dale(1915)

3.18 Pathological studies:

3.18.1 Absolute organ weight (g):

All the experimental birds from each group were sacrificed on 14th and 28th day of experimental trial. Organs like liver, kidneys, spleen and heart of experimental birds were separated carefully from the carcass and weighed and weights were expressed in grams (g).

3.18.2 Gross pathological examination:

The experimental Gramapriya birds were sacrificed on 14th day and 28th day of experimental trial and critically examined by conducting systematic post mortem examination and gross lesions observed were recorded**.**

3.18.3 Histopathological examination:

After recording the gross lesions the tissue pieces of suitable thickness of brain, heart, lungs, liver, kidneys, spleen, bursa and intestine were collected to evaluate microscopic toxicopathological alterations. The collected tissue samples were fixed and preserved in 10 percent neutral buffer formalin. After fixation the collected tissue pieces were processed as per the standard procedure. Paraffin embedded tissues were sectioned at 3-5µ thickness and stained with routine Haematoxylin and Eosin method (Culling, 1974).

3.19 Phytochemical estimation:

Tephrosia purpurea plant leaves powder examined for phytochemical constituents and some organoleptic properties. Chemical tests for the screening and identification of chemical constituents in the *Tephrosia purpurea* were carried out using the standard protocol (Table no. 3.6) for determination of tannins, saponins, anthraquinones, carbohydrates, steroids, flavonoids, alkaloids, s, terpenoids, phenolic compound and glycosides.

Table 3.6: Tests for the phytochemical analysis of the aqueous extract of *Tephrosia purpurea*

Sr. no.	Test performed	References
1	Tannins	Mace, 1963
2	Saponins	Kokate, 1999
3	Flavonoids	Gavhane, 2016
4	Steroids	Cuilci, 1994
5	Terpenoids	Gavhane, 2016
6	Glycosides	Evans, 1997
7	Alkaloids	Evans, 1997
8	Phenolic compounds	Mace, 1963
9	Anthraquinones	Gavhane, 2016
10	Carbohydrate	Ramakrishnan *et al.*,1994

3.20 Statistical Analysis

The data generated from various parameters were statistically analysed by Two way analysis and Completely Randomized Design (CRD) using WASP (Anonyms, 2018 WASP version 2.0 http: //www.ccari.res.in/wasp2.0/index.php.).

Results and Discussion

CHAPTER - 4

RESULTS AND DISCUSSION

4.1 Growth Performance:

4.1.1 Average weekly Feed consumption (gm/bird):

Mean (± SE) Average weekly Feed consumption (gm/bird) in birds at different intervals of study in different groups are depicted in table 4.1 & fig 4.1.

Table 4.1: Table showing values of weekly feed consumption (Mean ±SE, gm/bird) of experimental birds in different groups of study.

Groups of bird		Mean values of weekly feed consumption (gm/bird) at different intervals of study					CD Values		Statistics
		1st week	2nd week	3rd week	4th week	5th week	1%	5%	
Group I		^{q}84.42 ±1.91	^{p}153.45^{a} ±1.20	^{o}256.23^{b} ±1.86	^{n}493.03^{ab} ±0.95	^{m}579.13^{b} ±1.64	5.92	4.44	HS
Group II		^{q}82.64 ±1.61	^{p}133.24^{b} ±1.11	^{o}241.74^{c} ±2.21	^{n}490.19^{b} ±0.86	^{m}515.00^{d} ±1.80	6.05	4.54	HS
Group III		^{q}85.02 ±0.96	^{p}153.48^{a} ±2.05	^{o}275.68^{a} ±2.37	^{n}494.65^{a} ±1.30	^{m}607.65^{a} ±1.99	6.89	5.16	HS
Group IV		^{q}83.39 ±0.65	^{p}149.57^{a} ±1.65	^{o}238.00^{c} ±2.08	^{n}492.17^{ab} ±0.79	^{m}550.34^{c} ±1.64	5.58	4.17	HS
CD Value	1%	-	5.95	8.22	-	6.81			
	5%	-	4.44	6.14	2.85	5.09			
Statistics		NS	HS	HS	S	HS			

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).
Superscript (a, b, c, d) for column and superscripts (m, n, o, p, q) for rows.
Where,
Group I- Healthy control **Group II -** Profenofos control
Group III –*T. purpurea* control **Group IV-** Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

From 1st to 5th week of experiment the average feed consumption of birds in this group were 84.42±1.91, 153.45±1.20, 256.23±1.86, 493.03±0.95 and 579.13±1.64, respectively. The average feed consumption of birds in group I were statistically significant within weeks of experiment. The mean values of group I (healthy control group) were found in increasing trend throughout the study,

which showed the normal and physiological growth of the birds. It can be concluded that the birds of group I were considered as healthy status in feed consumption as compared to other groups throughout the experiment.

Group II (Profenofos Treatment group):

Week wise comparison:

On 1st to 5th week of experiment the mean feed consumption of birds in group II were 82.64±1.61, 133.24±1.11, 241.74±2.21, 490.19±0.86 and 515.00±1.80, respectively. The average feed consumption values of group II were statistically significant ($P<0.01$) within 1st to 5th week of experiment, which showed the increasing trend in feed consumption of bird as the age of the bird's progresses throughout the experiment.

Group wise comparison:

On 1st and 4th week of study interval the average feed consumption values (82.64 ±1.64 and 490.19±0.86, respectively) were found statistically comparable but numerically lower than mean values of healthy control group and other groups of experiment. However, on 2nd, 3rd and 5th week intervals of experiment the mean values of feed consumption of birds in group II (133.24±1.11, 241.74±2.21 and 515.00±1.80, respectively) were significantly ($P<0.01$) decreased as compare to values in birds of healthy control group and other groups of experiment.

Concluding the study results, the average feed consumption of birds in group II were significantly decreased throughout the experiment as compare to healthy control group and other treatment groups. Our findings were in accordance with Ghaffar *et al.,* (2014), Ahmad *et al.,* (2015), EL-Nahhal, (2018), Hussain *et al*., (2019), Wani *et al.,* (2017) and Begum *et al.,* (2015) who explained decreased feed consumption as a result of OP pesticide toxicity in birds.

Decrease in feed consumption of profenofos fed birds might be due to the toxic effects of OP pesticides (profenofos) and taste aversion caused due to that pesticide as stated by Ghaffar *et al.,* (2014) and Hussain *et al*., (2019). Also, the observations of histopathological alterations in liver and intestinal tissues also supports the reduction in feed consumption of profenofos fed birds.

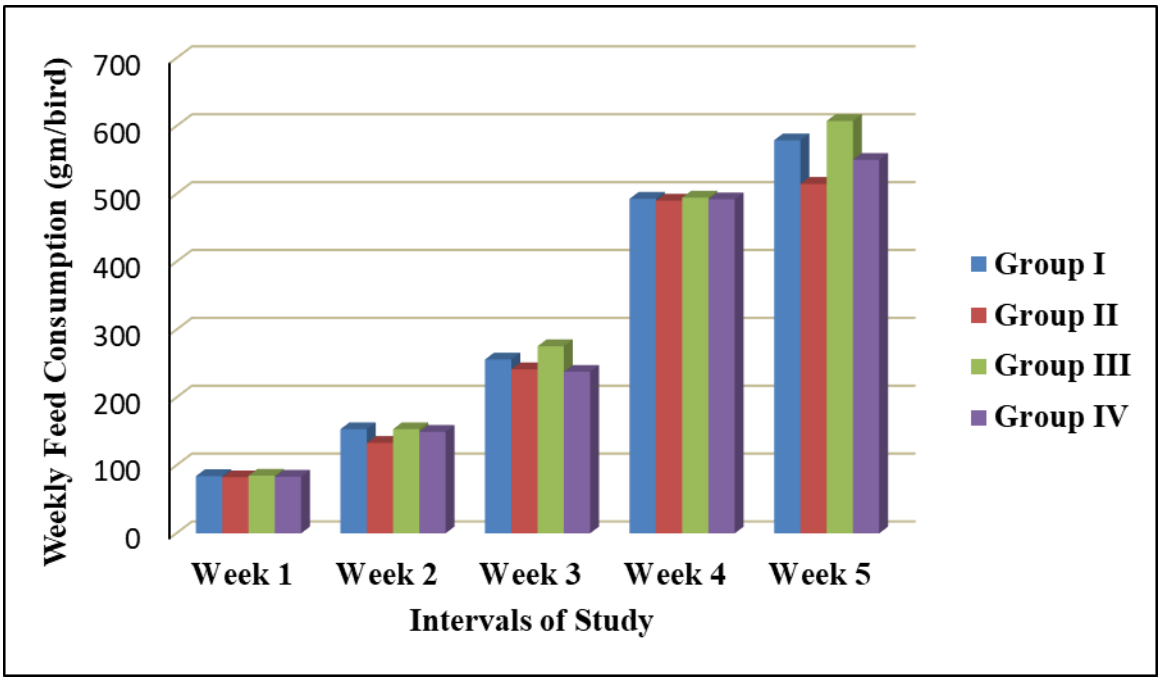

Fig 4.1: Mean values of Average weekly feed consumption (gm /bird) of experimental chicks in different groups of study

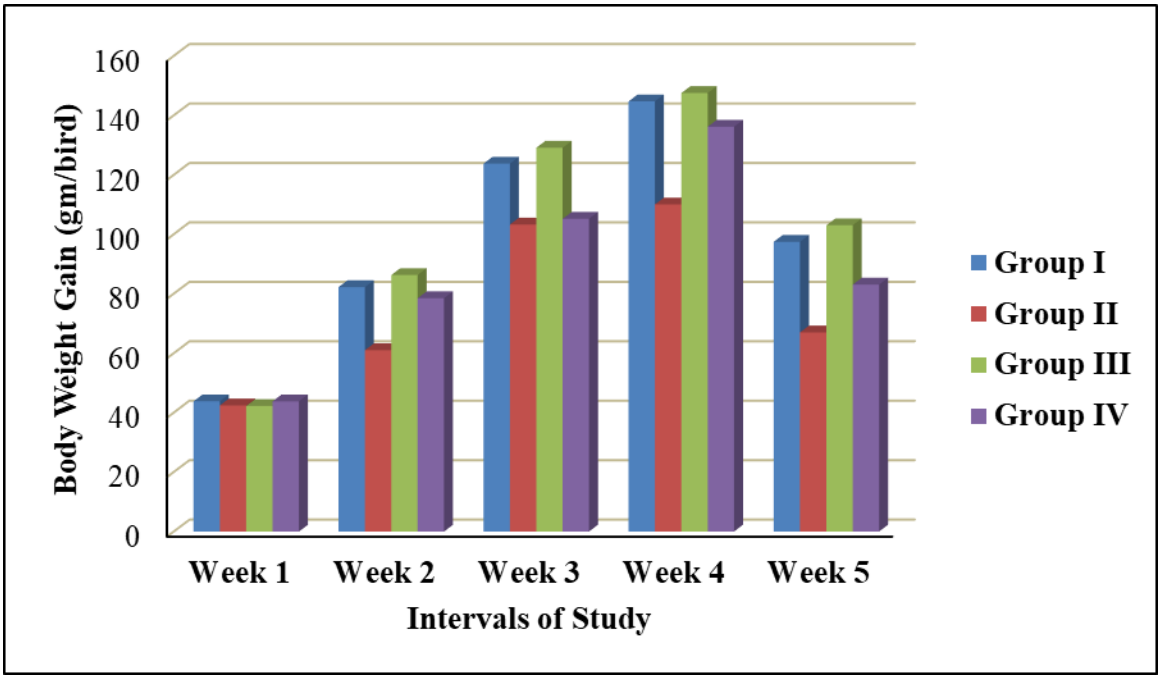

Fig 4.2: Mean values of Weekly Body weight gain (gm/bird) of experimental chicks in different groups of study.

Group III (*Tephrosia purpurea* treatment group):

Week wise comparison:

At 1st to 5th week of experiment the average feed consumption of birds in group III birds were 85.02 ±0.96, 153.48±2.05, 275.68±2.37, 494.65±1.30 and 607.65±1.99, respectively. The average feed consumption values of birds in group III were found statistically differ (P<0.01) within 1st to 5th week of experiment. And this could be due to the age and nutritional requirements of birds which progresses throughout the experiment.

Group wise comparison:

On 1st, 2nd and 4th week of experiment the average feed consumption of birds in group III were statistically at par with the values in healthy control group. Whereas, at 3rd and 5th week of study the average feed consumption values of group III (275.68±2.37 and 607.65±1.99, respectively) were found significantly increased than the values in healthy control group and other treatment groups of this study.

The numerical incline of average feed consumption values of birds in group III might be due to the feeding of *Tephrosia purpurea* through feed of group III birds, which might have exerted positive effects on liver and other visceral organs leads to significant increase in feed consumption of group III birds than healthy control group Lipinski *et al.,* 2019 and Gora *et al*., 2014 were observed significantly improved growth performance in broiler chickens and rats respectively, after addition of *T. purpurea* as feed additive

Group IV (Profenofos + *T. purpurea* treatment group):

Week wise comparison:

On 1st to 5th week of experiment the average feed consumption of birds in group IV birds were 83.39 ±0.65, 149.57±1.65, 238.00±2.08, 492.17±0.79 and 550.34±1.64, respectively. The average feed consumption values of group IV were statistically differ (P<0.01) within 1st to 5th week of experiment, and this accomplished by the increasing average feed consumption values of bird from 1st

to 5th week. This increasing trend could be were due to the age and nutritional requirements of bird which progresses throughout the experiment.

Group wise comparison:

At 1st , 2nd and 4th week of study the average feed consumption values of the birds in group IV (83.39 ±0.65, 149.57±1.65 and 492.17±0.79, respectively) were statistically at par with mean values of the birds in healthy control group and other groups of experiment. Whereas on 3rd and 5th week of experiment the mean values of feed consumption of group IV (238.00±2.08 and 550.34±1.64, respectively) were significantly ($P<0.01$) decreased as compare to values in healthy control group and plant control groups of experiment. Also, the mean feed consumption values of group IV showed numerical incline at 1st, 3rd and 4th week and statistically improved at 2nd and 5th week than values in birds of group II in this experiment.

Grossly, the average feed consumption of birds in group IV showed numerical and statistical incline as compare to toxin control group (Group II). As the *Tephrosia purpurea* leaves powder is hepatoprotective and antioxidant in nature, which might be ameliorate the toxic effects of the profenofos as seen in birds of group IV. Lipinski *et al.,* 2019 and Gora *et al*., 2014 were also explained the hepatoprotective and antioxidant properties of *T. purpurea* in arsenic toxicated rats. The observations of improved histoarchitecture of liver, intestine and kidney of the birds in group IV also supports the progressive increased results of feed consumption of group IV as compared to birds in group II.

4.1.2 Weekly Body weight gain (gm/bird):

Mean (± SE) Weekly Body weight (gm/bird) and Weekly Body weight gain (gm/bird) in birds at different weeks of study in different groups are represented in table 4.2 (A) and table 4.2 (B) & fig. 4.2 respectively.

Table 4.2 (A): Mean (± SE) values of Weekly Body weight (gm/bird) of experimental birds in different groups of study.

Groups of bird		Mean values of Weekly Body weight (gm/bird) in different interval of study					CD Values		Statistics
		1st week	2nd week	3rd week	4th week	5th week	1%	5%	
Group I		^{q}77.07 ±0.92	^{p}159.31^{b} ±0.55	^{o}283.1^{b} ±2.05	^{n}427.83^{b} ±2.93	^{m}525.29^{b} ±1.92	7.13	5.34	HS
Group II		^{q}76.82 ±0.54	^{p}137.83^{d} ±1.00	^{o}241.12^{d} ±1.67	^{n}351.18^{d} ±2.72	^{m}418.18^{d} ±1.69	6.45	4.83	HS
Group III		^{q}77.60 ±0.81	^{p}163.91^{a} ±0.98	^{o}293.04^{a} ±2.38	^{n}440.59^{a} ±1.00	^{m}543.64^{a} ±2.53	6.52	4.86	HS
Group IV		^{q}77.14 ±1.02	^{p}155.65^{c} ±0.67	^{o}260.87^{c} ±2.48	^{n}397.06^{c} ±2.27	^{m}480.20^{c} ±1.87	6.87	5.13	HS
CD Value	1%	-	3.15	8.34	9.04	7.79			
	5%	-	2.35	6.22	6.75	5.81			
Statistics		NS	HS	HS	HS	HS			

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).
Superscript (a, b, c, d) for column and superscripts (m, n, o, p, q) for rows.
Where,
Group I- Healthy control **Group II** - Profenofos control
Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

From 1st to 5th week of experiment the mean body weight gain of birds were 43.82±0.51, 82.24±1.15, 123.79±2.00, 144.72±1.76 and 97.47±1.02, respectively. The mean body weight gain of birds in group I statistically differ within weeks of experiment. The mean values weekly body weight gain in birds of group I (healthy control group) showed progressive increasing trend throughout the study, considered as normal and physiological growth and healthy status in body weight gain as compared to other groups throughout the experiment.

Table 4.2 (B): Average values (Mean±SE) of Weekly Body weight gain (gm/bird) of experimental birds in different groups of study.

Groups of bird		Mean values of Weekly Body weight gain (gm/bird) in different interval of study					CD Values		Statistics
		1st week	2nd week	3rd week	4th week	5th week	1%	5%	
Group I		^{q}43.82 ±0.51	^{p}82.24^{b} ±1.15	^{n}123.79^{a} ±2.00	^{m}144.72^{a} ±1.76	^{o}97.47^{b} ±1.02	5.30	3.98	HS
Group II		^{q}42.43 ±0.91	^{p}61.01^{d} ±0.84	^{n}103.29^{b} ±1.63	^{m}110.06^{c} ±1.66	^{o}67.01^{d} ±1.56	5.21	3.89	HS
Group III		^{q}42.25 ±0.77	^{p}86.31^{a} ±1.79	^{n}129.13^{a} ±2.52	^{m}147.54^{a} ±1.24	^{o}103.05^{a} ±2.01	6.76	5.05	HS
Group IV		^{q}43.80 ±1.14	^{p}78.52^{c} ±1.03	^{n}105.22^{b} ±1.49	^{m}136.19^{b} ±2.10	^{o}83.10^{c} ±1.77	5.92	4.44	HS
CD Value	1%	-	4.83	7.50	6.59	6.28			
	5%	-	3.61	5.59	4.92	4.68			
Statistics		NS	HS	HS	S	HS			

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).
Superscript (a, b, c, d) for column and superscripts (m, n, o, p, q) for rows.
Where,
Group I- Healthy control **Group II** - Profenofos control
Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

Week wise comparison:

At 1st to 5th week of experiment the mean body weight gain of group II birds were 42.43±0.91, 61.01±0.84, 103.29±1.63, 110.06±1.66 and 67.01±1.56, respectively. The mean body weight gain values of birds in group II differ significantly (P<0.01) within 1st to 5th week of experiment, and showed the increasing trend in body weight gain values of bird as the age of the bird's progresses throughout the experiment.

Group wise comparison:

At 1st week of experiment the average body weight gain of the birds (42.43±0.91) were statistically at par as compare to healthy control group and other groups of the experiment. Whereas, from 2nd to 5th week of experiment the

mean body weight gain values of birds in group II 61.01±0.84, 103.29±1.63, 110.06±1.66 and 67.01±1.56, respectively were significantly (P<0.01) decreased as compared to values in birds of healthy control group.

Grossly, the average weight gain of birds of group II were significantly decreased throughout the experiment as compare to healthy control group and other treatment groups. Our results were found similar with Ghaffar *et al.,* (2014), Ahmad *et al.,* (2015), EL-Nahhal, (2018), Hussain *et al*., (2019), Begum *et al.,* (2015) and Naraharisetti *et al.,* (2009), who explained decreased weight gain of birds as a result of OP pesticide toxicity in their studies.

Decreased body weight gain was the one of the significant indicator of OP pesticide toxicity (Naraharisetti *et al.,* 2009). Decrease in average body weight gain of profenofos fed group (group II) might be due to the toxic effects of OP pesticides (profenofos) resulted into decreased feed consumption and in body weight gain of these birds which can be supported by the observations of Ghaffar *et al.,* (2014); Hussain *et al*., (2019). Ahmad *et al.,* (2015) explained that the decrease in body weight might be due to OP pesticide (CPF) induced oxidative stress and the interaction of pesticide with the enzymes and hormones, which were essential for the normal metabolic process. Also, the observations of histopathological alterations in liver and intestinal tissues supports the decreased feed consumption of profenofos fed birds (group II) in this study.

Group III (*Tephrosia purpurea* treatment group):

Week wise comparison:

At 1st to 5th week of experiment the mean body weight gain of birds in group III birds were 42.25±0.77, 86.31±1.79, 129.13±2.52, 147.54±1.24 and 103.05±2.01, respectively. The mean body weight gain values of birds in group III were significantly (P<0.01) varied within 1st to 5th week of experiment, and showed progressively increasing trend in body weight gain values of birds as the age and nutritional requirements progresses throughout the experiment. Also,

mean body weight gain values of birds in group III were found at par with healthy control group.

Group wise comparison:

At 1st, 3rd and 4th week of experiment the average body weight gain of birds in group III were (42.25±0.77, 129.13±2.52 and 147.54±1.24, respectively) found statistically at par as compared to values in healthy control group at these intervals of study. However, at 2nd and 5th week of experiment the mean values of body weight gain of birds of group III were significantly inclined as compared to healthy control group.

Concluding the present study, the numerical incline in mean body weight gain values of group III might be due to the feeding of *Tephrosia purpurea* leaves powder through feed of group III birds, which might positively be the effects of improvement in histoarchitecture of liver and other visceral organs led to significant increase in body weight gain of group III birds. These findings can be well supported by the observations of Gora *et al*., (2014); Lipinski *et al*., (2019) who were observed significantly improved growth performance in broiler chickens and rats respectively, after addition of *T. purpurea* as feed additive.

Group IV (Profenofos + *T. purpurea* treatment group):

Week wise comparison:

At 1st to 5th week of experiment the mean body weight gain of group IV birds were 43.80±1.14, 78.52±1.03, 105.22±1.49, 136.19±2.10 and 83.10±1.77, respectively. The mean body weight gain values of the birds in group IV were found differ significantly throughout the experiment and showed marked progressive elevation throughout the study.

Group wise comparison:

At 1st week of experiment the average body weight gain of birds of group IV (43.80±1.14) were statistically at par as compared to values in birds of healthy control group and other groups of the experiment. Whereas, from 2nd to 5th week of experiment the mean body weight gain values of birds in group IV (78.52±1.03, 105.22±1.49, 136.19±2.10 and 83.10±1.77, respectively) were

significantly (P<0.01) decreased as compared to values in birds of healthy control group. Whereas, the values of body weight gain in birds of group IV were statistically elevated as compared to values in birds of group II throughout the experiment, indicating ameliorative effect of feeding of plant leaf powder in the diet of intoxicated birds.

The improved feed consumption and FCR values of birds in group IV in this study also supported the increased values of body weight gain as compared to body weight gain in birds of group II in experiment, which concluded that the *Tephrosia purpurea* showed the hepatoprotective properties to enhance the appetite and body weight gain positively than the toxin treated group.

As the *Tephrosia purpurea* leaves powder is hepatoprotective and antioxidant in nature which might ameliorate the toxic effects of the profenofos as seen birds of group IV which can be well supported by the Mathews *et al.*, (2012) and Lipinski *et al*., (2019), who were stated hepatoprotective properties of *T. purpurea* as feed additive.

Improved histoarchitecture of liver, intestine and kidney of birds in this group IV were also confirms the progressively inclined results of body weight gain of group IV as compared to birds in group II.

4.1.3 Weekly Feed Conversion Ratio:

Mean (± SE) Weekly Average Feed Conversion Ratio in birds at different weeks of study in different groups are represented in table 4.3 & fig.4.3

Group I (Healthy control group):

From 1st to 5th week of experiment mean values of feed conversion Ratio of birds were 1.93±0.04, 1.87±0.04, 2.07±0.09, 3.41±0.06 and 5.94±0.15, respectively. The average FCR of birds in group I were found statistically differed within weeks of FCR in birds of experiment. The mean values of group I (healthy control group) showed increasing trend throughout the study, and in normal range.

Table 4.3: Mean (± SE) values of Weekly Feed Conversion Ratio of experimental birds in different groups of study.

Groups of bird		Average Weekly FCR in different interval of study					CD Values		Statistics
		1st week	2nd week	3rd week	4th week	5th week	1%	5%	
Group I		^{o}1.93 ±0.04	^{o}1.87^{b} ±0.04	^{o}2.07 ±0.09	^{n}3.41^{c} ±0.06	^{m}5.94^{c} ±0.15	**0.34**	**0.25**	**HS**
Group II		^{p}1.95 ±0.03	^{o}2.18^{a} ±0.07	^{o}2.34 ±0.08	^{n}4.45^{a} ±0.05	^{m}7.69^{a} ±0.13	**0.29**	**0.22**	**HS**
Group III		^{o}2.01 ±0.02	^{p}1.78^{b} ±0.04	^{o}2.13 ±0.08	^{n}3.35^{c} ±0.06	^{m}5.90^{c} ±0.11	**0.26**	**0.21**	**HS**
Group IV		^{p}1.90 ±0.04	^{p}1.90^{b} ±0.06	^{o}2.26 ±0.10	^{n}3.61^{b} ±0.07	^{m}6.62^{b} ±0.07	**0.27**	**0.21**	**HS**
CD Value	**1%**	**-**	**0.21**	**-**	**0.23**	**0.45**			
	5%	**-**	**0.16**	**-**	**0.17**	**0.35**			
Statistics		**NS**	**HS**	**NS**	**HS**	**HS**			

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).
Superscript (a, b, c, d) for column and superscripts (m, n, o, p, q) for rows.
Where,
Group I- Healthy control **Group II -** Profenofos control
Group III –*T. purpurea* control **Group IV-** Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

Week wise comparison:

At 1st to 5th week of experiment the average FCR of birds in group II were 1.95 ±0.03, 2.18±0.07, 2.34±0.08, 4.45±0.05 and 7.69±0.13, respectively. The average FCR values of birds in group II differ significantly (P<0.01) at 1st, 4th and 5th week of experiment when compared to values in control group. Whereas, the average FCR values of birds in group II were statistically at par with the values in control group at 2nd and 3rd week of experiment indicating poor FCR values due to toxic effects and showed the growth in body weight gain, feed consumption and overall FCR values of bird as the age of the bird's progresses throughout the experiment.

Group wise comparison:

At 1st and 3rd week of experiment the average FCR values of birds in group II (1.95 ± 0.03 and 2.34 ± 0.08, respectively) were statistically at par with the values in birds of healthy control group. Whereas, at 2nd, 4th and 5th week of study the average FCR values of birds in group II were significantly elevated as compare to healthy control group and plant control group.

Grossly, the average FCR values of birds in group II were statistically and numerically inclined as compared to values in healthy control group and plant control group throughout the experiment. The results found in our experiment could be well supported by results of Wani *et al*., (2017), who noted significantly decreased average FCR in chlorpyrifos induced toxicity in broiler chicken.

The decreased average feed consumption and body weight gain of birds in toxin control group (group II) in this study might be responsible for the poor average FCR values in these birds is also stated by Hussain *et al*., (2019). Also, the observations of histopathological alterations in liver and intestinal tissues supports the decreased average FCR of profenofos fed birds (group II).

Group III (*Tephrosia purpurea* treatment group):

Week wise comparison:

At 1st to 5th week of experiment the average FCR values of birds in group III were 2.01 ±0.02, 1.78±0.04, 2.13±0.08, 3.35±0.06 and 5.90±0.11, respectively. The average FCR values of birds in group III were statistically significant ($P<0.01$) at 1st, 2nd, 4th and 5th week of experiment. However at 1st and 3rd week of experiment the average FCR of group III were not comparable, which showed the steady growth of group III birds at this week of experiment. The all average FCR values of group III were within normal range and at par with healthy control group.

Group wise comparison:

The average FCR of group III birds were statistically at par with healthy control group throughout the experiment, which might be due to the feeding of *Tephrosia purpurea* leaves powder through feed to birds of group III birds.

Group IV (Profenofos + *T. purpurea* treatment group):

Week wise comparison:

The average FCR values of the birds in group IV (1.90 ±0.04 and 1.90±0.06, respectively) were statistically at par on 1st and 2nd week of experiment. Whereas, at 3rd to 5th week of experiment the average FCR values of birds in group IV were 2.26±0.10, 3.61±0.07 and 6.62±0.07, respectively. The average FCR values of birds in group IV were showed progressive and marked elevation throughout the study.

Group wise comparison:

At 1st to 3rd week of the experiment the average FCR values of the birds in group IV were statistically at par with average values of FCR in birds of healthy control group. However, at 4th and 5th week of experiment the average FCR values of birds in group IV were significantly ($p<0.01$) comparable to values in healthy control group and plant control group. Also, at 4th and 5th week of experiment the average FCR values of birds in group IV showed significantly declined as compare to values in birds of healthy control group.

Overall, the birds of group IV were showed numerically and statistically reduction in values of average FCR as compared to group II birds throughout the experiment. The improved feed consumption and body weight gain of birds in group IV in this study supported the improved values of average FCR when compared to values in birds of group II in experiment, it can be concluded that the *Tephrosia purpurea* had hepatoprotective properties, which positively enhanced the appetite and body weight gain, which in turn led to improved average FCR of birds of group IV than toxin treated group as stated by Gora *et al*., (2014) and Lipinski *et al*., (2019) can support this findings. Moreover, improved histoarchitecture of liver, intestine and kidney of birds in group IV also confirms the inclined results of body weight gain and improved FCR values of group IV as compared to values in birds of group II.

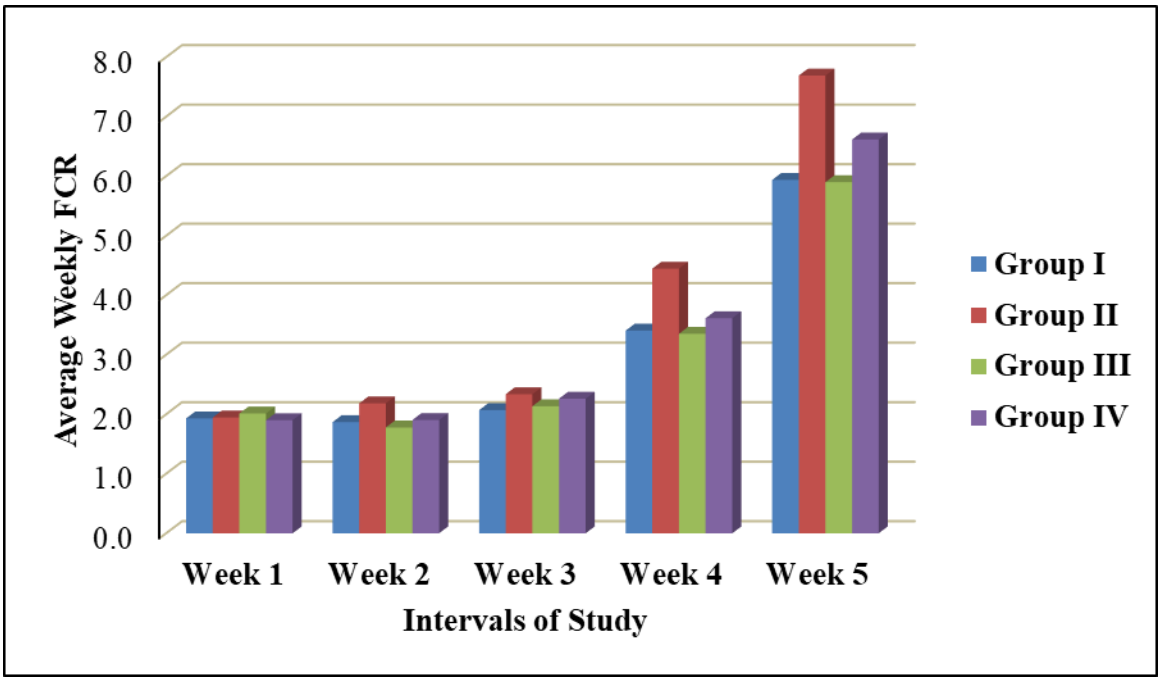

Fig 4.3: Mean values of Weekly Average Feed Conversion Ratio of experimental chicks in different groups of study.

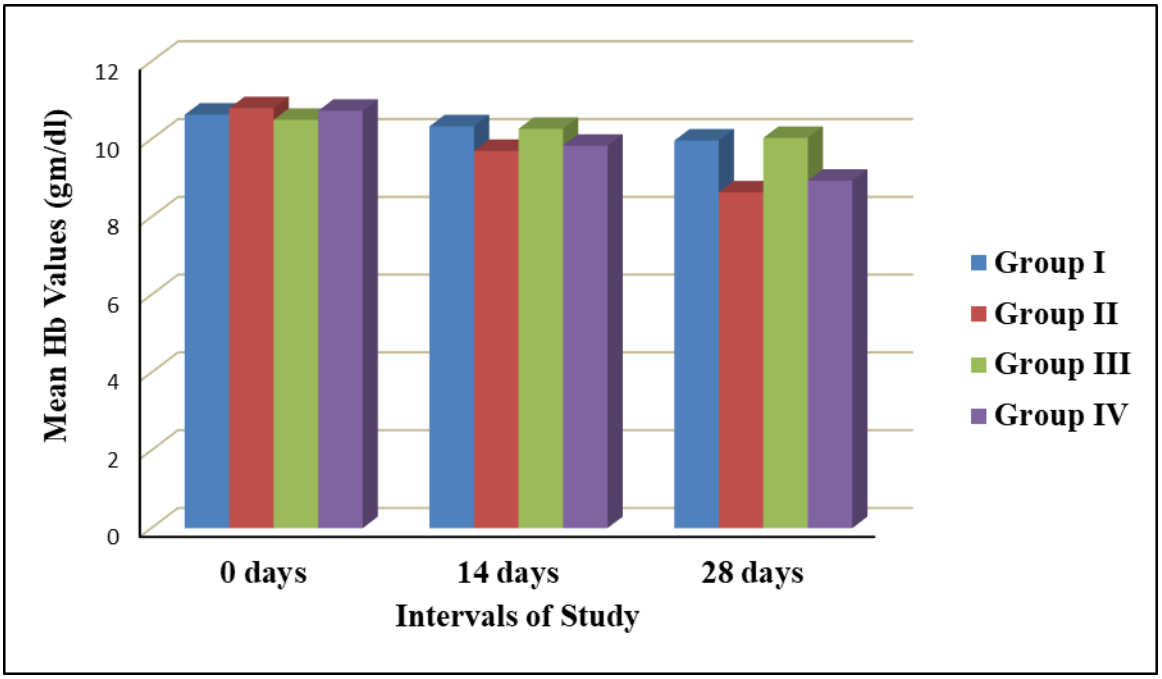

Fig. 4.4: Mean values of Haemoglobin (gm/dl) in different groups at different intervals of study

4.2 Heamatological parameter:

4.2.1 Heamoglobin Concentration (gm/dl):

Mean (± SE) values of Heamoglobin (gm/dl) in birds at different intervals of study in different groups are depicted in table 4.4 & fig.4.4

At 0th day of experiment:

At day 0 of experiment the mean (± SE) values of Heamoglobin concentration of experimental birds in group I to group IV were 10.63±0.17, 10.80±0.13, 10.50 ±0.12 and 10.73±0.11 gm/dl, respectively. The haemoglobin concentration at day 0 were not vary considerably different.

Group I (Healthy control group):

At day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) values of Heamoglobin were 10.33 ± 0.10 and 09.97 ± 0.11, respectively. This values were within normal range and didn't differ significantly. Which represent the healthy status of birds in group I during experimental duration.

Table 4.4: Table showing values of Heamoglobin (Mean±SE, gm/dl) concentration of experimental birds at different intervals of study

Groups of birds		Mean values of Haemoglobin (gm/dl) in different groups (Mean ± SE) at different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		10.63 ±0.17	10.33[a] ±0.10	09.97[a] ±0.11
Group II		10.80 ±0.13	09.70[b] ±0.14	08.63[b] ±0.16
Group III		10.50 ±0.12	10.27[a] ±0.11	10.03[a] ±0.12
Group IV		10.73 ±0.11	09.83[b] ±0.12	08.93[b] ±0.15
CD Values	**1 %**	**-**	**0.486**	**0.548**
	5 %	**-**	**0.351**	**0.404**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

At day 14th of experiment:

The mean (±SE) Heamoglobin value (09.70±0.14) of group II was markedly reduced as associated to both Group I and Group III values. This study showed that the heamoglobin value in group II was highly significant ($p<0.01$) to group I and group III on 14th day interval of experiment. Whereas the mean values of group II and group IV were numerically different to each other.

At day 28th of experiment:

Same as the 14th day results**,** the heamoglobin values at 28th day interval (08.63±0.16) were also significantly reduced ($p<0.01$) than the control group and group III. However mean value of the heamoglobin concentration was not significantly different but numerically decreased than group IV (Profenofos + *T. purpurea*).

The result of significantly decreased heamoglobin concentration in toxin treated groups during experimental trial was correlates with many studies like Rhayf *et al*., (2012); Ghaffar *et al.*, (2014); Ahmad *et al.,* (2015); Hussain *et al*., (2019); Also the study performed by Garg *et al*., (2004) showed numerically reduced haemoglobin concentrations in OP pesticide treated groups.

As the OP pesticide are the potent toxic for blood forming organs such as liver and bone marrow, which may negatively affects the many steps in erythropoiesis process Rhayf *et al*., (2012). Also due to vascular hemorrhages and congestion in visceral organs may cause decreased number of RBCs in blood vascular system Ghaffar *et al.*, (2014). The above reasons might cause the decrease in mean heamoglobin concentration of profenofos toxicity groups in present study. However the markedly decreased serum total protein concentration might resulted in reduced mean heamoglobin concentration (gm/dl) in profenofos treated groups.

Group III (*Tephrosia purpurea* treatment group):

At 14th and 28th day of experiment:

The mean values of the heamoglobin concentration (g/dl) of group III at 14th and 28th day were 10.27±0.11, 10.03 ±0.12, respectively. The mean values of group III were not significantly differ than control group at 14th and 28th day interval of study. This concluded that the mean heamoglobin values of group III were within normal range like the healthy control group. But group III mean heamoglobin values were highly significant to that of group II and Group IV, due to incorporated toxin in these groups.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th day of experiment:

The mean heamoglobin value of the group IV birds (09.83±0.12) was highly significant ($p<0.01$) than group I and group III. Numerically and statistically the mean heamoglobin values of group IV were reduced than control group and group III. However the mean heamoglobin values of group IV were statistically at par with group II but numerically increased than group II mean values.

At 28th day of experiment:

Like the previous interval results here also the mean heamoglobin concentration values of group IV (08.93±0.15) were numerically reduced and highly significant ($p<0.01$) to control group and group III. At 28th day the mean value of group IV was not statistically comparable to group II mean values, whereas the group IV showed numerically increased mean heamoglobin values than group II.

The study concluded that the mean heamoglobin values of group IV birds were numerically towards normal range than that of toxin control group II at 14th and 28th day of experiment. Due to the ameliorative effect and antioxidant property of *T. pupurea,* there might be decrease in oxidative stress in liver of group IV birds. Which might affect the haemoglobin concentration of this group IV. Gora *et al*., (2014) and Mathews *et al.,* (2012) were also reported the

hepatoprotective activity due to presence of poly-phenolic compounds in *T. purpurea* plant.

4.2.2 Packed Cell Volume (%):

Mean (± SE) values of Packed Cell Volume (%) in birds at different intervals of study in different groups are depicted in table 4.5 & fig. 4.5.

At 0 day of experiment:

At day 0 of experiment the mean (± SE) percentage values of Packed Cell Volume (PCV) of experimental birds in group I to group IV were 32.17±0.31, 32.67±0.21, 31.83±0.17, 32.33±0.33, respectively. The packed cell volume percentage values at day 0 did not differ significantly within experimental groups.

Table 4.5: Table showing values of Packed Cell Volume (Mean±SE, %) of experimental birds at different intervals of study

Groups of birds		Mean values (Mean ± SE) of PCV (%) in different groups at different intervals of study		
		0^{th} Day	**14^{th} Day**	**28^{th} Day**
Group I		32.17 ±0.31	32.83^{a} ±0.31	33.17^{a} ±0.31
Group II		32.67 ±0.21	29.00^{c} ±0.26	30.33^{b} ±0.42
Group III		31.83 ±0.17	32.33^{a} ±0.21	33.00^{a} ±0.26
Group IV		32.33 ±0.33	30.17^{b} ±0.31	31.00^{b} ±0.37
CD Values	**1 %**	**-**	**1.108**	**1.384**
	5 %	**-**	**0.809**	**1.016**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).
Where,
Group I- Healthy control **Group II** - Profenofos control
Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

At day 14 and day 28 of experiment:

At 14th day and 28th day of experiment the mean (±SE) percentage values of packed cell volume were 32.83±0.31 and 33.17±0.31, respectively. These values were within normal range and didn't differ significantly. Whereas, the PCV values of group I were at par with the group III at 14th and 28th day of experiment, which represent the healthy status of birds in group I and group III during experimental duration. The flavonoids present in *T. purpurea* leaves powder was with many medicinal, Antioxidant and hepatoprotective property and non-toxic to birds, which might be the reason for healthy liver consequences to normal PCV values in group III birds. Mathews *et al.,* 2012 and Dalwadi *et al*., 2014 were also stated the hepatoprotective activity of *T. purpurea* plant.

Group II (Profenofos Treatment group):

At day 14th of experiment:

Mean packed cell volume percentages of Group II birds were (29.00±0.26) significantly ($P<0.01$) lower than remaining three groups of birds (Group I, Group III and Group IV), at this stage of sacrifice.

At day 28th of experiment:

The mean values (30.33±0.42) of the group II were significantly reduced than group I and group III at 28th day of experiment. However, percentage PCV values of the group II birds were statistically at par with PCV values of the group IV but numerically decreased than PCV values of birds of group IV at 28th day of experiment.

Overall the mean percentage PCV values of group II were reduced than group I, group III and group IV at 14th day and 28th day of experiment.

Our findings were found in accordance with Garg *et al*., (2004), Ghaffar *et al.*, (2014), Ahmad *et al.,* (2015) and Hussain *et al*., (2019); who were also

observed the declined PCV values in OP pesticide toxicated birds. As the profenofos (OP pesticides) have their toxic effect on liver, kidney and bone marrow, which may negatively affects many steps in erythropoiesis process (Rhayf *et al*., 2012). Decreased number of RBCs in blood vascular system due to vascular haemorrhages observed in histopathological sections of various organs (Ghaffar *et al.*, 2014), are suggestive of reduction in mean PCV percentage value in profenofos toxicated group.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th day of experiment:

The mean percentage PCV values (30.17 ± 0.31) at this stage of sacrifice were highly significantly reduced than values in remaining three groups (group I, group II and group III) at this stage of experiment. However, mean PCV values of the group IV were numerically and statistically did not improved up to values in the control groups (Group I and group III) and were improved than the values of toxin treated group (Group II). This indicates that there was partial improvement in altered PCV values by toxin treatment after addition of *T. purpurea* leaves powder in the diet of birds.

At 28th day of experiment:

The mean percentage values of PCV (31.00±0.37) were highly significantly lower than the values in healthy control group (Group I) and plant control group (Group III) at this stage of experiment. However the mean value of group IV were statistically at par with the mean PCV values of the group II at this stage. Numerically the mean PCV values of group IV were improved than toxin treated group (group II) indicating not much effect of plant treatment in profenofos toxicity of birds at this stage of study.

Tephrosia purpurea have flavonoids with antioxidant and free radical scavenging property, which reduces the oxidative stress resulted in improved PCV values as compared to toxin control group. Ameliorative effect, anti-inflammatory and antioxidant property of *T. purpurea* protects the histo-architecture of liver and other visceral organs from profenofos toxicity causing mild decrease in

erythrocyte and erythropoiesis as compared to toxin treated group, as the medicinal properties of *T. purpurea* were stated by Mathews *et al.,* (2012).

4.2.3 Total Erythrocyte Count (10^6/mm^3):

Mean (± SE) values of Total Erythrocyte Counts (10^6/mm^3) in birds at different intervals of study in different groups are depicted in table 4.6.

At 0 day of experiment:

At day 0 of experiment the mean (± SE) total erythrocyte count (TEC) values of experimental birds in group I to group IV were 3.25±0.08, 3.17±0.07, 3.12±0.16 and 3.20±0.13, respectively and were found statistically and numerically at par within experimental groups.

Table 4.6: Mean (± SE) values of Total Erythrocyte Counts (10^6/mm^3) of experimental birds at different intervals of study

Groups of birds		Mean values of TEC (10^6/mm^3) in different groups (Mean ± SE) at different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		3.25 ±0.08	3.06 ±0.08	3.10^{a} ±0.08
Group II		3.17 ±0.07	2.72 ±0.12	2.64^{b} ±0.12
Group III		3.12 ±0.16	2.98 ±0.11	3.09^{a} ±0.09
Group IV		3.20 ±0.13	2.88 ±0.13	2.78^{ab} ±0.13
CD Values	**1 %**	**-**	**-**	**-**
	5 %	**-**	**-**	**0.317**
Statistics		**NS**	**NS**	**S**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

At day 14 of experiment:

At this stage of experiment the mean values of the total erythrocyte count of group I and group III were 3.06±0.08 and 2.98±0.11, respectively, which were statistically at par and not comparable with each other at this stage of experiment.

Also TEC values of this groups (Group I and group III) were statistically comparable to other treatment groups (Group II and group IV) at 14^{th} day of experiment. However, the mean TEC values in toxin treated group (group II) were found numerically lowest as compared to all other groups.

At day 28 of experiment:

At this stage of experiment the mean total erythrocyte counts of group I and group III were 3.10±0.08 and 3.09±0.09, respectively, which were statistically non-significant to each other and within physiological range at this stage of experiment.

Group II (Profenofos Treatment group):

At day 14^{th} of experiment:

Mean total erythrocyte counts (2.72±0.12) were numerically reduced than control healthy group (group I). However, the mean TEC values of group II were statistically non-significant to healthy control and other treatment groups at 14^{th} day of experiment.

At day 28^{th} of experiment:

At this stage of experiment the mean total erythrocyte counts (2.64±0.12) were statistically significantly reduced ($P<0.05$) as compare to healthy control group (group I) and plant control group (group III). However the mean TEC values of group II were statistically comparable but numerically reduced as compare to mean TEC values of group IV at 28^{th} day of experiment.

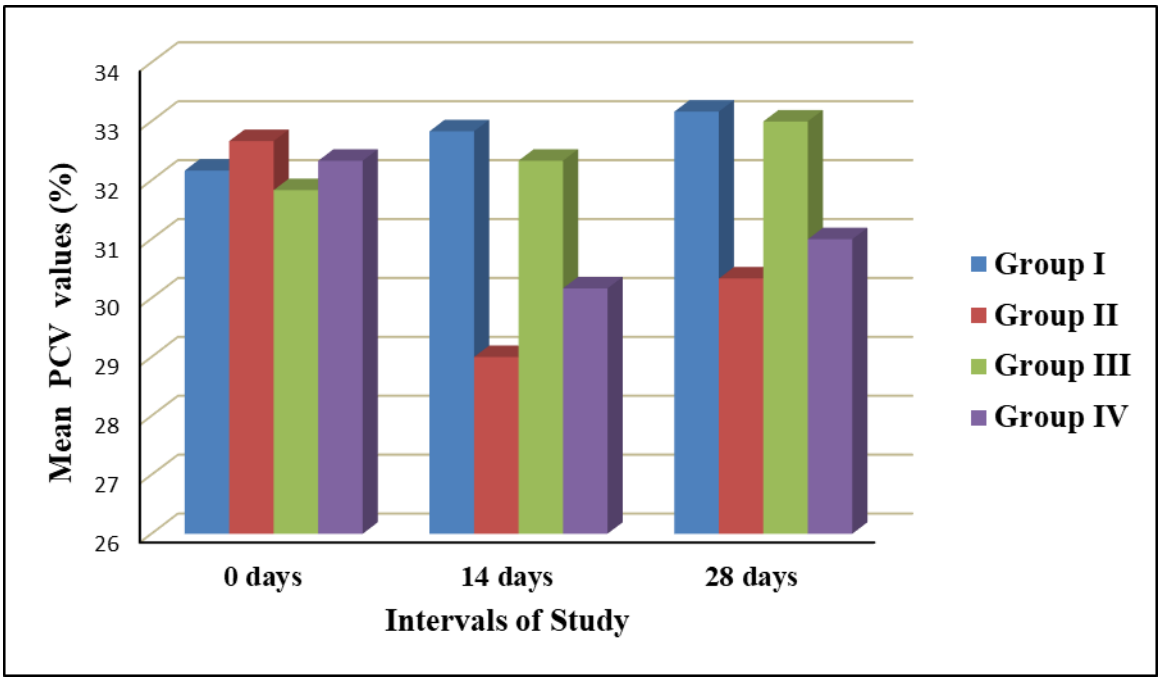

Fig. 4.5: Mean values (Mean ± SE) of PCV (%) in different groups at different intervals of study

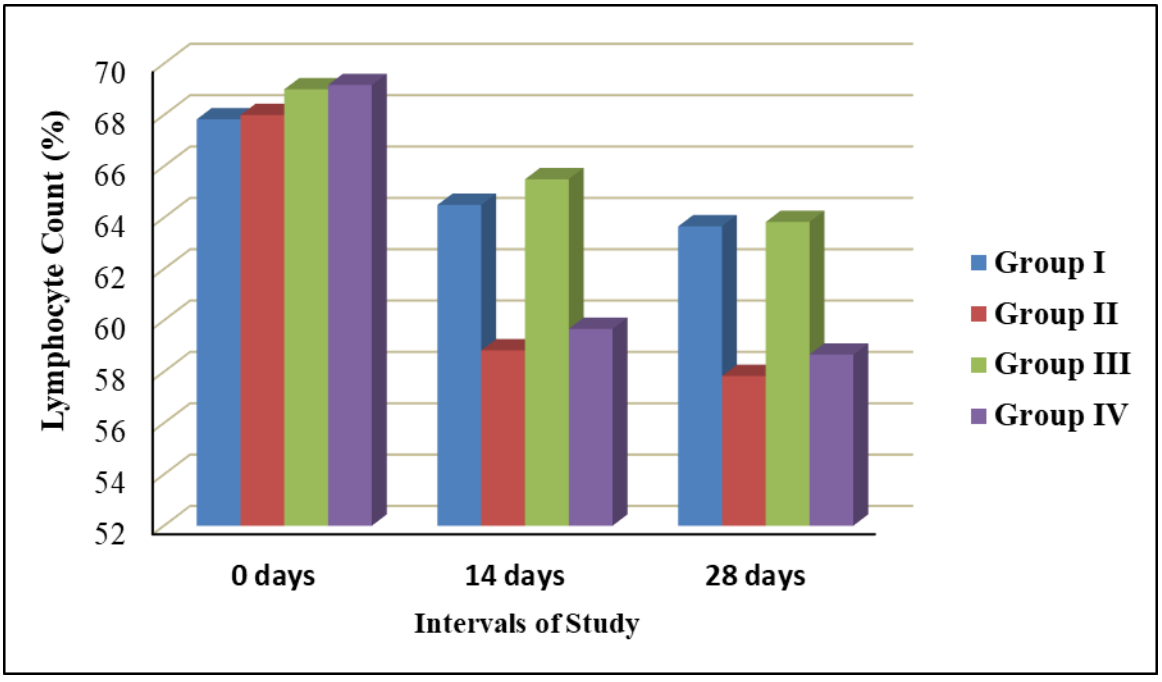

Fig 4.6: Mean values of Lymphocyte count (%) in experimental birds at different intervals of study

Overall, there was a decreasing trend in mean TEC counts of the birds treated with profenofos (group II), when compared to TEC values of other groups, indicating its toxic effects on circulating RBC'S.

Our findings are similar with Garg *et al*., (2004), Rhayf *et al*., (2012), Ghaffar *et al.*, (2014) and Ahmad *et al.,* (2015). As the OP pesticide are potent toxic for blood forming organs such as liver and bone marrow, which may negatively affects the many steps in erythropoiesis process (Rhayf *et al*., 2012). Also, due to vascular hemorrhages and congestion in visceral organs may cause decreased number of RBCs in blood vascular system (Ghaffar *et al.*, 2014). The above reasons might cause the reduction in mean total erythrocyte count in profenofos toxicity groups in present study.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th day of experiment:

At this stage of experiment the mean values of total erythrocytes count (2.88±0.13) were numerically reduced than healthy control group and plant control group (group III). Whereas, the mean TEC values were statistically non-significant to remaining two treatment groups (group-II and group-III) and healthy control group at 14th day of experiment.

At 28th day of experiment:

The mean values of total erythrocytes count of group IV were (2.78±0.13) numerically reduced as compared to healthy control group (group I) and group III. Whereas, the mean TEC values were statistically at par with remaining three groups at 28th day of experiment.

Data indicating that after addition of plant powder (*T.purpurea*) in the diet of profenofos treated birds (group IV), there was not much improvement in altered TEC counts in this study.

4.2.4 Total Leucocyte Count (10^3/mm^3):

Mean (± SE) values of Total Leucocyte Counts (10^3/mm^3) in birds at different intervals of study in different groups are depicted in table 4.7

At day 0 of experiment:

At day 0 of experiment, the mean (± SE) total leucocyte count (TLC) values of experimental birds in group I to group IV were 22.15±0.45, 23.07±0.94, 23.72±0.32, 22.52±0.37, respectively. At this stage of experiment the mean total leucocyte count values were statistically and numerically at par within their experimental groups.

Group I (Healthy control group):

At day 14 and day 28 of experiment:

At this stages of study the mean values of TLC were (23.10±0.97) and (24.12^{b}±0.94) at par with normal physiological range.

Table 4.7: Mean (± SE) values of Total Leucocyte Count (10^3/mm^3) of experimental birds at different intervals of study

Groups of birds		Mean values of TLC (10^3/mm^3) in different groups (Mean ± SE) at different intervals of study		
		0^{th} Day	14^{th} Day	28^{th} Day
Group I		22.15 ±0.45	23.10 ±0.97	24.12^{b} ±0.94
Group II		23.07 ±0.94	25.61 ±1.50	27.95^{a} ±0.89
Group III		23.72 ±0.32	22.21 ±0.80	23.70^{b} ±0.92
Group IV		22.52 ±0.37	24.86 ±1.19	25.42^{ab} ±1.31
CD Values	1 %	-	-	-
	5 %	-	-	3.040
Statistics		NS	NS	S

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

At day 14 of experiment:

At this stage of experiment the mean values of TLC were (25.61±1.50) numerically increased and statistically at par with healthy control group, plant control group (group III) and group IV, indicating not much effect of profenofos treatment on TLC counts at this stage of study.

At day 28 of experiment:

At this day of experiment the mean values of TLC were (27.95±0.89) statistically significantly ($p<0.05$) elevated than that of values in healthy control (group I) and plant control (group III) groups.

Grossly, the mean TLC values were statistically at par as compare to other groups at 14^{th} day of experiment and at 28^{th} day of experiment the mean values were numerically increased and statistically significant ($p<0.05$) as compare to healthy control. This finding was similar to observations of Kammon *et al*, (2011); Begum *et al*., (2015); Kafle *et al*., (2018 and Hussain *et al*., (2019), noted the altered TLC count due to OP pesticides toxicity in birds.

At 28^{th} day of experiment, the significant increase in total leukocyte number in profenofos treated group revealing visceral tissue injuries caused by profenofos pesticide consequences to oxidative stress and further inflammatory response in the respective tissues, which might be responsible for the significant increase in the leucocytes count.

Group III (*Tephrosia purpurea* treatment group):

At day 14 and day 28 of experiment:

The mean TLC values of the group III at 14^{th} and 28^{th} day of experiment were 22.21±0.80 and 23.70±0.92, respectively. The mean TLC values of group III were found at par with healthy control group (group I), indicating no toxic effects of feeding of plant powder alone in the diet of bird. As Verma *et al.,* (2017) were noted the safe dose of *T. purpurea* extract was upto 2000 mg/kg b.wt. PO in wistar rats.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th day of experiment:

At this interval of study, the mean TLC values (24.86±1.19) were statistically at par with healthy control and other treatment groups.

At day 28th of experiment:

At this interval of study the mean TLC values (25.42±1.31) were non-significant to healthy control (group I) and were comparable to toxin control group (group II). Numerically mean TLC values were lied between healthy control values and toxin control group at this time of experiment. Data indicating that there was not much effect of feeding of plant powder in the diet of birds against profenofos toxicity (group IV) treated birds.

4.2.5 Differential Leukocyte Counts (DLC, %):

4.2.5. (A) Lymphocyte count (%):

Mean (± SE) values of Lymphocyte count (%) in birds at different intervals of study in different groups are depicted in table 4.8 & fig. 4.6

At 0 day of experiment:

At day 0 of experiment the mean (± SE) lymphocyte count (%) values of experimental birds in group I to group IV were 67.83±0.65, 68.00±0.68, 69.00±0.63 and 69.17±0.83, respectively. At this stage of experiment, the mean lymphocyte counts (%) were statistically did not differ significantly within experimental groups.

Group I (Healthy control group):

At day 14th and day 28th of experiment:

At this stage of study the mean values of lymphocyte counts (%) were 64.50±1.18 and 64.00±1.15 comparable with values in all other groups.

Table 4.8: Average values of Lymphocyte count (Mean±SE, %) in DLC examination of experimental birds at different intervals of study

Groups of birds		Mean values of lymphocyte count (%) in different groups (Mean ± SE) at different intervals of study		
		0^{th} Day	**14^{th} Day**	**28^{th} Day**
Group I		67.83 ±0.65	64.50^{a} ±1.18	64.00^{a} ±1.15
Group II		68.00 ±0.68	58.83^{b} ±0.87	57.83^{b} ±1.19
Group III		69.00 ±0.63	65.50^{a} ±0.76	63.83^{a} ±0.79
Group IV		69.17 ±0.83	59.67^{b} ±0.61	58.67^{b} ±1.12
CD Values	**1 %**	**-**	**3.541**	**4.330**
	5 %	**-**	**2.593**	**3.175**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

<u>On 14^{th} and 28^{th} day of experiment:</u>

On this both the intervals of experiment the mean values of lymphocyte counts (%) were 58.83±0.87 and 57.83±1.19, respectively. There were marked decline (p<0.01) in mean lymphocytes (%) as compared to values in healthy control group (group I) and plant control group (group III). Moreover, mean lymphocytes counts (%) values of group II were statistically at par but numerically decreased to mean values of group IV at this intervals of study.

Grossly, the mean counts of lymphocyte percentages of group II showed marked decline (lymphocytopenia) throughout the experiment due to pesticide toxicity in birds. Our findings were similar with observations noted by Garg *et al*., (2004), Kafle *et al*., (2018), Kulthe *et al*., (2018), Begum *et al.,* (2015) and Wani *et al.,* (2017) in OP pesticide toxicated birds. These all earlier studies noted that the OP pesticides toxicity might resulted into the significant lymphocytopenia in birds.

Organophosphate toxicity was characterized by significant decrease in lymphocyte count. The T-lymphocyte count reflected the total lymphocyte count

which were the precursor of cell mediated immune response in chicks (Garg *et al*., 2004), so cytotoxic effect of OP pesticides might be the reason for immunosuppression and lymphocytopenia in birds (Kulthe *et al*., 2018). Also, due to oral feeding of profenofos (OP pesticide) in Gramapriya birds, the generalised intoxication stress and necrotic effects were noticed in visceral and lymphoid organs in the histopathological examination, which might be the reason for decreased production of number of circulating lymphocytes in profenofos toxicated birds and consequences to declined mean lymphocytes percentage in group II (Begum *et al.,* 2015). A reduction in the number of total lymphocyte counts might be a signal of dropping of the immune competency of the birds (Garg *et al*., 2004). Findings of the present study can be well corroborated with the earlier observations recorded by these workers.

Group III (*Tephrosia purpurea* treatment group):

At day 14 and day 28 of experiment:

At 14^{th} and 28^{th} day of experiment the mean values of lymphocyte counts (%) were 65.50±0.76 and 63.83±0.79, respectively. The mean values of lymphocyte percentages of group III were statistically at par and numerically higher as compared to values in healthy control group at both intervals of study. Whereas, the mean values of group III were statistically significantly ($P<0.01$) improved than the values in toxin treated group (group II) and in birds of group IV at both the intervals of experiment.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14^{th} and 28^{th} day of experiment:

At both 14^{th} and 28^{th} day of experiment the mean value of percentage lymphocytes counts of birds in group IV were 59.67±0.61 and 58.67±1.12, respectively. The mean percentages of lymphocyte counts of group IV were found statistically significantly decreased than the values of healthy control group (Group I) and plant control group (group III). However, the mean counts of group IV were found statistically at par but numerically slight elevated as compared to values 58.83±0.87, 57.83±1.19, respectively, in toxin treated group (group II).

Grossly, the mean counts of lymphocytes percentage of birds in group IV were numerically somewhat inclined than toxin control group (group II) throughout the experiment. As *T. purpurea* was used as feed additive throughout the experiment, the moderately improved histo-architecture of lymphoid tissues were observed which outcomes to numerical improvement in production of lymphocytes counts in birds of group IV.

4.2.5. (B) Heterophil count (%):

Mean (± SE) values of heterophil counts (%) in birds at different intervals of study in different groups are depicted in table 4.9 & fig. 4.7.

At 0^{th} day of experiment:

At day 0 of the experiment the mean (± SE) heterophil count (%) values of experimental birds in group I to group IV were 24.50±0.56, 24.00±0.58, 23.67±0.67 and 23.50±0.43, respectively. At this stage of experiment the mean heterophil counts (%) were statistically and numerically not comparable within experimental groups.

Group I (Healthy control group):

At day 14 and day 28 of experiment:

At this stage of study the mean values of heterophil counts (%) were 27.33±0.80 and 26.83±0.83 were in normal physiological range. Which were not comparable within groups at this interval of experiment, representing the healthy status of birds of all groups.

Group II (Profenofos Treatment group):

At 14^{th} and 28^{th} day of experiment:

On 14^{th} and 28^{th} day of experiment the mean values of heterophil counts (%) were 31.33±0.88 and 32.00±0.77, respectively. These values were markedly increase ($p<0.01$) as compared to mean heterophil (%) in healthy control group (group I) and plant control group (group III). However, mean heterophil counts (%) values of group II were statistically at par but numerically improved than mean values of group IV, at this intervals of study.

Grossly, the mean counts of heterophil percentages of group II showed marked elevation (heterophilia) throughout the experiment due to pesticide toxicity in birds. Our findings were similar with Garg *et al*., (2004), Kafle *et al*., (2018) and Begum *et al.,* (2015). These all studies were noted that the OP pesticides toxicity might resulted into the significant heterophilia in birds.

Garg *et al*., (2004) noted that the neutrophilia was the characteristic feature of the OP pesticide toxicity in rats**.** The present study noted the degenerative and necrotic changes in visceral organs and lymphoid tissues, which might be the reason of increased heterophils (as toxin elicited inflammatory response) in toxin treated group II (Kafle *et al*., 2018).

Table 4.9: Average values of Heterophil counts (Mean±SE, %) in DLC examination of experimental birds at different intervals of study

Groups of birds		**Mean values of Heterophil counts (%) in different groups (Mean ± SE) at different intervals of study**		
		0th Day	**14th Day**	**28th Day**
Group I		24.50 ±0.56	27.33^{b} ±0.80	26.83^{b} ±0.83
Group II		24.00 ±0.58	31.33^{a} ±0.88	32.00^{a} ±0.77
Group III		23.67 ±0.67	26.00^{b} ±0.73	26.33^{b} ±0.61
Group IV		23.50 ±0.43	30.67^{a} ±0.61	31.00^{a} ±0.63
CD Values	**1 %**	**-**	**3.073**	**2.896**
	5 %	**-**	**2.253**	**2.123**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group III (*Tephrosia purpurea* treatment group):

<u>At day 14 and day 28 of experiment:</u>

At 14th and 28th day of experiment the mean values of heterophil counts (%) were 26.00±0.73 and 26.33±0.61, respectively. The mean values of heterophil counts of birds in group III were statistically non-significant at par to values in healthy control group at both intervals of study. Whereas the mean values of group III were statistically significantly (P<0.01) decreased than the values in

toxin treated group (group II) and group IV at both interval of experiment. As the *T. purpurea* plant has the hepatoprotective effect and reported non-toxic (Gora *et al*., 2014), there was not any significant alterations in heterophil percentage throughout the experiment in birds of this group (group III).

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean percentage counts of heterophil of group IV were 30.67±0.61 and 31.00±0.63, respectively. The mean percentage of heterophil counts of group IV were statistically increased than the values in healthy control group (Group I) and plant control group (Group III). Moreover the mean counts of group IV were statistically at par but numerically somewhat declined as compared to toxin treated group (group II), indicating not much ameliorative action of the feeding of plant powder on heterophil count.

Grossly, the mean counts of heterophil percentage of group IV were numerically somewhat declined than toxin control group (group II) throughout the experiment. As *T. purpurea* was used as feed additive throughout the experiment, the moderately improved histo-architecture of liver lymphoid tissues were observed which contributes to improve the production of heterophil count in group IV.

4.2.5. (C) Eosinophil count (%), Basophil count (%) and Monocyte count (%):

Mean values of Eosinophil, basophil and monocyte counts (%) of birds in different groups (Group I to Group IV) at 0th day, 14th day and 28th day interval of study did not differ significantly. The findings of our study were in similar with Kulthe *et al.,* (2018) and Begum *et al.,* (2015).

Table 4.10: Mean (± SE) values of Eosinophil, Basophil and Monocyte count (%) in DLC examination of experimental birds at different intervals of study

Groups of birds	Intervals of study		
	0 Day	14th Day	28th Day
	A. Mean values of Eosinophil count (%) (Mean ± SE)		
Group I	2.67 ±0.21	2.33 ±0.33	2.50 ±0.22
Group II	2.83 ±0.17	3.33 ±0.21	3.67 ±0.42
Group III	2.50 ±0.22	3.17 ±0.40	3.33 ±0.33
Group IV	3.00 ±0.26	3.50 ±0.22	3.50 ±0.43
CD value	-	-	-
Statistics	NS	NS	NS
	B. Mean values of Basophil count (%) (Mean ± SE)		
Group I	0.67 ±0.21	1.00 ±0.37	1.33 ±0.21
Group II	1.00 ±0.37	1.33 ±0.33	1.50 ±0.22
Group III	0.83 ±0.17	0.83 ±0.17	1.00 ±0.26
Group IV	0.50 ±0.22	1.17 ±0.31	1.50 ±0.34
CD value	-	-	-
Statistics	NS	NS	NS
	C. Mean values of Monocyte count (%) (Mean ± SE)		
Group I	4.33 ±0.33	4.83 ±0.48	5.67 ±0.33
Group II	4.17 ±0.31	5.17 ±0.31	5.00 ±0.37
Group III	4.00 ±0.26	4.50 ±0.43	5.50 ±0.34
Group IV	3.83 ±0.31	5.00 ±0.37	5.33 ±0.33
CD value	-	-	-
Statistics	NS	NS	NS

Means bearing similar superscripts in column do not differ significantly ($P<0.05$) ($P<0.01$).

Where,

Group I- Healthy control, **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

4.2.6 Blood Clotting time (Sec.):

Mean (± SE) values of Blood Clotting time (Sec.) in birds at different intervals of study in different groups are depicted in table 4.11 & fig.4.8.

At 0th day of experiment:

At day 0 of experiment the mean (± SE) blood clotting time (BCT) values of experimental birds in group I to group IV were 60.00±1.29, 61.67±1.05, 60.83±0.83 and 59.17±1.54, respectively. At this stage of experiment, the mean

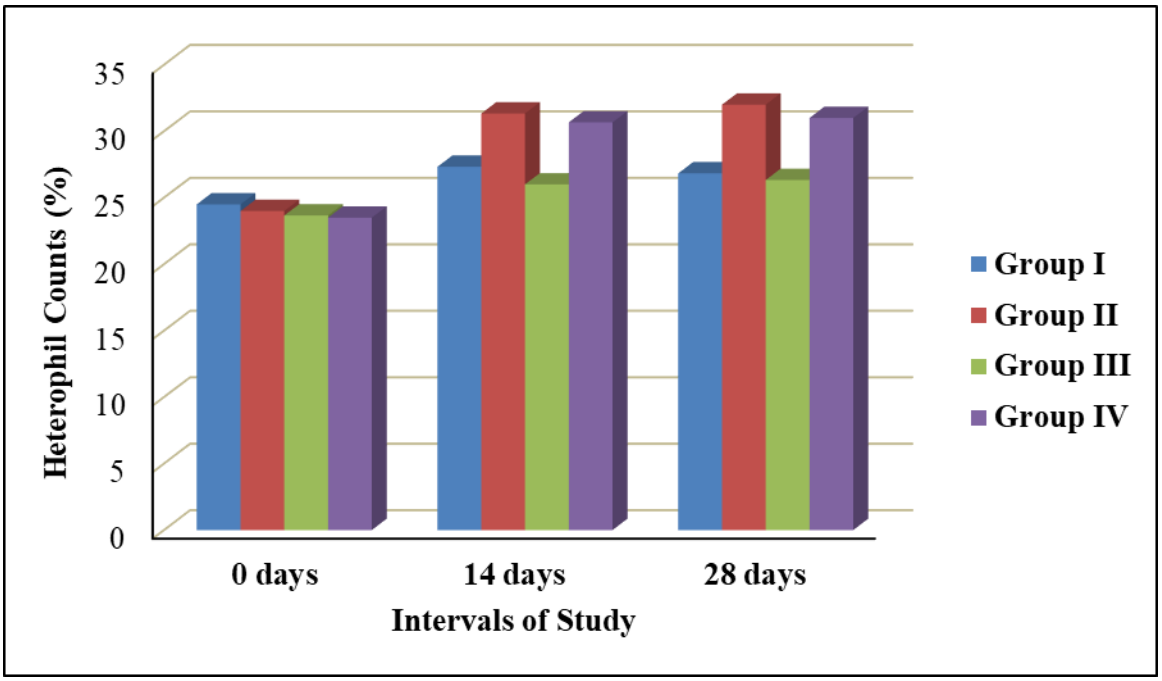

Fig 4.7: Mean values of Heterophil counts (%) in experimental birds at different intervals of study

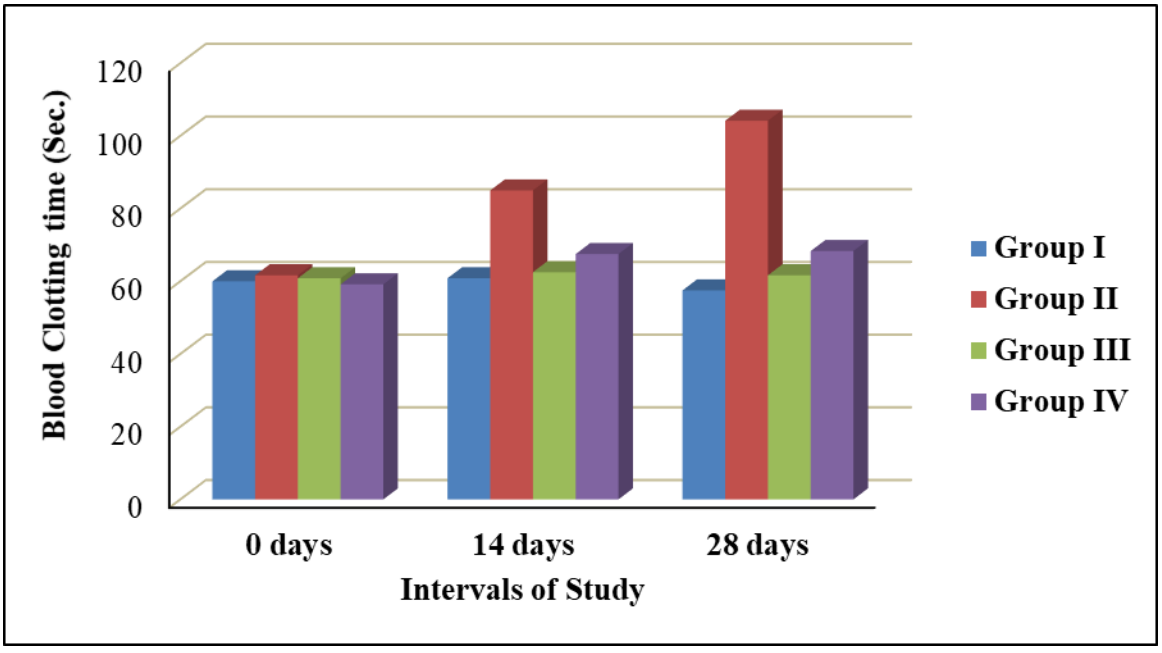

Fig 4.8: Mean values of Blood Clotting time (Sec.) of experimental birds at different intervals of study

blood clotting time values were statistically and numerically not comparable within experimental groups.

Table 4.11: Table showing values of Blood Clotting time (Mean±SE, Sec.) of experimental birds at different intervals of study

Groups of birds		Mean values of BCT (Sec.) in different groups (Mean ± SE) at different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		60.00 ±1.29	60.83^{c} ±1.54	57.50^{c} ±1.71
Group II		61.67 ±1.05	85.00^{a} ±1.83	104.17 ^{a}±1.54
Group III		60.83 ±0.83	62.50^{c} ±1.12	61.67^{c} ±1.05
Group IV		59.17 ±1.54	67.50^{b} ±1.71	68.33^{b} ±1.67
CD Values	**1 %**	**-**	**6.312**	**6.091**
	5 %	**-**	**4.639**	**4.466**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column and rows do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

<u>At day 14 and day 28 of experiment:</u>

At this stage of study the mean values of blood clotting time were 60.83±1.54 and 57.50±1.71 and were at par with normal physiological range.

Group II (Profenofos Treatment group):

<u>On 14th and 28th day of experiment:</u>

At this stages of experiment, the mean values of blood clotting time 85.00±1.83 and 104.17±1.54, respectively, were highly significantly elevated (P<0.01) than values in healthy control (group I), plant control group (group III) and treatment group (group IV).

Grossly, on 14th and 28th day experiment the blood clotting time of group II were significantly increased as compare to healthy control group and treatment

groups. As profenofos pesticides (OP pesticides) are the potent toxic to liver and kidney tissue, which were mentioned in the results of histopathological examination of this study. This revealed that profenofos also induced vascular damage and permeability of various visceral organs consequence to hemorrhages in visceral tissues. This might be the reason for loss of platelets and erythrocytes in blood vascular system and might affect the blood clotting time in the toxicated group. Our findings were similar with Moregaonkar (1990), Shingumare (2018) and Londhe (2018), in which they observed the increased prothrombin time in OP pesticide toxicated laboratory animals in their studies.

Group III (*Tephrosia purpurea* treatment group):

At day 14 and day 28 of experiment:

At 14th and 28th day of experiment, the mean values of blood clotting time 62.50±1.12 and 61.67±1.05, respectively, were statistically at par with the values in healthy control group, indicating non-toxic effects of feeding of profenofos powder in the diet of birds (Verma *et al.,* 2017).

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th and 28th day of experiment:

On 14th and 28th day of experiment the mean blood clotting time values 67.50±1.71 and 68.33±1.67, respectively, were numerically lied between mean values of healthy control group (group I) and toxin treated group (group II). However at both interval of study the mean value of BCT were highly significantly lower ($P<0.01$) as compare to toxin treated group (group II) but were higher as compared to values in control groups (groups I & II), indicating partial improvement in BCT values after treatment with plant powder (*T. purpurea*) against profenofos toxicity.

Overall, due to free radical scavenging activity and hepatoprotective activity of the *T. purpurea* was significantly affect to decrease the oxidative stress in liver and other vascular organs. Which might be helpful to normalize the mean blood clotting time values of group IV as compare to toxin treated group (group II). As Verma *et al.,* (2017) and Gora *et al*., (2014) were also noted the

hepatoprotective nature of *T. purpurea* CCl4 and arsenic toxicated rats respectively.

4.3 Serum Biochemical Alterations:

4.3.1 Serum glucose (mg/dl):

Mean (± SE) serum glucose levels (mg/dl) in birds at different intervals of study in different groups are depicted in table 4.12 & fig. 4.9

At 0^{th} day of experiment:

At day 0 of experiment the mean (± SE) serum glucose levels (mg/dl) of experimental birds in group I to group IV were 279.67±1.31, 281.60±1.52, 282.65±1.30 and 284.53±1.97, respectively. At this stage of experiment, the mean serum glucose levels were statistically and numerically comparable within all experimental groups.

Group I (Healthy control group):

At day 14 and day 28 of experiment:

At this stage of study the mean serum glucose levels were (289.89±2.45 and 259.18±2.29, respectively) at par with normal physiological range.

Table 4.12: Mean (± SE) values of serum glucose levels (mg/dl) of experimental birds at different intervals of study

Groups of birds		Mean values of serum glucose levels (mg/dl) in different groups (Mean ± SE) at different intervals of study		
		0^{th} Day	14^{th} Day	28^{th} Day
Group I		279.67 ±1.31	289.89^{b} ±2.45	259.18^{b} ±2.29
Group II		281.60 ±1.52	304.54^{a} ±1.92	279.48^{a} ±1.12
Group III		282.65 ±1.30	290.28^{b} ±0.99	260.96^{b} ±3.40
Group IV		284.53 ±1.97	293.17^{b} ±2.69	275.88^{a} ±2.86
CD Values	1 %	-	8.503	10.306
	5 %	-	6.237	7.557
Statistics		NS	HS	HS

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

On 14th day of experiment:

The mean serum glucose levels at this interval of study (304.54±1.92) showed marked elevation as compared to healthy control group and other treatment groups. The mean values of group II were extremely significantly ($P<0.01$) increased when compared to healthy control (group I) and other treatment groups (group III and group IV) at this stage of experiment.

On 28th day of experiment:

On 28th day of study the mean serum glucose levels (279.48±1.12) were statistically significantly reduced as compared to healthy control group (group I) and plant control group (group III). However, the mean serum glucose levels of group II were found statistically at par but numerically increased as compare to group IV values at this stage of experiment.

Grossly the results of mean serum glucose levels found in our experiment similar with Garg *et al.,* (2004), Kammon *et al.,* (2010) and Kammon *et al*, (2011). Hyperglycemia is the outcome of different mechanisms involved in body glucose metabolism, which was might have caused due to acute or chronic exposure of OP pesticides.

The elevation of blood sugar clearly indicated that pesticide toxicity had adverse effects on glycolysis (Garg *et al.,* 2004). OP pesticides provoke metabolic pathways in brain, skeletal muscles, and liver, which increases glucose production. In addition insulin resistance, disturbed insulin secretion, and damaged pancreatic Langerhans islets are the consequences of OP pesticides exposure which might affect the glucose levels in toxicated birds (Rahimi and Abdollahi, 2007). In the first stages of OP pesticide toxicity, carbohydrates (glucose) are used to supply energy and to meet the stress situation. However in

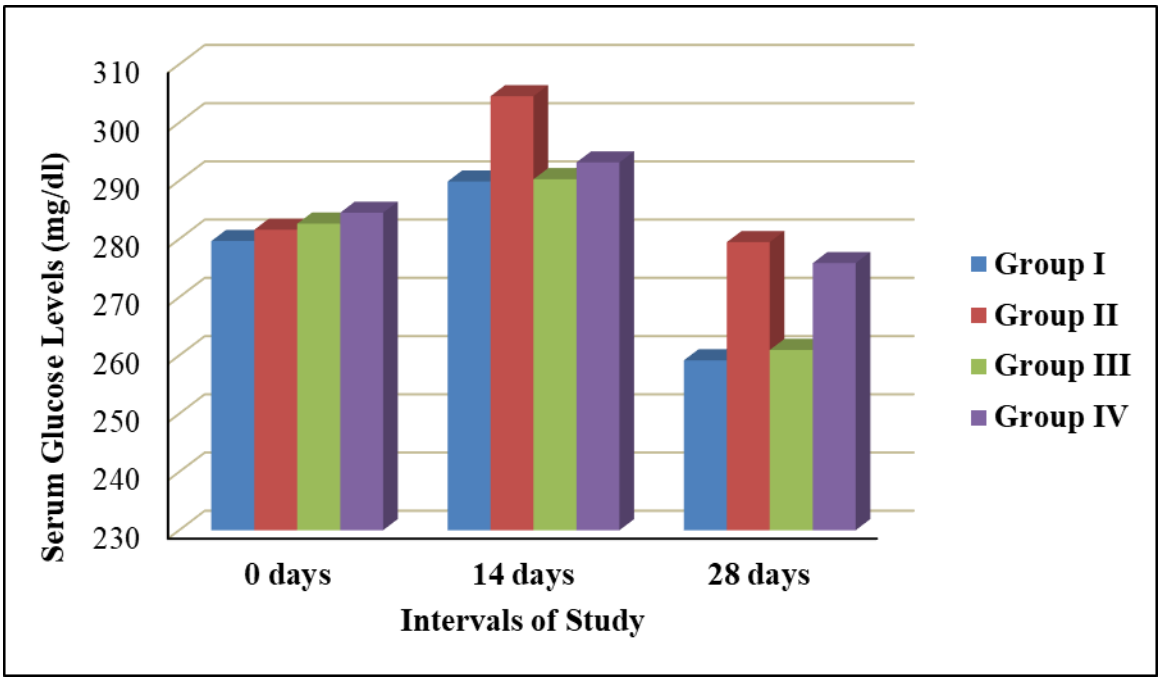

Fig. 4.9: Mean values of Serum Glucose Levels (mg/dl) of experimental birds at different intervals of study

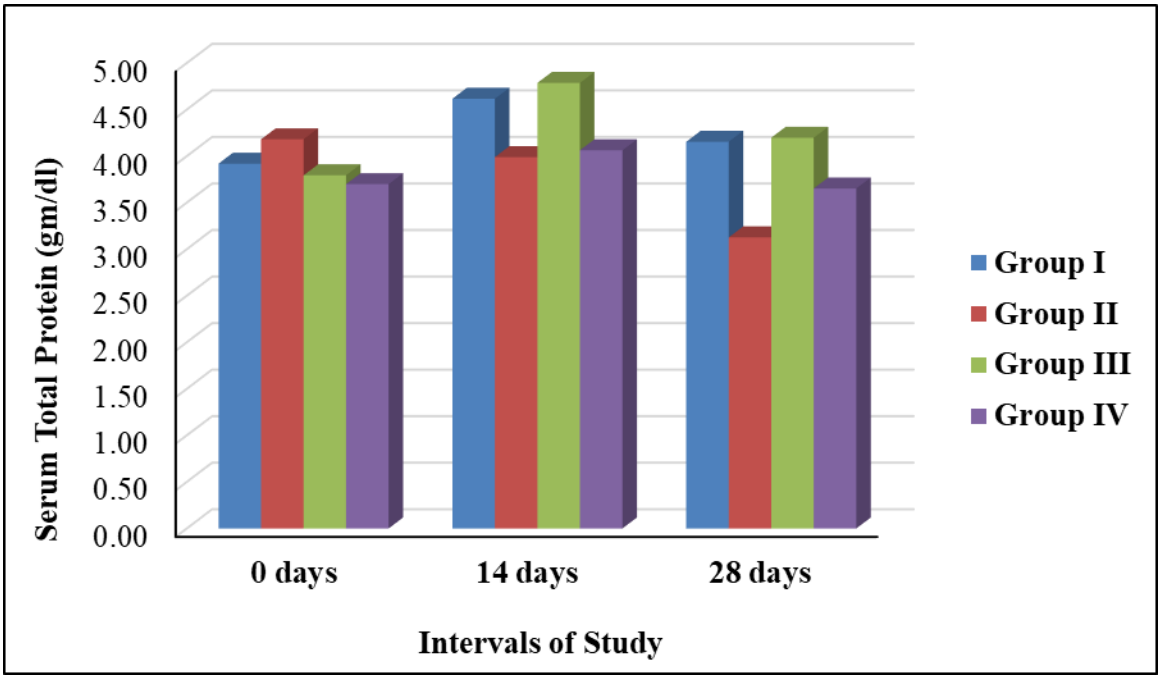

Fig. 4.10: Mean values of Serum Total Protein (gm/dl) of experimental birds at different intervals of study

last phases of toxicity, lipids and proteins were the major source of energy as gluconeogenesis was increased due to OP pesticide toxicity, that all are in support of hyperglycemia in profenofos toxicated birds (Mohajeri and Abdollahi, 2010).

Group III (*Tephrosia purpurea* treatment group):

At 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean levels of serum glucose of group III 290.28±0.99 and 260.96±3.40 respectively were at par with mean glucose values of healthy control group, indicating non toxicity of feeding of plant powder (*T. purpurea*) in the diet of birds.

Group IV (Profenofos + *T. purpurea* treatment group):

At 14th of experiment:

Mean values of serum glucose (293.17±2.69) were statistically at par with the values of healthy control group and plant control group (group III) at this stage of experiment. Moreover, the serum glucose levels of birds of this group (group IV) were found statistically improved than the values of profenofos treated group (group II, 304.54 ± 1.92), indicating ameliorative effects of plant treatment on profenofos toxicity.

On 28th day of experiment:

At this stage of experiment the mean values of serum glucose were statistically elevated than the values of healthy control group. Whereas the mean serum glucose values were significantly improved than the values of toxin treated group (group II).

Grossly in case of mean serum glucose levels of group IV, the mild ameliorative effect of *T. purpurea* plant was seen. *T. purpurea* plant powder might be helpful for decrease the oxidative stress in liver which might be helpful for normalizing the mean glucose levels in group IV as compare to group II. This can be supported by Pavana *et al*., (2007), in which they noted the significantly improved blood glucose levels in streptozotocin induced diabetic rats.

4.3.2 Serum total protein (gm/dl):

Mean (± SE) serum total protein (gm/dl) in birds at different intervals of study in different groups are depicted in table 4.13 & fig. 4.10.

On 0th day of experiment:

At day 0 of experiment the mean (± SE) serum total protein (gm/dl) of experimental birds in group I to group IV were 3.92±0.09, 4.18±0.08, 3.80±0.08 and 3.70±0.25, respectively. At this stage of experiment, the mean serum total protein (gm/dl) values were statistically at par within experimental groups.

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) serum total protein (gm/dl) values were 4.62±0.11 and 4.16±0.06, respectively. This mean serum total protein (gm/dl) levels of group I were within normal range and not significant within groups of experiment. Which represent the healthy status of birds in group I throughout experimental duration.

Table 4.13: Mean (± SE) values of serum total protein (gm/dl) of experimental birds at different intervals of study

Groups of birds		Mean values of serum total protein (gm/dl) in different groups (Mean ± SE) in different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		3.92 ±0.09	4.62^{a} ±0.11	4.16^{a} ±0.06
Group II		4.18 ±0.08	3.99^{b} ±0.09	3.13^{c} ±0.08
Group III		3.80 ±0.08	4.79^{a} ±0.07	4.20^{a} ±0.14
Group IV		3.70 ±0.25	4.06^{b} ±0.05	3.65^{b} ±0.11
CD Values	**1 %**	**-**	**0.331**	**0.408**
	5 %	**-**	**0.247**	**0.293**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

On 14th day of experiment:

At this stage of study, the mean serum total protein values of group II (3.99±0.09) were statistically significantly (P<0.01) reduced than the values in birds of healthy control group and plant control group (group III). Whereas, the mean values of group II were statistically at par but numerically decreased than the values in birds of group IV at this stage of experiment.

On 28th day of experiment:

The mean serum total protein values were (3.13±0.08) statistically significantly (P<0.01) reduced than the values of birds in healthy control group and other treatment groups (group III and group IV).

Our findings were in accordance with Garg *et al.,* (2004); Begum *et al.,* (2015); Singh *et al.,* (2016); Kulthe *et al.,* (2018) and Wani *et al.,* (2017). These all studies noted hypoproteinemia induced due to OP pesticide toxicity in chickens.

Oxidative injury caused to liver parenchyma by OP pesticide consequences to decreased potential of liver to synthesis of protein Kulthe *et al.,* (2018). Also, Garg *et al.,* (2004) reported that rough surfaces endoplasmic reticulum is the primary organelle for globulin synthesis, which might be affect during pesticide toxicity. Singh *et al.,* (2016) reported that the OP compound might suppress the growth of cellular protein and RNA synthesis resulting in low level of serum protein. Mohajeri and Abdollahi, (2010) stated that the proteins might be used as primary energy source through gluconeogenesis process in OP pesticide toxicated animals, that all are in support of hypoproteinemia in profenofos toxicated birds.

Also, Present study recorded significant reduction in body weight gain, feed consumption of birds of this group (group II) throughout the experiment, which might have affected the protein concentration in profenofos toxicated birds (Begum *et al.,* 2015). The histo-architectural changes observed in kidney and liver

in this study could also be responsible for deformed synthesis and excretion of the serum protein in group II of this study.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of the study the mean serum total protein values were 4.79±0.07 and 4.20±0.14, respectively. Which were statistically at par as compare to healthy control group. As the *T. purpurea* plant is having the hepatoprotective effect (Mathews *et al.*, 2012), there was not any significant effect on serum total protein throughout the experiment in plant control group.

Group IV (Profenofos + *T. purpurea* treatment group):

On 14th day of experiment:

Mean total protein values of group IV (4.06±0.05) were statistically comparable with healthy control group and plant control group (group III). Whereas the mean values of TP of group IV were statistically increased as compared to toxin treated group (group II).

On 28th day of experiment:

The mean serum total protein values of group IV were (3.65±0.11) not statistically significantly improved up to values of healthy control group and plant control group. However the mean values of birds in group IV were significantly improved than mean values of group II, indicating partial ameliorative effects of plant treatment in profenofos intoxicated birds.

Increased feed consumption and body weight gain as observed in group IV birds as compared to group II birds in this experiment supports this findings. The present study also noted mild to moderate improvement in histoarchitecture of liver parenchyma and kidney tissue of group IV as compare to group II, which might be positively effected on increased protein synthesis and decreased protein excretion, which resulted to the near to normal values of total protein in group IV birds.

4.3.3 Serum Albumin (gm/dl):

Mean (± SE) Serum Albumin (gm/dl) in birds at different intervals of study in different groups are depicted in table 4.14

On 0th day of experiment:

At day 0 of experiment the mean (± SE) serum albumin values (gm/dl) of experimental birds in group I to group IV were 2.19±0.12, 2.27±0.08, 2.08±0.08 and 2.07±0.18, respectively. At this stage of experiment the mean serum albumin values (gm/dl) were statistically at par within all experimental groups.

Table 4.14: Mean (± SE) values of serum albumin (gm/dl) of experimental birds at different intervals of study

Groups of birds		Mean values of serum albumin (gm/dl) in different groups (Mean ± SE) in different intervals of study		
		0th Day	14th Day	28th Day
Group I		2.19±0.12	2.39^{a}±0.07	2.03^{a}±0.10
Group II		2.27±0.08	1.99^{b}±0.04	1.58^{b}±0.10
Group III		2.08±0.08	2.46^{a}±0.12	2.02^{a}±0.06
Group IV		2.07±0.18	2.02^{b}±0.13	1.81^{ab}±0.08
CD Values	1 %	-	0.387	0.355
	5 %	-	0.288	0.257
Statistics		NS	HS	HS

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) serum albumin (gm/dl) values of birds in this group were 2.39±0.07 and 2.03±0.10, respectively. Mean

serum albumin (gm/dl) levels of group I were found within normal range, which represent the healthy status of birds in group I throughout experimental duration.

Group II (Profenofos Treatment group):

On 14th and 28th day of experiment:

The mean serum albumin values were (1.99±0.04) significantly ($P<0.01$) reduced when compare to healthy control group (group I) and plant control group (group III). Whereas, mean albumin values of group II were found numerically reduced but statistically comparable with the values of birds in group IV at this time of experiment.

Our findings were in accordance with Begum *et al.,* (2015); Singh *et al.,* (2016); Kulthe *et al.,* (2018) and Wani *et al.,* (2017). These all studies noted hypoproteinemia induced due to OP pesticide toxicity in chickens.

Oxidative injury caused to liver parenchyma by OP pesticide consequences to decreased potential of liver to synthesis of protein (Kulthe *et al.,* 2018). Singh *et al.,* (2016) reported that the OP compound might suppress the growth of cellular protein and RNA synthesis resulting in low level of serum albumin. Mohajeri and Abdollahi, (2010) stated that the proteins might be used as primary energy source through gluconeogenesis process in OP pesticide toxicated animals, that all are in support of decreased albumin concentration in profenofos toxicated birds.

Also, Present study recorded significant decreased body weight gain, feed consumption of birds of this group throughout the experiment, which might have affected the serum albumin concentration in profenofos toxicated birds (Begum *et al.,* 2015). Also, decreased levels of serum total protein and globulin of group II recorded in present study, might be resulted into decreased concentration of albumin in group II. The histo-architectural changes observed in kidney and liver of birds of this group are also responsible for deformed synthesis and excretion of the serum albumin in group II of this study, which further confirms the relation between profenofos and declined serum albumin in this group as stated by Singh *et al.,* (2016).

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of the study, the mean serum albumin values were 2.46±0.12 and 2.02±0.06, respectively, which were found statistically at par as compared to healthy control group. As the *T. purpurea* plant was with the hepatoprotective effect (Mathews *et al.*, 2012), there was not any significant effect on serum albumin throughout the experiment.

Group IV (Profenofos + T. purpurea treatment group):

On 14th day of experiment:

The mean serum albumin values of group IV (2.02±0.13) were numerically decreased and statistically significant to healthy control group and plant control group (group III) and comparable to value in birds of group III at 28th day interval. However, the mean values of group IV were showed numerically improved than values in birds of group II, but statistically were comparable to group II at this stage of experiment.

On 28th day of experiment:

At this stage of experiment the mean albumin values of group IV (1.81±0.08) were statistically comparable to values in birds of healthy control group and other treatment groups (group III and group II). However the mean values of group IV were found numerically improved than the levels in toxin control group (group II) at this stage of experiment, indicating partial improvement of these values after treatment of plant powder in profenofos intoxicated birds.

There was increased feed consumption and body weight gain were observed in group IV birds as compared to group II birds in this experiment. The present study also noted the mild to moderate improvement in histo-architecture of liver parenchyma and kidney tissue of group IV as compared to group II, which might be positively reflected on increased protein synthesis and decreased protein excretion resulted the near to normal values of albumin in group IV birds.

4.3.4 Serum Globulin (gm/dl):

Mean (± SE) Serum Globulin (gm/dl) in birds at different intervals of study in different groups are depicted in table 4.15.

Table 4.15: Mean (± SE) values of serum globulin (gm/dl) of experimental birds at different intervals of study

Groups of birds		Mean values of serum globulin (gm/dl) in different groups (Mean ± SE) in different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		1.73 ± 0.08	2.23 ± 0.08	2.13^{a} ± 0.13
Group II		1.91 ± 0.06	2.01 ± 0.10	1.55^{b} ± 0.10
Group III		1.72 ± 0.10	2.33 ± 0.13	2.17^{a} ± 0.14
Group IV		1.64 ± 0.11	2.05 ± 0.09	1.85^{ab} ± 0.18
CD Values	**1 %**	**-**	**-**	**-**
	5 %	**-**	**-**	**0.419**
Statistics		**NS**	**NS**	**S**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV-** Profenofos + *T. purpurea* treatment

On 0th day of experiment:

At day 0 of experiment the mean (± SE) serum globulin values (gm/dl) of experimental birds in group I to group IV were 1.73±0.08, 1.91±0.06, 1.72±0.10 and 1.64±0.11, respectively. At this stage of experiment, the mean serum globulin values (gm/dl) were statistically and numerically at par within experimental groups.

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) serum globulin (gm/dl) values in birds of group were 2.23±0.08 and 2.13±0.13, respectively. Mean serum globulin (gm/dl) levels of group I were within normal range, which represent the healthy status of birds throughout experimental duration.

Group II (Profenofos Treatment group):

On 14th day of experiment:

At this stage of experiment mean serum globulin values of group II (2.01±0.10) did not differ significantly from the values of birds in healthy control group and other treatment groups (group III and group IV).

On 28th day of experiment:

Mean serum globulin values of birds in group II (1.55±0.10) were significantly ($P<0.05$) lower than the values in birds of healthy control group and plant control group (group III). Whereas, mean values of serum globulin in birds of group II were statistically comparable with group IV at this interval of study. Data indicated that there was reduction in serum globulin levels at these birds at later stage of the experiment.

Our findings were in accordance with Garg *et al.,* (2004), Singh *et al.,* (2016) and Kulthe *et al.,* (2018), who noted decreased globulin concentration induced due to OP pesticide toxicity in chickens.

Oxidative injury caused to liver parenchyma by OP pesticide consequences to decreased potential of liver to synthesis of protein (Kulthe *et al.,* 2018). Also Garg *et al.,* (2004) reported that rough endoplasmic reticulum is the primary organelle for globulin synthesis, which might be affect during pesticide toxicity. Singh *et al.,* (2016) reported that the OP compound might suppress the growth of cellular protein and RNA synthesis resulting in low level of serum globulin. Mohajeri and Abdollahi, (2010) stated that the proteins might be used as primary energy source through gluconeogenesis process in OP pesticide toxicated animals, which all are in support of decreased protein and subsequently decreased globulin concentration in profenofos toxicated birds.

Also, present study recorded significant decreased body weight gain, feed consumption of birds throughout the experiment, which might be affect the serum globulin concentration in profenofos toxicated birds as stated by Begum *et al.,* (2015). Decreased levels of serum total protein and albumin birds recorded in

present study of group II, might be resulted into decreased concentration of globulin.

Group III (*Tephrosia purpurea* treatment group):

On 14th and 28th day of experiment:

At this both intervals of the study the mean serum globulin values were 2.33±0.13 and 2.17±0.14, respectively. Which were statistically at par with the values in birds of healthy control group. As the *T. purpurea* plant is having hepatoprotective effect (Mathews *et al.*, 2012), there was not any significant effect on serum globulin throughout the experiment.

Group IV (Profenofos + T. purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean serum globulin values of birds in group IV were 2.05±0.09 and 1.85±0.18, respectively. The mean serum globulin values of group IV were statistically at par with the values in birds of healthy control group and other treatment groups (group II and group III). Whereas, at 28th day of experiment the mean serum globulin values of birds in group IV were comparable to, values in healthy control group and toxin control group (group II).

Data indicated that there was not much improvement in serum globulin levels in birds of group IV at both the intervals of study when compared to values in birds of group II, indicating failure of treatment with plant in improvement of serum globulin levels in this experiment.

4.3.5 Serum aspartate transaminase (AST, IU/L):

Mean (± SE) serum aspartate transaminase (IU/L) in birds at different intervals of study in different groups are depicted in table 4.16 & fig. 4.11

Table 4.16: Mean (± SE) values of Serum aspartate transaminase levels (IU/L) of experimental birds at different intervals of study

Groups of birds		Mean values (Mean ± SE) of serum AST (IU/L) in different groups in different intervals of study		
		0^{th} Day	**14^{th} Day**	**28^{th} Day**
Group I		155.19 ± 1.30	141.76^{c} ± 1.72	143.19^{c} ± 1.05
Group II		158.32 ± 1.62	178.49^{a} ± 1.85	157.36^{a} ± 1.56
Group III		154.47 ± 1.05	138.82^{c} ± 1.46	144.43^{c} ± 1.26
Group IV		156.66 ± 2.65	169.89^{b} ± 1.36	149.82^{b} ± 0.94
CD Values	**1 %**	**-**	**6.475**	**4.934**
	5 %	**-**	**4.758**	**3.614**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group** IV- Profenofos + *T. purpurea* treatment

On 0^{th} day of experiment:

At day 0 of experiment the mean (± SE) serum AST (IU/L) values of experimental birds in group I to group IV were 155.19±1.30, 158.32±1.62, 154.47±1.05 and 156.66±2.65, respectively. At this stage of experiment, the mean serum AST levels were statistically comparable within all experimental groups.

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At this intervals of study, the mean AST values of birds in group I were 141.76±1.72 and 143.19±1.05, respectively, which were statistically at par with values in all other experimental groups (Group II, group III and group IV).

Group II (Profenofos Treatment group):

On 14^{th} and 28^{th} day of experiment:

At 14^{th} and 28^{th} day of experiment the mean serum AST values were 178.49±1.85 and 157.36±1.56, respectively. At both the intervals of study the mean AST values of group II were found significantly (P<0.01) elevated as

compared to healthy control group and other treatment groups (group III and group IV).

Grossly, at both the intervals of experiment mean AST values of group II birds were significantly elevated as compared to other groups in experiment. Our findings were in accordance with Kammon *et al*, (2011); Kammon *et al.,* (2010); Begum *et al.,* (2015); Singh *et al.,* (2016); Wani *et al.,* (2017); Khudair *et al.,* (2017) and EL-Nahhal and Lubbad*,* (2018), noted elevated AST levels in OP pesticides toxicity in birds. The histopathological changes in liver sections of this group correlates the damage caused by profenofos pesticide to liver parenchyma.

Generally, increased hepatic enzyme concentrations are the indicator of parenchymatous degeneration of visceral organs resulting into leakage of enzymes from the cell (Singh *et al.,* 2016). Due to histoarchitectural damage caused to liver tissue, there might be leakage of hepatic enzymes in blood vessel (Begum *et al.,* 2015). This might be the reason for increased AST levels in toxin treated groups as compared to healthy control group and treatment control group.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment, the mean AST values of the birds in group III were 138.82±1.46 and 144.43±1.26, respectively. The mean AST values of this group were found statistically at par with healthy control group at both the interval. As poly phenolic compounds and flavonoids in leaves of *T. purpurea* plant having hepatoprotective properties and thus decreases the oxidative stress causing cellular damage to liver, which might be help to maintain the hepatic cellular architecture at par with normal. As the various medicinal properties of *T. purpurea* were stated by Dalwadi *et al*., (2014) and Mathews *et al.*, (2012).

Group IV (Profenofos + T. purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of study, mean values of AST in birds of group IV 169.89±1.36 and 49.82±0.94 were significantly improved ($P<0.01$) than AST levels of birds in group II at both the intervals of study. However, this

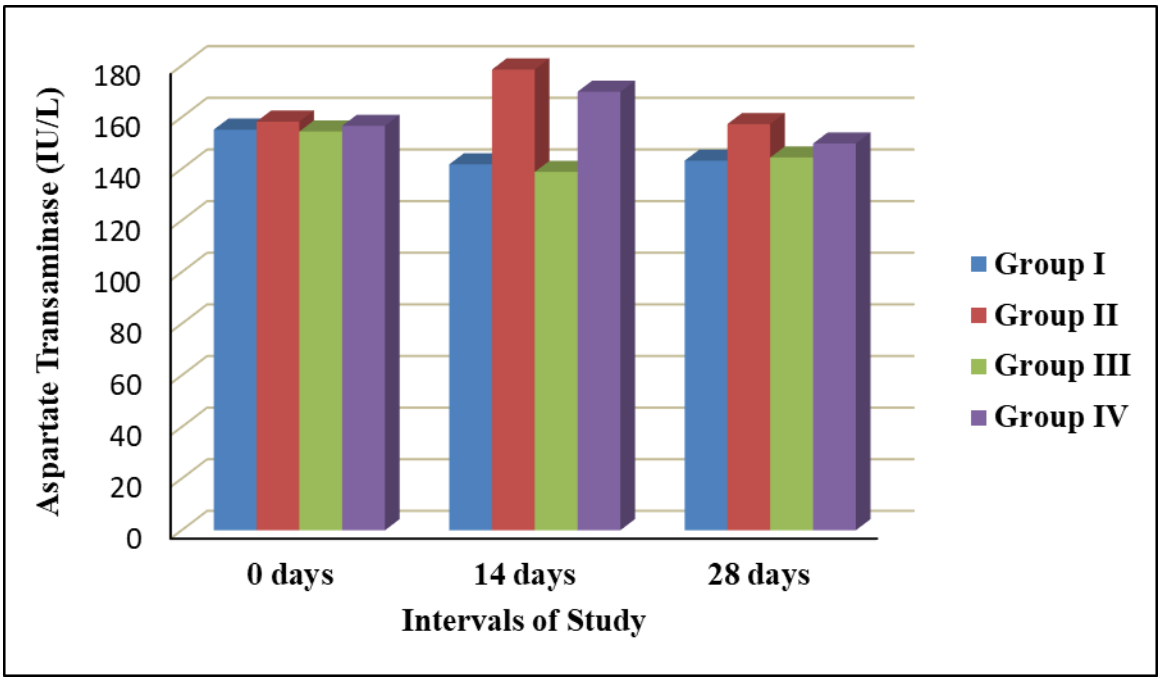

Fig 4.11: Mean values of Serum Aspartate Transaminase levels (IU/L) of experimental birds at different intervals of study

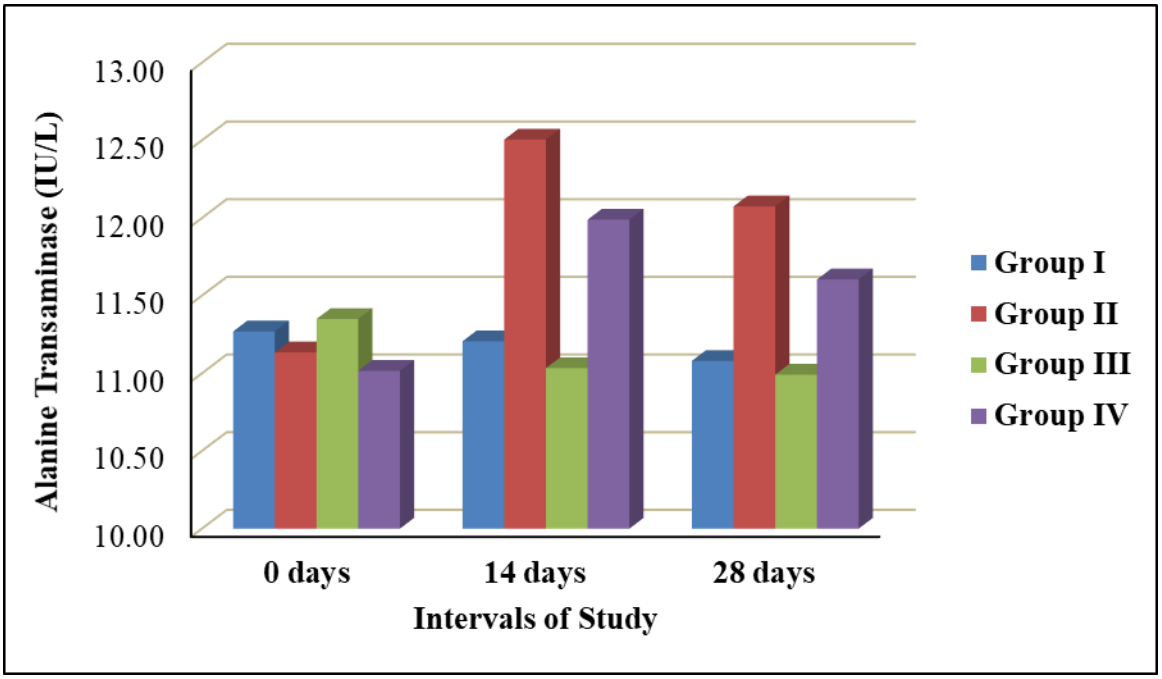

Fig 4.12: Mean values of Serum Alanine Transaminase (IU/L) of experimental birds at different intervals of study

improvement was partial, as the values were not improved up to the levels in control group.

As poly phenolic compounds and flavonoids present in leaves of *T. purpurea* plant is having hepatoprotective properties and significantly decreases the oxidative stress causing cellular damage to liver, which might be help to maintain the hepatic cellular architecture at par with normal as stated by Gora *et al*., (2014); Verma *et al.,* (2017) and Khatri *et al.,* (2009). So the mean AST values of group IV were numerically towards healthy control group. Which showed partial improvement in liver enzyme assay due to use of *T.purpurea* leaves powder in group IV.

4.3.6 Serum alanine transaminase (ALT, IU/L):

Mean (± SE) serum alanine transaminase (ALT, IU/L) in birds at different intervals of study in different groups are depicted in table 4.17 & fig.4.12

On 0^{th} day of experiment:

At day 0 of experiment the mean (± SE) serum ALT levels (IU/L) of experimental birds in group I to group IV were 11.27±0.13, 11.14±0.12, 11.35±0.11 and 11.02±0.11, respectively. At this stage of experiment, mean serum ALT levels (IU/L) were not significantly vary within experimental groups.

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14^{th} and 28^{th} day of experiment the mean (±SE) serum alanine transaminase (IU/L) levels in this group were 11.21±0.13 and 11.08±0.15, respectively. Mean serum ALT levels of birds in group I were within normal range and represent the healthy status of birds throughout experimental duration.

Table 4.17: Mean (±SE) values of serum alanine transaminase (IU/L) of experimental birds at different intervals of study

Groups of birds		Mean values of serum ALT (IU/L) in different groups (Mean ± SE) at different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		11.27 ± 0.13	11.21^{c} ± 0.13	11.08^{c} ± 0.15
Group II		11.14 ± 0.12	12.51^{a} ± 0.08	12.08^{a} ± 0.11
Group III		11.35 ± 0.11	11.03^{c} ± 0.10	10.99^{c} ± 0.13
Group IV		11.02 ± 0.11	11.99^{b} ± 0.09	11.61^{b} ± 0.09
CD Values	**1 %**	**-**	**0.412**	**0.498**
	5 %	**-**	**0.309**	**0.366**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

On 14th and 28th day of experiment:

At both the intervals of study, the mean serum alanine transaminase (ALT) levels of group II were 12.51±0.08 and 12.08±0.11, respectively. Which were markedly elevated (P<0.01) than the mean ALT values of healthy control group and other treatment groups (group III and group IV).

Finally, at 14th and 28th day of experiment the mean ALT values of group II birds were significantly increased as compare to other groups in experiment. Our findings were similar with Kammon *et al*, (2011); Kammon *et al.,* (2010); Begum *et al.,* (2015); Singh *et al.,* (2016); Wani *et al.,* (2017); Khudair *et al.,* (2017) and EL-Nahhal and Lubbad, (2018), who observed significant elevation in mean ALT values in OP pesticide toxicated birds in their study.

Elaveted hepatic enzyme concentrations are the indicator of parenchymatous degeneration of visceral organs resulting into leakage of enzymes from the cell (Singh *et al.,* 2016). The histopathological changes in liver of this group correlates the damage caused by profenofos pesticide to liver parenchyma

resulting in elevated levels of ALT. Due to vascular damage, necrosis and degeneration observed in liver parenchyma, the leakage of hepatic enzymes might be carried out from liver parenchyma to blood vessels (Begum *et al.,* 2015). This might be the reason for increased ALT levels in toxin treated groups as compared to healthy control group.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At both intervals of experiment, mean ALT values of birds in the group III were 11.03±0.10 and 10.99±0.13, respectively. The mean ALT values of this group were within normal physiological range and statistically at par with the values in birds of healthy control group. As poly phenolic compounds and flavonoids present in leaves of *T. purpurea* plant is having hepatoprotective properties and significantly decreases the oxidative stress causing cellular damage to liver, which might be helpful to maintain the hepatic cellular architecture at par with normal. As the various medicinal properties of *T. purpurea* were stated by Dalwadi *et al*., (2014) and Mathews *et al.*, (2012).

Group IV (Profenofos + T. purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of study mean ALT values of birds in group IV (11.99±0.09 and 11.61±0.09, respectively) were significantly elevated than mean values of birds in healthy control group and toxin control group (group II). However, mean ALT levels of birds in this group at both the intervals were significantly improved the levels in birds of group II (12.51 ± 0.08 and 12.08 ± 0.11, respectively), data indicated that there was partial ameliorative effects of the plant treatment as the ALT levels were not improved up to the levels in healthy control and plant treatment control group (Group III).

As poly phenolic compounds and flavonoids present in leaves of *T. purpurea* plant is having hepatoprotective properties and significantly decreases the oxidative stress, which subsequently minimize the cellular damage to liver. This might be help to maintain the hepatic cellular architecture at par with normal

and minimal leakage of hepatic enzymes in vascular system as stated by Gora *et al*., (2014); Verma *et al.,* (2017) and Khatri *et al.,* (2009) in their study.

4.3.7 Serum Alkaline Phosphatase (ALP, IU/L):

Mean (± SE) Serum Alkaline Phosphatase (ALP, IU/L) in birds at different intervals of study in different groups are depicted in table 4.18 & fig. 4.13

On 0^{th} day of experiment:

At day 0 of experiment the mean (± SE) serum ALP (IU/L) values of experimental birds in group I to group IV were 2857.33±192.60, 2901.33±68.62, 2985.50±77.20 and 2940.83±100.83, respectively. At this stage of experiment the mean serum ALP levels were statistically not comparable within experimental groups, this showed the healthy status of the birds at pre-treatment interval of study.

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14^{th} and 28^{th} intervals of study, the mean ALP values were 2880.67 ± 80.01 and 2948.00 ±124.34, respectively, and were statistically at par with each other and within normal physiological range representing the healthy status of birds throughout the experimental period.

Table 4.18: Table showing (Mean ± SE) values of serum alkaline phosphatase (ALP, IU/L) of experimental birds at different intervals of study

Groups of birds		Mean values (Mean ± SE) of serum Alkaline phosphatase (ALP, IU/L) in different groups of different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		2857.33 ±192.60	2880.67^{b} ±80.01	2948.00^{b} ±124.34
Group II		2901.33 ±68.62	3312.00^{a} ±112.59	3537.00^{a} ±155.88
Group III		2985.50 ±77.20	2846.83^{b} ±74.67	2837.00^{b} ±111.30
Group IV		2940.83 ±100.83	3223.83^{a} ±58.76	3389.00^{a} ±96.98
CD Values	**1 %**	**-**	**337.27**	**499.10**
	5 %	**-**	**247.29**	**365.95**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group II (Profenofos Treatment group):

<u>On 14th and 28th day of experiment:</u>

At 14th and 28th day of experiment the mean serum ALP values were (3312.00 ±112.59 and 3537.00 ±155.88, respectively) significantly (P<0.01) elevated than the values in healthy control group (Group I) and plant control group (group III).

Grossly, throughout the experiment the mean ALP values of group II birds were significantly increased as compare to other groups in experiment. Our findings were in accordance with Kammon *et al.,* (2010); Begum *et al.,* (2015); Singh *et al.,* (2016); Wani *et al.,* (2017); Khudair, (2017) and EL-Nahhal and Lubbad., (2018). The histopathological changes in liver of this group correlates the damage caused by profenofos pesticide to liver parenchyma.

Generally, increased hepatic enzyme concentrations are the indicator of cellular degeneration of liver parenchyama resulting into leakage of enzymes from the cell Singh *et al.,* (2016); Kammon *et al.,* (2010). Begum *et al.,* (2015) and EL-Nahhal and Lubbad, (2018) also explained that the liver exert an ability to

detoxify any toxin like OP pesticides, as a result the large fraction of ALT may be leaked in the blood. Then again, hepatocytes might be damaged due to cellular oxidative stress; subsequently, leakage of hepatic enzyme might take place. This might be the reason for increased AST levels in toxin treated groups, as compared to healthy control group, in this group.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean ALP values of the group III were 2846.83 ±74.67 and 2837.00 ±111.30, respectively. The mean ALP values of this group were within normal physiological range and statistically were at par with the ALP values of healthy control group. As poly phenolic compounds and flavonoids present in *T. purpurea* plant leaf powder, which are known to possess hepatoprotective properties, therefore significantly decreases the oxidative stress causing cellular damage to liver, which might be help to maintain the hepatic cellular architecture at par with normal. As the many medicinal properties of *T. purpurea* were stated by Dalwadi *et al*., (2014) and Mathews *et al.*, (2012).

Group IV (Profenofos + T. purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean serum ALP values were (3223.83 ±58.76 and 3389.00 ±96.98, respectively) significantly higher than the values of healthy control group and plant control group (group III). Whereas, the mean values of serum ALP of birds in group IV were statistically at par but numerically declined than mean values of toxin control group (group II).

Grossly, the mean serum ALP values of the group IV were partially improved the altered values of toxin control group (group II). As poly phenolic compounds and flavonoids present in leaves of *T. purpurea* plant is having hepatoprotective properties and significantly decreases the oxidative stress causing cellular damage to liver, which might be helpful to maintain the hepatic cellular architecture at par with normal. Gora *et al*., (2014); Verma *et al.,* (2017) and Khatri *et al.,* (2009) were noted significant hepatoprotective activity of *T.*

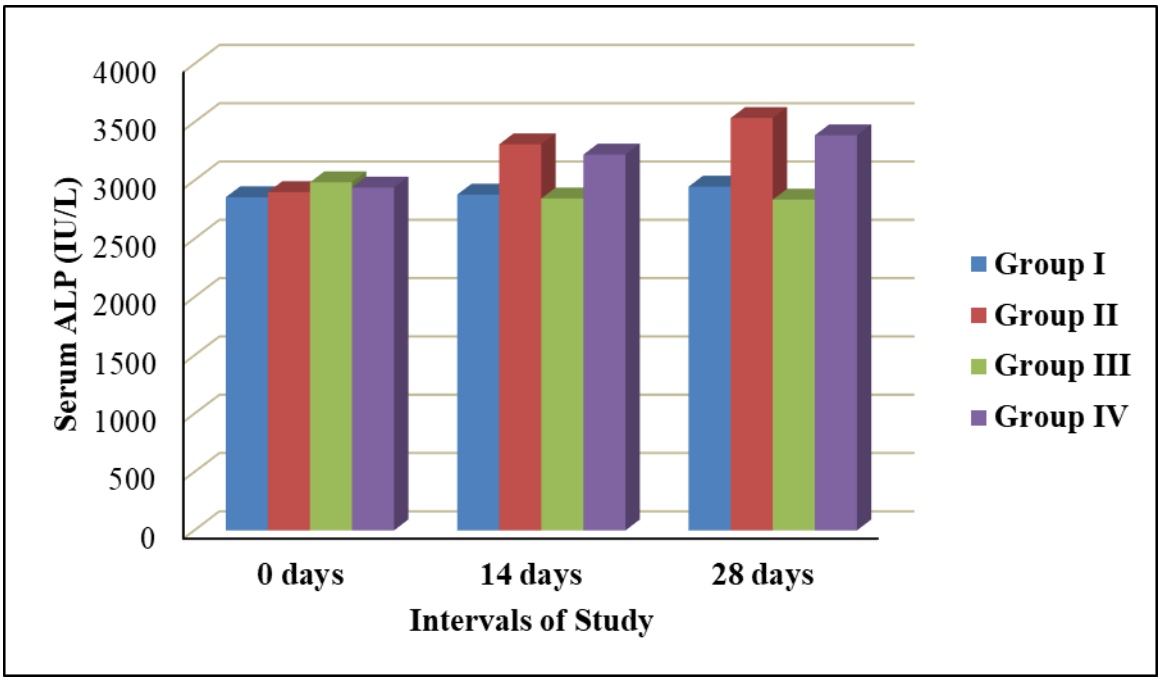

Fig 4.13: Mean values of Serum Alkaline Phosphatase (ALP, IU/L) of experimental birds at different intervals of study

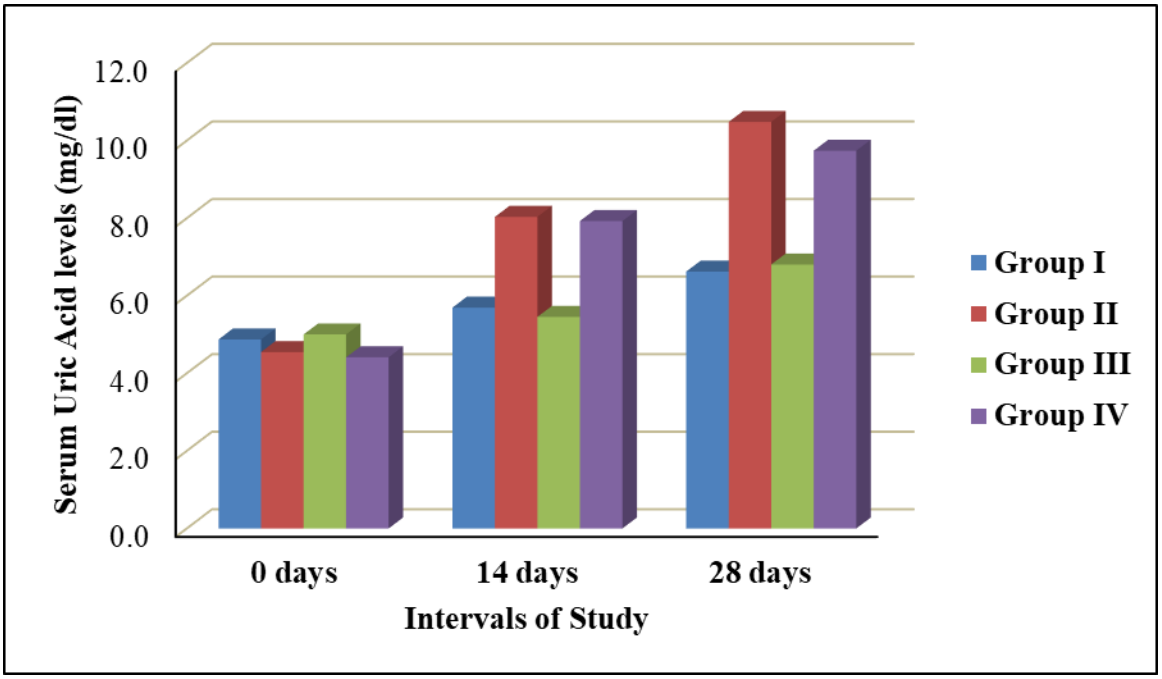

Fig 4.14: Mean values of Serum Uric Acid levels (mg/dl) of experimental birds at different intervals of study

purpurea in arsenic, CCl_4 and thioacetamide induced hepatotoxicity in rats. Also, observations of improved histoarchitecture of liver and kidney of group IV than group II, in this study also supports the results of mean serum ALP values of group IV in this experiment.

4.3.8 Serum Uric Acid (SUA, mg/dl):

Mean (± SE) serum uric acid levels (mg/dl) in birds at different intervals of study in different groups are depicted in table 4.19 & fig. 4.14

On 0^{th} day of experiment:

At day 0 of experiment the mean (± SE) serum uric acid levels (mg/dl) of experimental birds in group I to group IV were 4.88±0.16, 4.55±0.18, 5.01±0.08 and 4.42±0.22, respectively. At this stage of experiment the mean serum uric acid levels (mg/dl) were statistically not significant within experimental groups.

Table 4.19: Mean (± SE) values of serum uric acid levels (mg/dl) of experimental birds at different intervals of study

Groups of birds		Mean values of serum uric acid levels (mg/dl) in different groups (Mean ± SE) at different intervals of study		
		0^{th} Day	**14^{th} Day**	**28^{th} Day**
Group I		4.88 ±0.16	5.70^{b} ±0.12	6.62^{c} ±0.16
Group II		4.55 ±0.18	8.04^{a} ±0.05	10.49^{a} ±0.13
Group III		5.01 ±0.08	5.46^{b} ±0.09	6.81^{c} ±0.06
Group IV		4.42 ±0.22	7.93^{a} ±0.14	9.74^{b} ±0.17
CD Values	**1 %**	**-**	**0.427**	**0.550**
	5 %	**-**	**0.305**	**0.400**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly ($P<0.05$) ($P<0.01$).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean serum uric acid levels were 5.70±0.12 and 6.62±0.16, respectively. This values were within normal range and didn't differ significantly, which represent the healthy status of birds in group I during experimental duration.

Group II (Profenofos Treatment group):

On 14th day of experiment:

At this interval of study, the mean serum uric acid levels (8.04±0.05) significantly (P<0.01) elevated than the SUA levels in to healthy control group (Group I) and plant control group (Group III). Moreover, mean serum uric acid levels of group II were numerically higher but statistically at par with group IV.

On 28th day of experiment:

At this stage of experiment the mean serum uric acid levels (10.49±0.13) were numerically significantly elevated (P<0.01) as compared to healthy control group (Group I) and other treatment groups (group III and group IV).

Study concluded that the mean uric acid levels of toxin treated group (group II) showed marked elevation throughout the experiment as compare to other groups. These results were with in accordance with the findings of Kammon *et al.,* (2010); Singh *et al.,* (2016) and Khudair *et al.,* (2017) of OP pesticides toxicity in birds.

Blood serum levels of uric acid mainly depends protein catabolism and renal function of that bird. The present findings of uric acid levels were indicative of marked damage to kidney function and protein metabolism in this group (group II) of birds as stated by Singh *et al.,* (2016). Histopathological observations of necrosis and degeneration in kidney section of respective group (group II) in the present study also support this findings.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean uric acid values (5.46±0.09 and 6.81±0.06, respectively) were statistically at par with healthy control group (group I), which was showed the healthy status of the birds in group III throughout the experiment. This balance in levels of SUA in birds of this group indicates that feeding of plant leaves powder alone is not harmful.

Group IV (Profenofos + T. purpurea treatment group):

On 14th day of experiment:

At this stage of experiment the mean uric acid values of the birds in group IV were (7.93±0.14) significantly elevated than the values in healthy control group and plant control group (group III). However, the mean uric acid values of group IV were numerically decreased but statistically at par as compared to levels in toxin control group (group II), indicating partial improvement.

On 28th day of experiment:

At this interval of the study the mean uric acid values were (9.74±0.17) numerically differ and statistically significantly elevated than the value of healthy control group and toxin control group (group II). Moreover, mean uric acid values of birds in group IV showed marked decline in SUA levels as compared to toxin control group (group II), indicating partial improvement.

Grossly, the mean uric acid values of group IV showed partial improvement throughout the experiment, as compared to levels in toxin control group (group II). Which can be approved by improved histoarchitecture of kidneys of group IV birds as compared to group II birds in this study. Jain *et al.,* (2013) observed the nephroprotective activity of *T. purpurea* in which they noted the improvement in BUN and creatinine levels in gentamicin induced renal toxicity in rats.

4.3.9 Blood urea nitrogen (mg/dl):

Mean (± SE) levels of blood urea nitrogen (mg/dl) in birds at different intervals of study in different groups are depicted in table 4.20 & fig.4.15.

On 0 day of experiment:

At day 0 of experiment the mean (± SE) blood urea nitrogen levels (mg/dl) of experimental birds in group I to group IV were 6.07±0.13, 5.98±0.11, 6.26±0.09 and 6.35±0.12, respectively. At this stage of experiment, mean blood urea nitrogen levels (mg/dl) were statistically not significant within different experimental groups.

Table 4.20: Mean (± SE) values of blood urea nitrogen levels (mg/dl) of experimental birds at different intervals of study

Groups of birds		Mean values (Mean ± SE) of blood urea nitrogen levels (mg/dl) in different groups at different intervals of study		
		0th Day	**14th Day**	**28th Day**
Group I		6.07 ±0.13	5.87^{c} ±0.11	6.22^{b} ±0.08
Group II		5.98 ±0.11	7.50^{a} ±0.15	7.18^{a} ±0.06
Group III		6.26 ±0.09	6.01^{c} ±0.12	6.16^{b} ±0.07
Group IV		6.35 ±0.12	6.99^{b} ±0.10	6.98^{a} ±0.06
CD Values	**1 %**	**-**	**0.485**	**0.278**
	5 %	**-**	**0.354**	**0.204**
Statistics		**NS**	**HS**	**HS**

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) blood urea nitrogen levels were 5.87±0.11 and 6.22±0.08, respectively. This mean blood urea nitrogen levels of group I were represents the healthy status of birds in group I during experimental duration.

Group II (Profenofos Treatment group):

On 14th day of experiment:

The mean Blood Urea Nitrogen (BUN) levels of birds in group II (7.50±0.15) were numerically elevated significantly (P<0.01) as compared to levels in birds of healthy control group (Group I) and other treatment groups (group III and group IV).

On 28th day of experiment:

At this stage of experiment the mean blood urea nitrogen levels of birds in group II (7.18±0.06) were increased statistically (P<0.01) than the levels in birds of healthy control group and plant control group (group III). However, the mean blood urea nitrogen levels of group II were found numerically elevated but statistically at par as compare to mean values of group IV, indicating partial improvement of levels of BUN after addition of plant powder in the diet of profenofos intoxicated birds.

Grossly, the mean blood urea nitrogen levels of toxin treated group (group II) showed considerable elevation throughout the experiment as compare to other treatment groups. These findings were similar with the observation of Singh *et al.,* (2016) and EL-Nahhal and Lubbad, (2018), who observed decreased levels of BUN in chlorpyrifos toxicated chickens.

Mean blood urea nitrogen levels mainly depends upon protein catabolism and renal function of that bird. The present findings of blood urea nitrogen levels showed marked damage to kidney function and protein metabolism in this group (group II) of birds Singh *et al.,* (2016). Which was proved by the histopathological observations of necrosis and degeneration in kidney section of respective group (group II) in the present study.

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean blood urea nitrogen levels (6.01±0.12) and (6.16±0.07) were statistically at par with that of values in birds of

healthy control group (group I), which showed the healthy status of the birds in group III throughout the experiment.

Group IV (Profenofos + T. purpurea treatment group):

On 14th day of experiment:

The mean blood urea nitrogen levels of birds in group IV (6.99±0.10) showed marked increase ($P<0.01$) as compare to values in birds of healthy control group and plant control group (group III). However, the mean BUN values of birds in group IV were significantly lower than the levels in toxin control group (group II).

On 28th day of experiment:

Same as the earlier 14th day result the mean BUN values of birds in group IV (6.98 ±0.06) were significantly higher than values in healthy control group and plant control group (group III). However the mean BUN values of group IV were statistically at par, but numerically decreased as compared to toxin control group (group II).

To conclude the results, mean BUN values of group IV showed partial improvement throughout the experiment as compare to levels in toxin control group (group II). Which can be proved by mild to moderate improvement in histoarchitecture of kidney and liver of group IV birds, as compared to group II birds. Jain *et al.,* (2013) observed the nephroprotective activity of *T. purpurea* in which they noted the improvement in BUN and creatinine levels in gentamicin induced renal toxicity in rats.

4.3.10 Serum Acetyl Choline Esterase (U/L):

Mean (± SE) Serum acetyl choline esterase (AchE) (U/L) in birds at different intervals of study in different groups are depicted in table 4.21 & fig. 4.16.

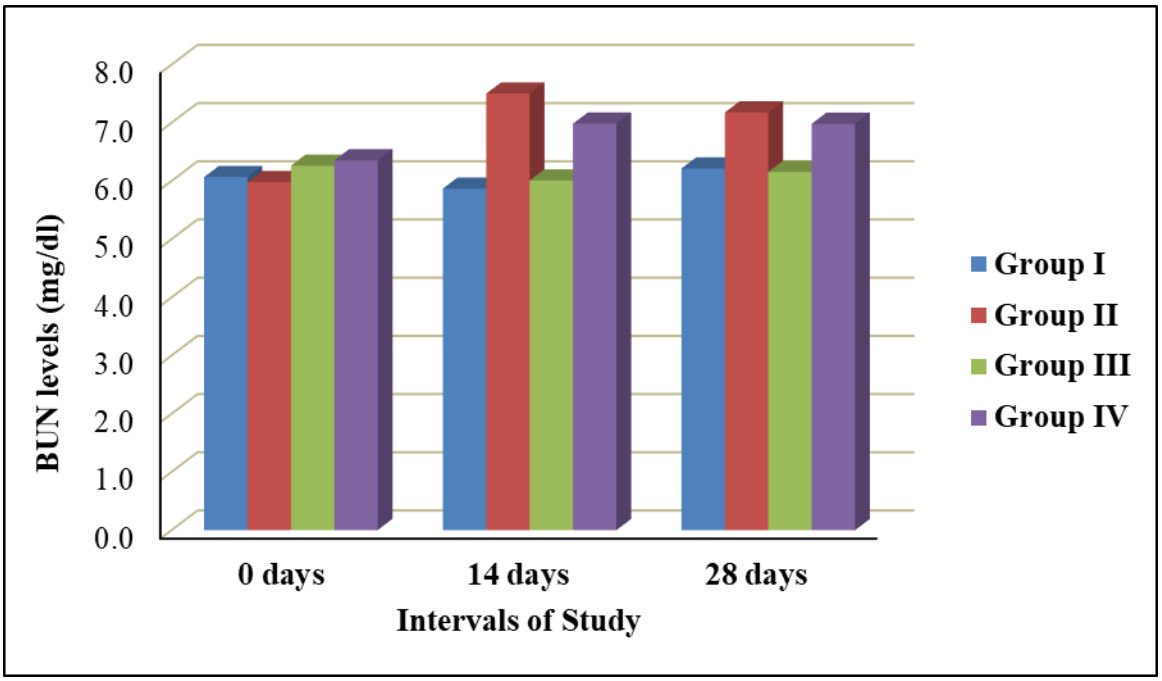

Fig 4.15: Mean values of Blood Urea Nitrogen levels (mg/dl) of experimental birds at different intervals of study

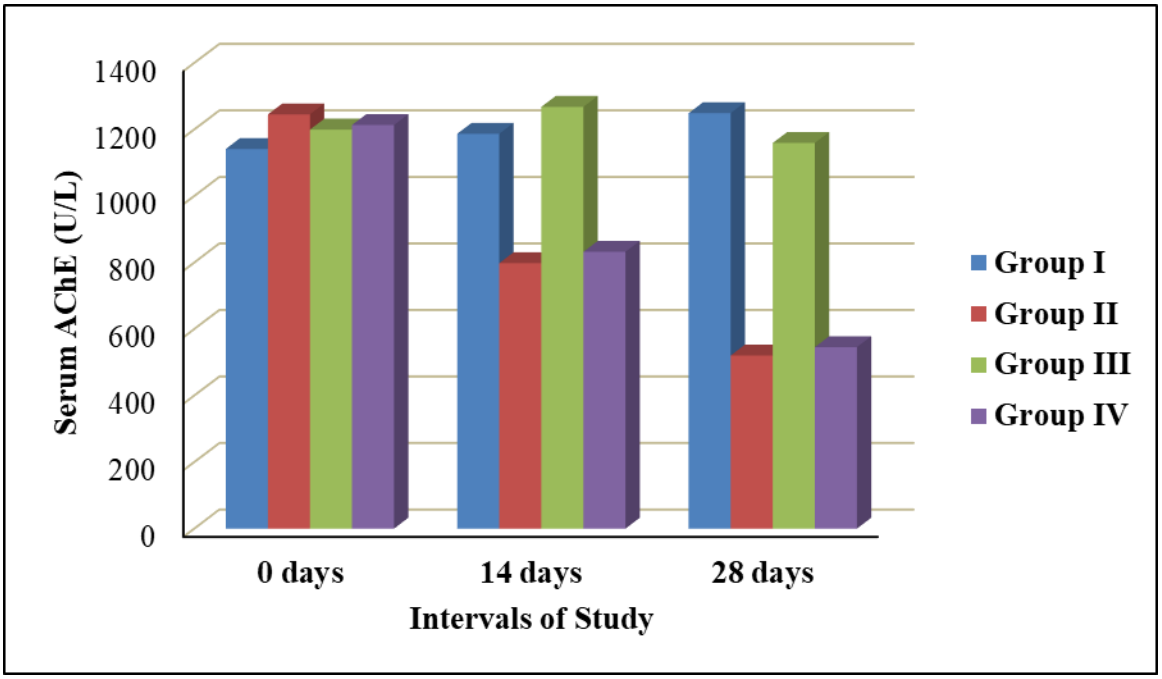

Fig. 4.16: Mean (± SE) values of Serum Acetyl Choline Esterase (U/L) of experimental birds at different intervals of study

On 0th day of experiment:

At day 0th of experiment the mean (± SE) serum acetyl choline esterase (U/L) of experimental birds in group I to group IV were 1141.33±49.32, 1245.33±67.61, 1199.67±63.14 and 1213.50±40.98, respectively. At this stage of experiment, the mean serum acetyl choline esterase were found at par within experimental groups.

Table 4.21: Mean (± SE) values of acetyl choline esterase (U/L) of experimental birds at different intervals of study

Groups of birds		Mean values (Mean ± SE) of serum acetyl choline esterase (AchE, U/L) in different groups at different intervals of study		
		0th Day	14th Day	28th Day
Group I		1141.33 ±49.32	1187.03^{a} ±86.22	1249.00^{a} ±58.53
Group II		1245.33 ±67.61	798.17^{b} ± 64.21	520.30^{b} ±80.68
Group III		1199.67 ±63.14	1268.00^{a} ±61.87	1159.40^{a} ±57.00
Group IV		1213.50 ±40.98	832.07^{b} ±49.08	545.47^{b} ±28.89
CD Values	1 %	-	268.35	238.18
	5 %	-	196.76	174.64
Statistics		NS	HS	HS

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

Group I (Healthy control group):

On day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean (±SE) serum acetyl choline esterase (U/L) values were 1187.03±86.22 and 1249.00±58.53, respectively. Mean serum acetyl choline esterase (U/L) levels of group I were within normal range and represent the healthy status of birds.

Group II (Profenofos Treatment group):

On 14th and 28th day of experiment:

At 14th and 28th day of experiment, the mean serum acetyl choline esterase (AchE) values were 798.17±64.21 and 520.30±80.68, respectively. The mean

AchE values of the birds in group II were significantly (P<0.01) reduced as compared to healthy control group and plant control group (group III). Also mean AchE values of birds in group II were statistically at par but numerically lowers at this time of interval of experiment.

Our findings were correlated with the fingings of Garg *et al.,* (2004); Kammon *et al.,* (2010); Begum *et al.,* (2015) and Wani *et al.,* (2017), who observed that the OP pesticides might be responsible for the decreased serum AchE concentration in chickens.

Normally, the cholinesterase rapidly hydrolyze the neurotransmitter acetylcholine into inactive fragments of choline and acetic acid after the completion of neurochemical transmission at terminal endings of postganglionic nerves. The major toxicity of organophosphate compounds is the covalent binding of phosphate radicals to the active sites of the cholinesterases, transforming them into enzymatically inert proteins. The inhibition of cholinesterase activity leads to the accumulation of acetylcholine at synaptic ends, causing overstimulation and subsequent disruption of transmission in both the central and peripheral nervous systems (Kumar *et al*,. 2010).

Group III (Tephrosia purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of the study the mean serum acetyl choline esterase values of birds were 1268.00±61.87 and 1159.40±57.00, respectively, which were statistically at par as compare to healthy control group. As the *T. purpurea* plant possess the hepatoprotective properties (Mathews *et al.*, 2012), there was not any significant effect on serum acetyl choline esterase levels in birds of this group.

Group IV (Profenofos + T. purpurea treatment group):

On 14th and 28th day of experiment:

At both the intervals of study, the mean AchE values were 832.07±49.08 and 545.47±28.89, respectively. The mean AchE values of group IV were statistically comparable (P<0.01) with the values in birds of healthy control group and plant control group (group III). Mean values of group IV were statistically at

par with mean values of group II, but here numerically slightly elevated than the values in birds of group II at both intervals of experiments, indicating beneficial effects of addition of plant powder in the diet of intoxicated birds.

4.4 Clinical signs, symptoms and Mortality:

All experimental groups were observed and examined daily throughout the experimental period for general clinical signs and symptoms seen in all birds. There was no single mortality the group wise changes observed in clinical signs and symptoms of experimental birds were mentioned below

Group I (Healthy control group):

During experimental trial the birds in healthy control group (group I) were active, healthy and with normal physiological growth parameters. Our findings were supported by observations obtained in growth, Heamatological, biochemical and histopathological parameters of group I in this study.

Group II (Profenofos Treatment group):

Throughout the experimental trial the birds of group II were showed mild clinical symptoms, in which sluggishness, dullness, depression, mild hurdling and ruffled feather were observed at last weeks of experimental trial. However the present study noted the clinical signs like markedly decreased water and feed intake, reduced appetite, mild diarrhoea, reduced growth and listlessness were observed in group II birds at last weeks of experiment.

Our findings were in accordance with (Begum *et al*., 2015), (Singh *et al*., 2016), (Wani *et al*., 2017) and (Kafle *et al.,* 2018). OP pesticide might be affect the normal physiological processes which are evident as the clinical signs (Singh *et al*., 2016). These clinical and behavioral signs observed in present study might be due to accumulation of acetylcholine at nerve endings which potentiates cholinergic toxicity by inhibition of acetylcholinesterase enzyme well known for its muscarinic, nicotinic and central nervous system effects (Ghaffar *et al.,* 2013).

Group III (Tephrosia purpurea treatment group):

The birds of group III were active and healthy throughout the experimental period. As the Tephrosia purpurea leaves powder was beneficial to liver and kidney health and non-toxic to birds, the birds of group III were not showing any significant clinical signs and symptoms throughout the experiment (Mathews *et al.,* 2012 and Khatri *et al.,* 2009).

Group IV (Profenofos + *T. pupurea* treatment):

The birds of group IV were showed mild clinical signs which were mentioned in group II but the severity of signs and symptoms were much lesser than the toxin control group (group II). As the Tephrosia purpurea leaves powder was beneficial to liver and kidney health and non-toxic to birds, the birds of group IV were showing mild improvement in clinical signs and symptoms as compare to toxin control group (group II) throughout the experiment (Mathews *et al.,* 2012 and Verma *et al.,* 2017).

4.5 Pathological Studies:

4.5.1 Absolute Organs Weight (gm):

4.5.1. (A) Absolute weights of liver (gm):

Mean (± SE) values of absolute weights of liver (gm) of birds in at different intervals of study are depicted in table 4.22.

Group I (Healthy control group):

<u>At day 14 and day 28 of experiment:</u>

At 14^{th} and 28^{th} day of experiment the mean absolute weights of liver were (10.06 ±0.19 and 13.95±0.20) statistically at par and represent normal range as compared to other groups in experiment.

Group II (Profenofos Treatment group):

At 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean absolute weights of liver (8.98±0.24) were statistically significantly (P<0.01) decreased than the weights of liver of birds in healthy control group and plant control group.

Grossly, the absolute liver weights of birds in group II were significantly decreased in profenofos intoxicated birds (Group II) throughout the experimental period. Our findings were found similar to Naraharisetti *et al.,* (2009), Al-Baggou, (2014) and EL-Nahhal and Lubbad, (2018). The decreased body weight and body weight gain of group II observed in this experiment also supports the results of decreased absolute liver weight.

Group III (Tephrosia purpurea treatment group):

At 14th and 28th day of experiment:

At 14th and 28th day of experiment the mean absolute liver weights of birds in group III (9.98 ±0.21 and 13.44±0.21, respectively) were statistically comparable with healthy control group. The use of *Tephrosia purpurea* (hepatoprotective) as feed additive in the diet of birds in group III might be useful for maintainance of the integrity and histoarchitecture of the liver. (Verma *et al.,* 2017 and Gora *et al*., 2014).

Group IV (Profenofos + *T. pupurea* treatment):

At 14th day of experiment:

At this stage of experiment, the mean values of absolute liver weights (9.09±0.27) were statistically at par and numerically increased as compare to mean values of liver weights in birds of group II. Whereas, the mean absolute liver weights of group IV were significantly lower than healthy control group and plant control group.

At 28th day of experiment:

At this interval of experiment the mean absolute liver weights (11.00±0.26) of birds in this group were significantly (P<0.01) improved than the

liver weights of birds in group II, however this improvement was not up to the levels in healthy control group and other treatment groups.

Grossly, throughout the experiment, the mean absolute liver weights of the birds in group IV were partially improved than the values of mean absolute liver weights of toxin control group. The use of *Tephrosia purpurea* (hepatoprotective) might be useful for maintain the integrity and histoarchitecture of the liver of birds in group IV birds as compared to birds in group II.

Table 4.22: Mean (± SE) Absolute organ weights of experimental birds at different intervals of study

Groups of birds		Absolute weights of Liver, Kidney, Heart, spleen, testis and ovary (Mean ±S.E, gm)											
		Weight of liver		Weights of kidneys		Weight of heart		Weight of spleen		Weight of testis		Weight of ovary	
		14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day
Group I		10.06[a] ±0.19	13.95[a] ±0.20	3.61 ±0.28	6.17[a] ±0.15	2.66 ±0.15	3.69 ±0.11	0.70 ±0.06	1.13 ±0.14	0.10 ±0.01	0.21 ±0.02	0.18 ±0.05	0.36 ±0.03
Group II		8.98[b] ±0.24	10.32[c] ±0.12	3.06 ±0.21	4.58[b] ±0.28	2.18 ±0.17	2.94 ±0.27	0.54 ±0.04	0.88 ±0.12	0.08 ±0.01	0.13 ±0.03	0.13 ±0.01	0.29 ±0.05
Group III		9.98[a] ±0.21	13.44[a] ±0.21	3.49 ±0.27	6.10[a] ±0.25	2.51 ±0.09	3.48 ±0.26	0.70 ±0.09	0.99 ±0.09	0.09 ±0.03	0.18 ±0.01	0.19 ±0.05	0.35 ±0.04
Group IV		9.09[b] ±0.27	11.00[b] ±0.26	3.37 ±0.24	4.80[b] ±0.27	2.30 ±0.11	3.35 ±0.15	0.62 ±0.02	0.91 ±0.10	0.09 ±0.01	0.15 ±0.03	0.14 ±0.01	0.31 ±0.01
CD Value	1%	0.928	0.829	-	0.984	-	-	-	-	-	-	-	-
	5%	0.676	0.609	-	0.715	-	-	-	-	-	-	-	-
Statistics		HS	HS	NS	HS	NS	NS	NS	NS	NS	NS	NS	NS

Means bearing similar superscripts in column do not differ significantly (P<0.05) (P<0.01).

Where,

Group I- Healthy control **Group II** - Profenofos control

Group III –*T. purpurea* control **Group IV**- Profenofos + *T. purpurea* treatment

4.5.1. (B) Absolute weights of kidney (gm):

Mean (± SE) values of absolute weights of kidney (gm) of birds at different intervals of study in different groups are depicted in table 4.22

Group I (Healthy control group):

At day 14 and day 28 of experiment:

At 14th and 28th day of experiment the mean absolute weights of kidney in birds of this group were 3.61 ±0.28 and 6.17±0.15, statistically at par and considered as within normal range as compare to other groups in experiment.

Group II (Profenofos Treatment group):

At day 14th of experiment:

At 14th day of experiment, the mean absolute weights of kidneys in birds of group II (3.06 ±0.21) were numerically lower but statistically at par with all experimental groups.

At day 28th of experiment:

At 28th day of experiment, the mean absolute weights of kidneys of birds in group II (4.58±0.28) were significantly ($P<0.01$) decreased as compare to kidney weights in birds of healthy control group and plant control group.

Grossly, the absolute kidney weights of group II were decreased throughout the experimental period. Our findings were found similar to Naraharisetti *et al.,* (2009), and EL-Nahhal and Lubbad, (2018). The decreased body weight gain of birds in group II in this experiment also supports the results of decreased absolute kidney weight.

Group III (Tephrosia purpurea treatment group):

At 14th of experiment:

At 14th day of experiment the mean absolute weights of kidneys of birds in group III (3.49±0.27) were non comparable within all groups of experiment.

At 28th day of experiment:

At 28th day of experiment the mean absolute weights of kidneys in birds of group III (6.10±0.25) were significantly (P<0.01) increased improved as compare to weights in birds of toxin control group whereas were statistically at par with values in healthy control group.

Group IV (Profenofos + *T. pupurea* treatment):

At 14th day of experiment:

At 14th day of experiment the mean absolute weights of kidneys in birds of group IV were (3.37±0.24) non comparable within all groups of experiment.

At 28th day of experiment:

At 28th day of experiment the mean absolute weights of kidneys were (4.80±0.27) significantly (P<0.01) decreased as compared to weights in birds of healthy control control group, whereas were statistically at par with toxin control group, indicating not much ameliorative effects of *T. purpurea.*

4.5.1. (C) Absolute weights of heart (gm):

Mean (± SE) values of absolute weights of heart (gm) of birds in different groups at different intervals of study are depicted in table 4.22. At 14th and 28th day of experiment the mean absolute heart weights did not differ significantly within all groups of experiments.

4.5.1. (D) Absolute weights of spleen (gm):

Mean (± SE) values of absolute weights of spleen (gm) of birds at different intervals of study in different groups are depicted in table 4.22. At both the intervals of study (14th and 28th day) mean absolute spleen weights not comparable within all groups of experiments.

Group II (Profenofos Treatment group)

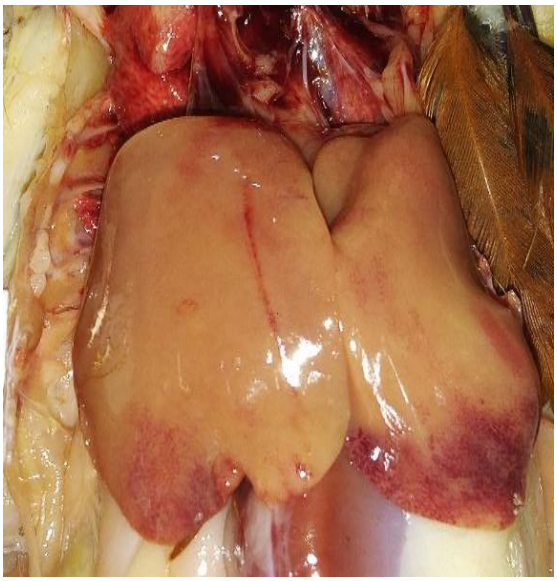

Plate 5: Diffuse necrosis with congestion and Petechial hemorrhages at the borders of liver of a bird from group II at 14th day

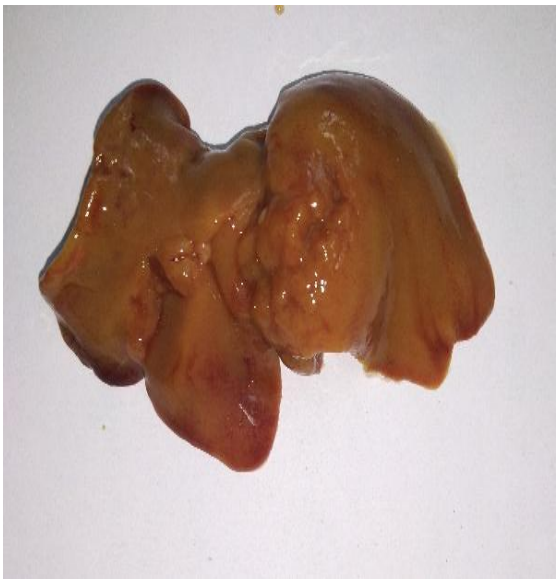

Plate 6: Note fragile liver in a bird of group II at 28th day of experiment

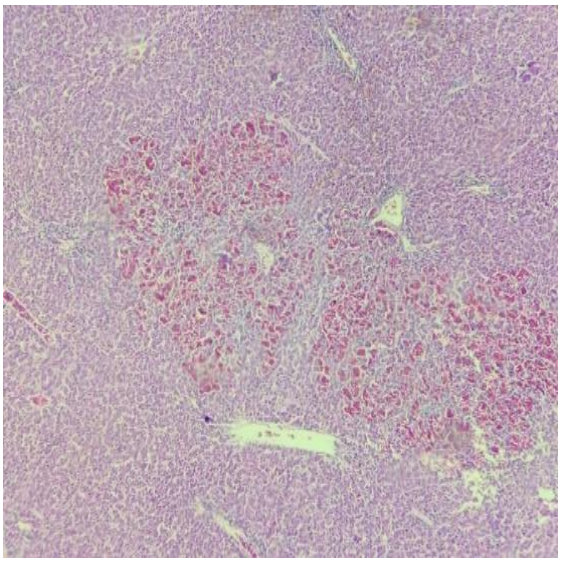

Plate 7: Areas of congestion of hepatic capillaries in liver parenchyma of bird of group II at 14th day (H & E × 100)

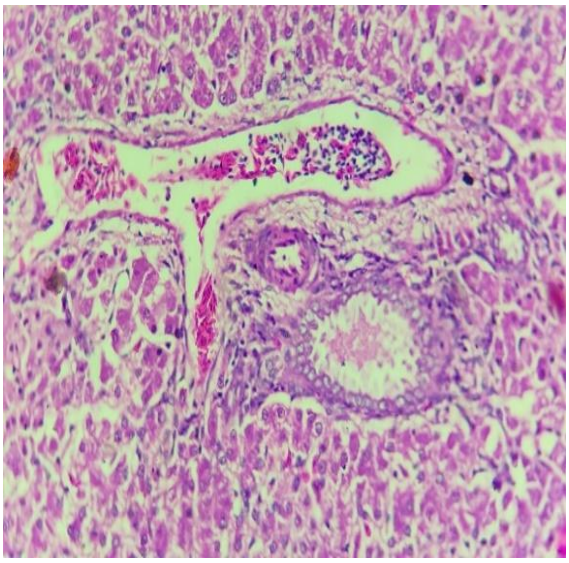

Plate 8: Dilatation of portal vein and hyperplasia of bile duct in a section of liver of bird in group II at 14th day interval (H & E × 400)

4.5.1. (E) Absolute weights of testis (gm):

Mean (± SE) values of absolute weights of testis (gm) of birds in different groups at different intervals of study are depicted in table 4.22. At 14^{th} and 28^{th} day of experiment the mean absolute testis weights were statistically not comparable within all groups of experiments.

4.5.1. (F) Absolute weights of ovary (gm):

Mean (± SE) values of absolute weights of ovary (gm) of birds at different intervals of study in different groups are depicted in table 4.22. At both the intervals of study (14^{th} and 28^{th} day) of mean absolute ovary weights were statistically not significant within all groups of experiments.

4.5.2 Gross and Histo-pathological alterations:

4.5.2. (A) Liver:

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Gross pathological alterations:

At both 14^{th} and 28^{th} day of experiment, the gross pathological examination of liver of sacrificed birds of group I and group III did not show any considerable gross pathological changes.

Histo-pathological alterations:

Histo-architecture of liver of birds sacrificed from group I & group III at 14^{th} and 28^{th} day of experiment appeared near to normal. The plant powder of *T. purpurea* in non-toxic and had various medicinal properties through antioxidental action at cellular level in tissues (Gora *et al*., 2014 and Khatri *et al.,* 2009).

Group II (Profenofos Treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At these intervals of study liver showed diffuse congestion, areas of hemorrhages (Petechial) at the borders of liver, diffuse pale areas and appeared flaccid in consistency (Plate 5 – 6). Our findings were in accordance with Kafle *et al.,* (2018), Kammon *et al*, (2011) and Kammon *et al.,* (2010), who reported OP pesticide toxicity studies in chickens.

Histo-pathological alterations:

14th day of experiment:

At this day of sacrifice, histopathological examination of liver section revealed moderate to severe congestion of hepatic capillaries, congested and dilated centrilobular vein in the liver parenchyma and dilated central hepatic vein (Plate-7). There were focal cystic changes in the hepatic parenchyma. Dilated portal vein, fibrous connective tissue proliferation, hyperplasia of bile duct, newly formed bile ducts and lymphocytic aggregations around triad were the significant changes observed in portal triad areas of liver (Plate 8 - 10). There were diffuse degenerative changes (Both granular and vacuolar) in hepatocytes and small and rounded focal areas of coagulative necrosis in the liver parenchyma, particularly at the border areas indicative of infarction.

28th day of experiment:

At this stage of experiment, congestion was not much marked as compared to findings noted at 14th day liver sections. However, there were diffuse necrotic changes in hepatic cells, particularly of hepatocytes around portal triad (Periportal necrosis) in liver parenchyma (Plate 11 & 12). Also, some sections of liver showed dilated and congested central hepatic vein & hepatocytes around it were showing diffuse degenerative & necrotic changes.

Group II (Profenofos Treatment group)

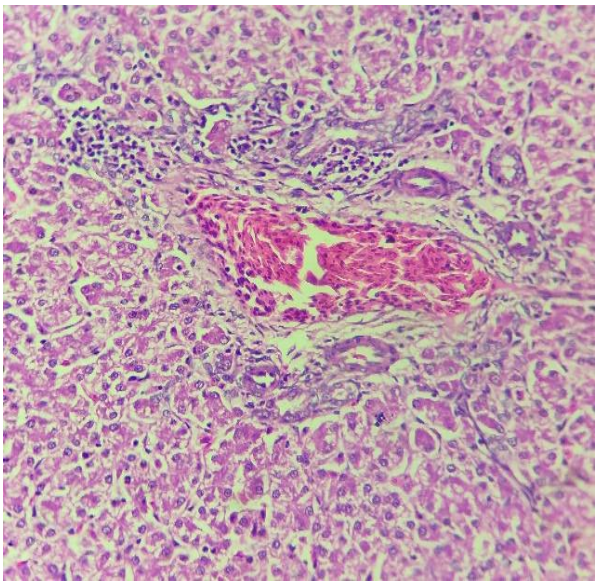

Plate 9: Section of liver showing newly formed bile duct and lymphocytic aggregations around hepatic triad of bird of group II at 14th day of experiment (H & E × 400)

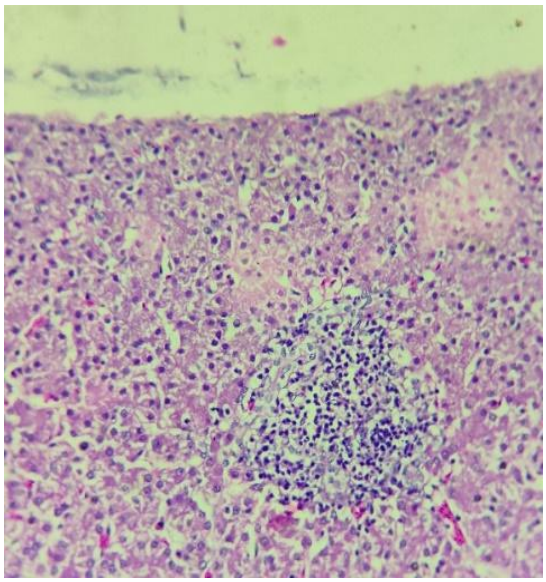

Plate 10: Section showing focal areas of infarction with lymphocytic aggregations in liver parenchyma of a bird in group II at 14th day interval (H & E × 400)

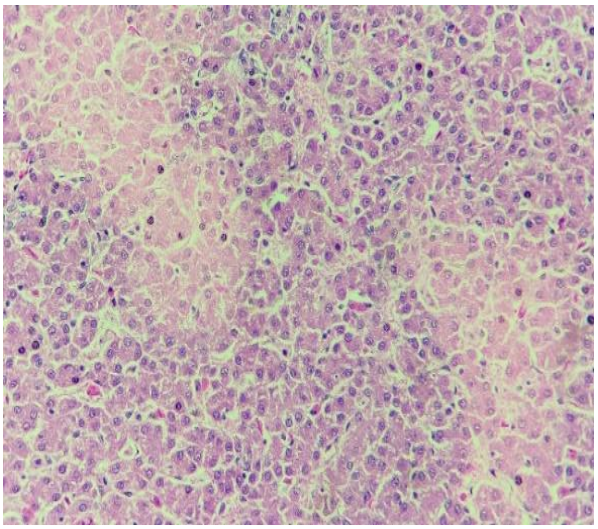

Plate 11: Note focal areas of coagulative necrosis in the liver parenchyma of bird of group II on 28th day of study (H & E × 400)

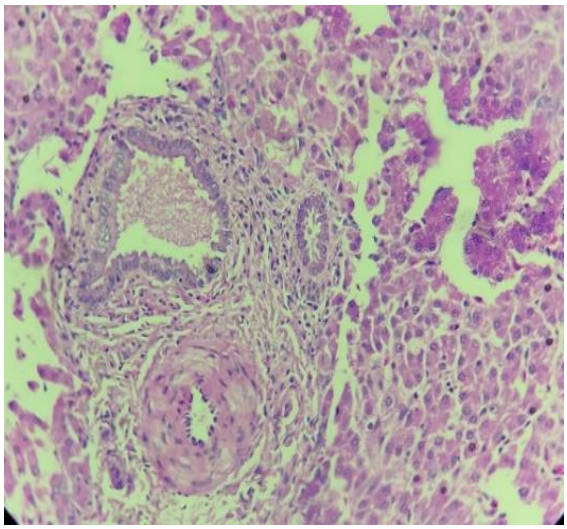

Plate 12: Section showing hyperplasia of bile duct in liver parenchyma of bird of group II at 28th day of experiment (H & E × 400)

Findings of our study were found similar with Kammon *et al.,* (2010), Kammon *et al*, (2011), Kafle *et al.,* (2018), Sodhi et al., (2008) and Begum *et al.,* (2015). Rhayf *et al.,* (2012) in their study on dimethoate induced histopathological changes in local layer chickens the study stated that OP pesticide toxicity can cause oxidative stress by the generation of free radicals and induce hepatic lipid peroxidation in mice.

Liver might be affected due to the irritant and toxic nature of the OP compound, as it was the major organ for biotransformation and detoxification of most of the xenobiotics in body (Rhayf *et al.,* 2012). Misshapen nucleus, condensation of chromatin, residues of cell organelles in cytoplasm, lipid vacuoles, loss of endoplasmic reticulum and mitochondrial swelling noted in hepatocytes in OP toxicity. Also, disturbances in oxido-reduction processes in mitochondria might be responsible for cellular degenerative changes in liver parenchyma as reported by Ghodke *et al.,* (2019) in subacute Acephate toxicated broiler chickens.

Group IV (Profenofos + *T. purpurea* treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At these stages of sacrifice, focal areas of congestion and haemorrhages were the only changes observed in the liver of birds of group IV.

Histo-pathological alterations:

14th day of experiment:

In the liver section of birds of this group at 14th day, it was observed that the degenerative & necrotic changes in hepatocytes around central vein were minimal as compare to profenofos treated group (group II). However, the congestion and dilatation of hepatic vein in portal area was consistent at this stage of sacrifice (Plate 37).

28th day of experiment:

At this stage of experiment, congestion in liver parenchyma was found comparatively reduced. Ameliorative changes viz. reduction in congestion of blood vessels and improvement of histo-architecture of liver was noticed.

When comparison of histological alterations in liver section between Group II & group IV were made, it is observed that overall histoarchitecture was maintained in this group at both the sacrifices. Also, the degenerative and necrotic changes in liver parenchyma were minimal. However, the changes in and around the portal triad such as dilatation of portal vein , hyperplasia of bile duct, moderate lymphocytic proliferation in the area were still observed. Overall, ameliorative effects of feeding of plant leaf powder was evident on improvement of altered histoarchitecture caused by toxin in the liver section (Plate 38).

Khatri *et al*., (2009) also noted that the flavanoids present in *Tephrosia purpurea* could be responsible for the membrane stabilizing activity in hepatic cell, through potent antioxidative action. Antioxidant activity of *T. purpurea* helps to reduce increased oxidative stress and protects the tissues from the oxidative damage (Gora *et al.,* 2014). Also, the observations of improved serum AST, ALT and ALP (Hepatic health indicator) values in birds of group IV throughout the study period, supported the improved histoarchitecture observed in birds of group IV.

4.5.2. (B) Kidney:

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

<u>Gross pathological alterations:</u>

At both **14th and 28th day** of experiment, the gross pathological examination of kidneys of sacrificed birds in group I and group III did not showed any considerable gross pathological changes, except for congestion.

Group II (Profenofos Treatment group)

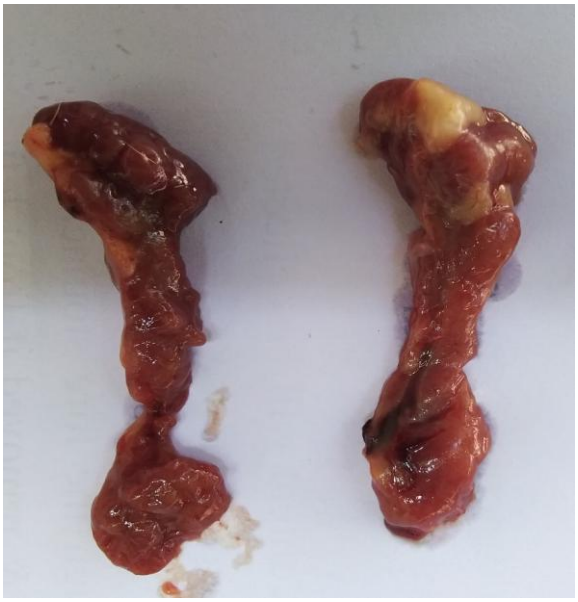

Plate 13: Congested kidneys of a bird in group II at 28th day

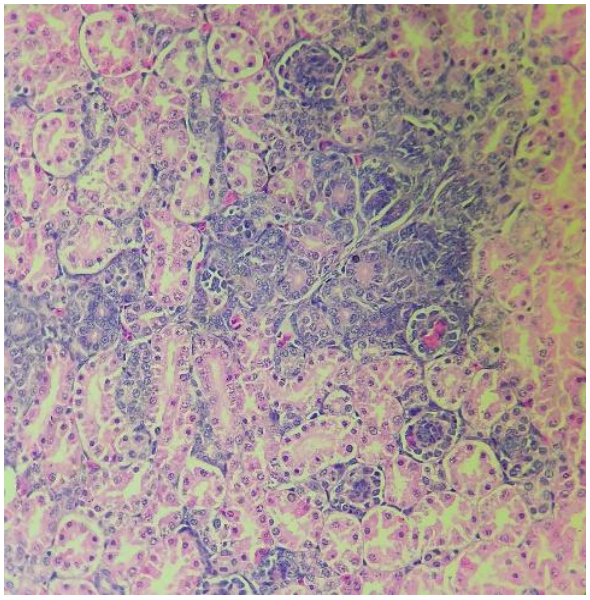

Plate 14: Hyperplasia in few tubular epithelial cells and degenerative changes in many tubules in kidney of bird in group II at 14th day (H & E × 400)

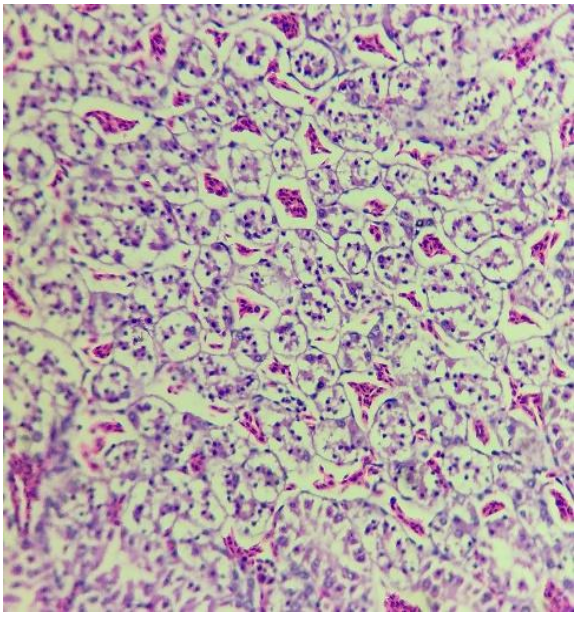

Plate 15: Note diffuse congestion of interstitial capillaries in kidney & necrotic changes in tubular epithelia of bird in group II at 14th day interval (H & E × 400)

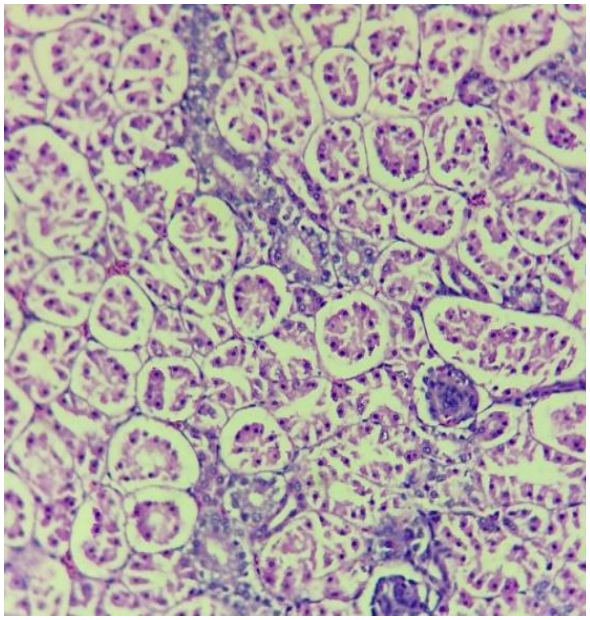

Plate 16: : Note varied degree of necrotic changes in tubular epithelial cells in kidney of birds of group II at 14th day interval (H & E × 400)

Histo-pathological alterations:

Histo-architecture of kidneys of birds sacrificed from group I & group III at 14th and 28th day of experiment appeared near to normal. The *Tephrosia purpurea* plant powder was also found non-toxic and had various medicinal properties through antioxidental action at cellular level in tissues (Mathews *et al.,* 2012).

Group II (Profenofos Treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At this interval of study kidney revealed mild, diffuse congestion and focal hemorrhages (Plate 13). Our findings were in accordance with Kafle *et al.,* (2018), Kammon *et al*, (2011) and Kammon *et al.,* (2010).

Histo-pathological alterations:

14th day of experiment:

At this stage of sacrifice of experiment histopathological examination of kidney section revealed diffuse degenerative changes (Acute cellular swelling & hydropic degeneration) and multifocal areas of necrotic changes in tubular epithelial cells of kidney (Plate 15 & 16). At places, tubular epithelial hyperplasia was evident. Diffuse congestion of intertubular capillaries, and focal areas of hemorrhages and increased glomerular cellularity were also noticed in some kidney sections (Plate 17- 19).

28th day of experiment:

In addition to the changes noticed in earlier interval, there were widely distributed areas of coagulative necrosis in renal parenchyma and areas of infarction were also evident. Tubular necrotic changes were more marked at this sacrifice (Plate 20). Also, there were cystic spaces and hyalinized masses (protein casts) in the center of few tubules of kidney.

Our observations can be well supported by the findings of Kammon *et al.,* (2010), Kafle *et al.,* (2018), Rhayf *et al*., (2012), Begum *et al.,* (2015) and Hussain *et al*., (2019).

Lesions in the kidneys were revealing of nephrotoxic effects of the profenofos and its metabolites as kidneys are the major route for elimination for most of the OP compounds (Kafle *et al.,* 2018). Tubular lesions observed due to the direct toxic effect of OP pesticide on the cell function in kidney tissue as well as the reactive free radical or oxidative stress caused due to OP pesticides were also responsible for altered histopathological changes in kidney. Renal tubules have high oxygen consumption capacity, vulnerable enzyme systems and toxin transport (excretion) mechanisms that might be responsible for damages caused by OP pesticide (Kafle *et al.,* 2018). Also Kafle *et al.,* (2018) were justified that the necrotic changes might be related to the depletion of ATP consequences to the death of the cells in tissue.

Group IV (Profenofos + *T. purpurea* treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At 14th and 28th day of experiment the mild congestion and focal heamorrhages were noticed over the kidneys of birds in group IV as compared to kidneys in birds of toxin control group (group II).

Histo-pathological alterations:

14th day of experiment:

Histoarchitectural changes in renal section of birds in this group at 14th day interval revealed not much improvement after addition of *T. purpurea,* in the diet of toxicated birds. However, at places some hyperplastic changes were evident in renal tubular epithelia & necrotic changes were moderate in tubular epithelia as compared to purely toxin treated group (Plate 39).

Group II (Profenofos Treatment group)

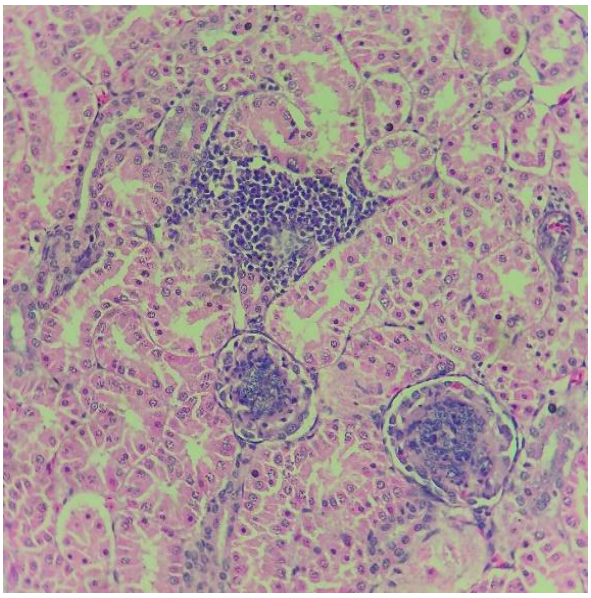

Plate 17: Note increased glomerular cellularity & focal lymphocytic aggregations in kidney of bird in group II at 14th day interval (H & E × 400)

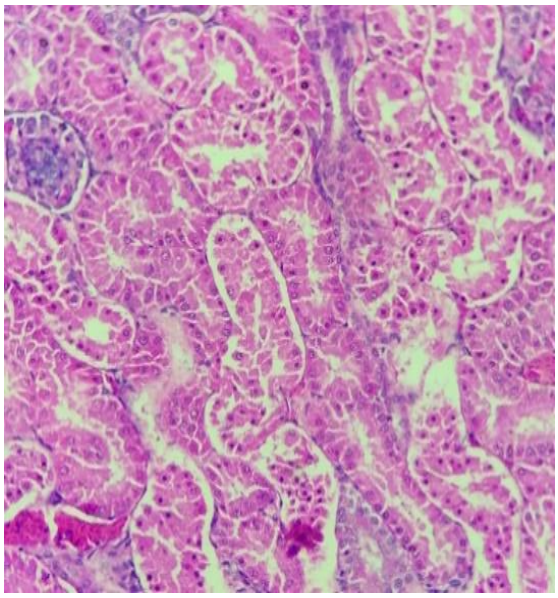

Plate 18: Note the acute cellular swelling in a section of kidney in group II bird at 14th day interval (H & E × 400)

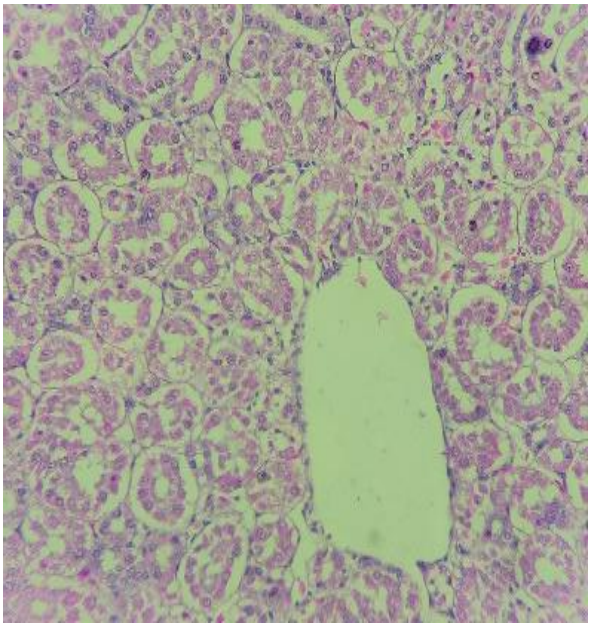

Plate 19: Microphotograph of kidney with cellular swelling and cystic degenerative changes in a bird from group II at 14th day (H & E × 400)

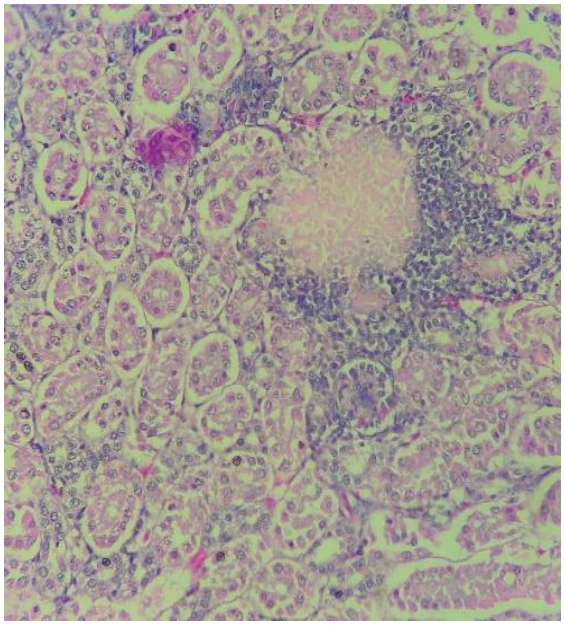

Plate 20: Note an area of coagulated necrosis in renal parenchyma surrounded by MNC infiltration in a bird from group II at 28th day of study (H & E × 400)

28th day of experiment:

At this stage of experiment the histoarchitecture of kidneys were moderately improved. Some sections showed focal lymphocytic aggregations. At places some hyperplastic changes were also evident in renal tubular epithelia (Plate 40). Necrotic changes were found moderate as compared to purely toxin treated group (group II).

Poly phenolic compounds and flavonoids in leaves of *T. purpurea* having free radical scavenging activity protects cellular damage through antioxidant activity of by *T. purpurea* as reported by Gora *et al.,* (2014) and Mathews *et al.,* (2012). The improved heamatological and biochemical parameters (BUN, uric acid and total protein) of birds in group IV, supported the histological improvement in kidney tissues of group IV birds.

4.5.2. (C) Heart:

Gross pathological alterations:

At 0, 14th and 28th day of experiment, the gross pathological examination of heart of sacrificed birds of all groups did not show any significant gross pathological changes.

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Histo-pathological alterations:

Histo-architecture of heart sections of birds sacrificed from group I & group III at 14th and 28th day of experiment appeared near to normal. It is recorded that plant powder also non-toxic and had various medicinal properties through antioxidental action at cellular level in tissues (Dalwadi *et al*., 2014), which supports the observations recorded in plant treatment control group (Group III).

Group II (Profenofos Treatment):

Histo-pathological alterations:

14th day of experiment:

At this time of sacrifice, the histopathological examination of heart sections revealed mild to moderate focal areas of zenkers degeneration and necrosis of heart muscle cells (Plate 21).

28th day of experiment:

Histopathological examination of myocardium revealed focal areas of zenkers degeneration, focal to diffuse areas of zenkers necrosis, increase spaces between muscles bundles which consequence to fragmentation and lysis of myocardial muscles, were the predominant changes were noticed at this stage of sacrifice (Plate 22).

The findings in our study were similar with Kammon *et al*., (2011). Similarly, observations of altered biochemical (ALP) and hematological parameters in profenofos toxicated birds (group II) supports the histological findings in heart tissues in this experiment.

Group IV (Profenofos + *T. pupurea* treatment group):

Histo-pathological alterations:

14th day and 28th day of experiment:

Histomorphological observations of myocardium in the birds of this group revealed near to normal myocardial structure, except for focal areas of zenkers degeneration (Plate 41).

Tephrosia purpurea possess potent antioxidant, anti-inflammatory and free radical scavenging activity. Which helped to maintain the histoarchitectural properties of heart in group IV birds. Also, the observations of improved heamatological and biochemical parameters in group IV birds in this study

Group II (Profenofos Treatment group)

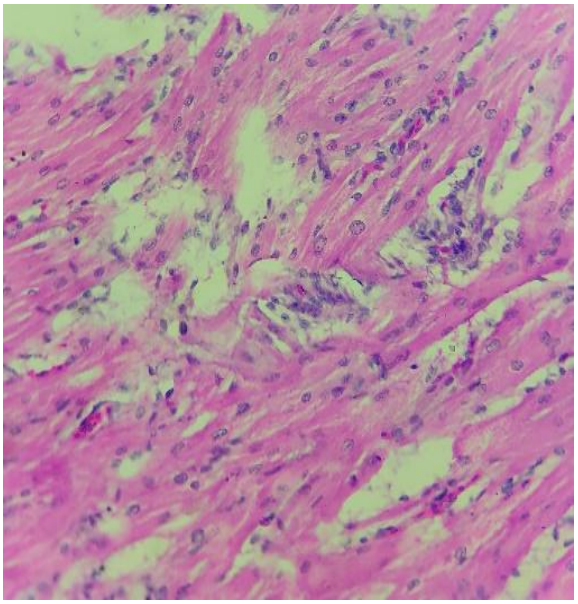

Plate 21: Zenkers degeneration, fragmentation & lysis of myocardial muscles in heart of a bird from group II at 14th day interval (H & E × 400)

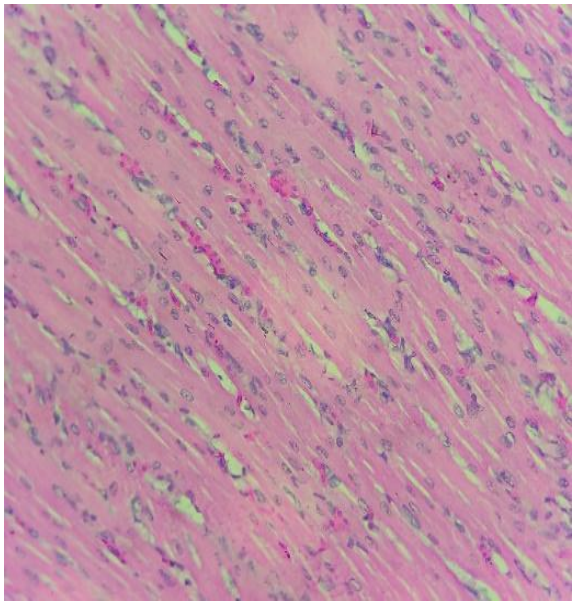

Plate 22: Myocardium with focal areas of zenkers necrosis with MNC infiltration in a bird of group II at 28th day (H & E × 400)

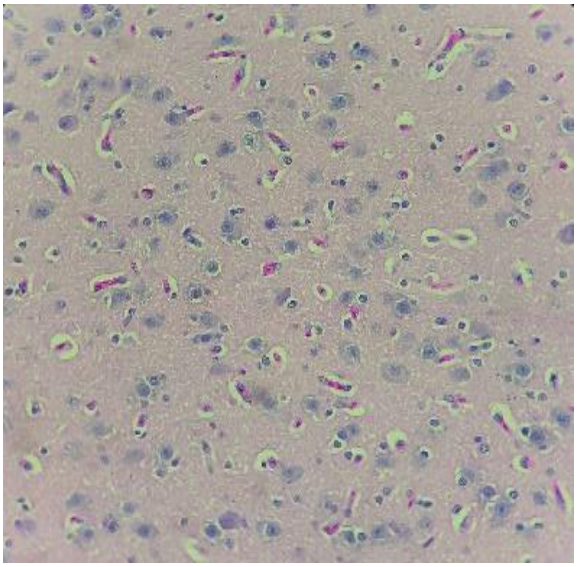

Plate 23: Congestion of microcapillaries and neuronal degenerations in brain section of a bird from group II at 14th day (H & E × 400)

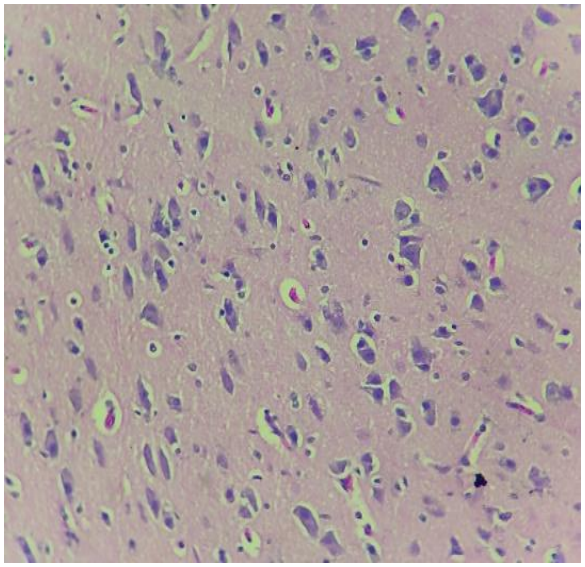

Plate 24: Vacuolation in the cytoplasm of neurons, neuronal degeneration & increased glial cell population in brain of a bird from group II at 28th day (H & E × 400)

supports the gross and microscopical lesions in heart tissue (Gora *et al*., 2014 and Dalwadi *et al*., 2014).

4.5.2. (D) Brain:

Gross pathological alterations:

At **0th, 14th and 28th day** of experiment, the gross pathological examination of brains of sacrificed birds of all groups did not show any marked gross pathological changes

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Histo-pathological alterations:

Histo-architecture of brain sections of birds sacrificed from group I & III at **14th and 28th day** of experiment appeared near to normal.

Group II (Profenofos Treatment group):

Histo-pathological alterations:

14th day of experiment:

At this sacrifice of experiment, histopathological examination of brain section revealed mild to moderate congestion of microcapillaries, vacualation in the cytoplasm of few neurons and condensation of nuclei in many neurons, many neurons showed neuronal degeneration and necrosis of brain tissue. At places, mild to moderate gliosis were also noticed (Plate 23).

28th day of experiment:

In addition to histoarchitectural changes observed at earlier interval, satellitosis was more marked throughout the brain tissue. Also, there were focal pale areas of necrosis in the brain parenchyma at 28th day of experiment (Plate 24).

Our findings were found in accordance with Rhayf *et al*., (2012) and Wani *et al.,* (2017). Oxidative stress caused by OP pesticides, might injures brain tissue consequences to impairments in metabolism of lipids, carbohydrates and proteins. As the brain was most susceptible to oxidative damage, the impairment in mitochondrial energy metabolism induced a delayed neurodegenerative condition which was a kind of apoptotic neuronal degeneration (Mohajeri and Abdollahi, 2010).

The lipid nature of the brain and lipophilic property of the OP pesticides might be responsible for crossing the blood brain barrier by the OP pesticide. Highest oxygen metabolic rate and inadequate defense system against oxidative stress such as considerably lower catalase activity in the brain makes the brain tissue highly susceptible for OP toxicity as stated by Wani *et al.,* (2017).

Group IV (Profenofos + *T. purpurea* treatment group):

Histo-pathological alterations:

14^{th} day and 28^{th} day of experiment:

On the basis of histo pathological alterations observed in brain tissue of bird sacrificed at 14^{th} and 28^{th} day, it can be concluded that there was not much improvement after addition of *T. Purpurea* plant in profenofos toxicated birds, as the changes were persistent (Plate 42).

4.5.2. (E) Intestine:

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Gross pathological alterations:

At both **14^{th} and 28^{th} day** of experiment, the gross pathological examination of intestinal loop of sacrificed birds of group I and group III did not show any noticeable gross pathological changes.

Group II (Profenofos Treatment group)

Plate 25: Intestine revealed mild, diffuse congestion and focal hemorrhages in a bird of a group II at 14th day

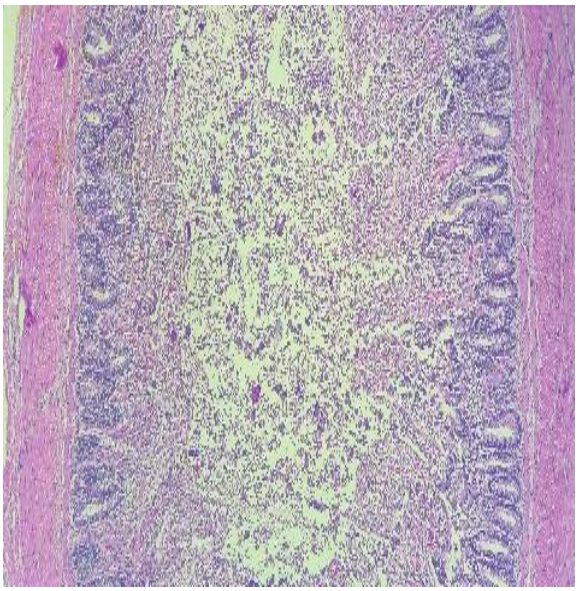

Plate 26: Necrotic enteritis with full of desquamated epithelial cells, scanty exudate and inflammatory cells in lumen of a bird from group II at 28th day of study (H & E × 100)

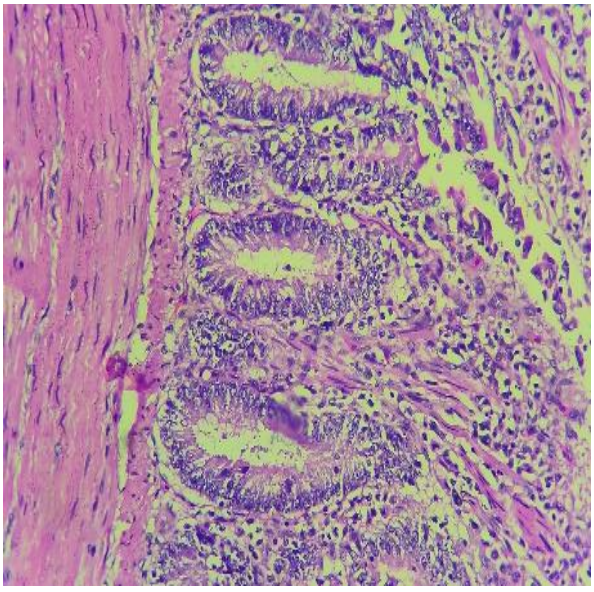

Plate **27:** Desquamation of tops of the villi of intestine in bird from group II at 28th day of study (H & E × 400)

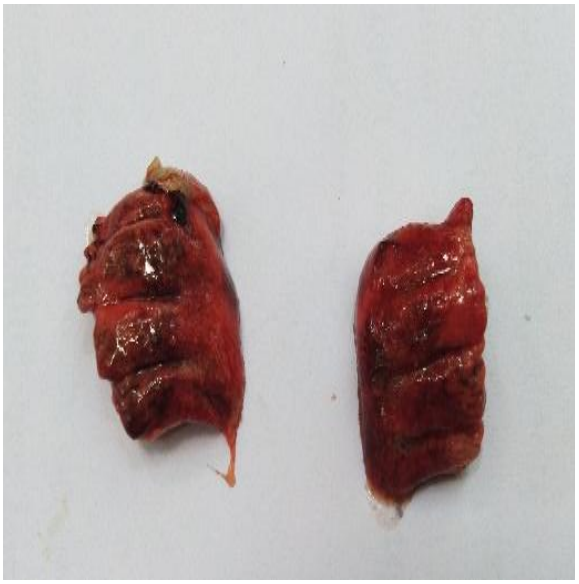

Plate 28: Diffuse congestion and hemorrhages and focal pneumonic patches over lungs of birds in group II at 28th day

Histo-pathological alterations:

Histo-architecture of intestinal sections of birds sacrificed from group I & group III, at 14th and 28th day of experiment appeared near to normal.

Group II (Profenofos Treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At this interval of study intestinal loop revealed mild, diffuse congestion and focal hemorrhages (Plate 25). Our findings were in accordance with Kammon *et al*, (2011).

Histo-pathological alterations:

14th day of experiment:

At this stage of experiment, the sections of intestine revealed desquamation of tops of the villi, scanty exudate and infiltration of inflammatory cells (mostly mononuclear cells) along with few erythrocytes in the lumen are the changes noticed in mucosa of intestine. Whereas, there were widely distributed focal areas of necrosis of muscles with scanty inflammatory cells around it, was evident in lamina muscularis (in the inner circular layer) of the intestine.

28th day of experiment:

Histopathological changes in intestinal sections of birds of this group at this sacrifice were similar to those observed in the intestinal sections at 14th day, however the focal areas of necrosis in lamina muscularis was not evident. Also, there were hyperplastic changes in the glandular epithelium of intestinal glands (Plate 26 & 27).

Our findings were similar with Kammon *et al*, (2011) and Khudair, (2017) and kafle *et al.,*(2018). As the profenofos was administered orally, intestinal mucosa is the first tissue in body to deal with the toxic effects of profenofos during its absorption through intestinal mucosa.

Group IV (Profenofos + *T. purpurea* treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At 14th and 28th day of experiment the mild diffuse congestion and focal heamorrhages in imtestine of a bird from group II, whereas mild heamorrhagic lesions were observed in intestine of bird from group IV as compared to intestine in birds from toxin control group (group II).

Histo-pathological alterations:

14th day of experiment:

At this stage of the experiment, histomorphological changes in intestinal section did not reveal any improvement, when compared to changes noticed in section of intestines of birds in group II at this stage of sacrifice.

28th day of experiment:

The **i**ntestinal sections revealed some improvement in the form of maintenance of villi structure. Deshpande *et al.,* (2003) were also noted the antiulcer activity in gastrointestinal epithelial in rats, which supports the observations of improved historchitecture of intestinal villi of birds in group IV birds (Plate 43).

4.5.2. (F) Lungs:

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Gross pathological alterations:

At both **14th and 28th day** of experiment, the gross pathological examination of lungs of sacrificed birds of group I and group III did not show any detectable gross pathological alteration.

Group II (Profenofos Treatment group)

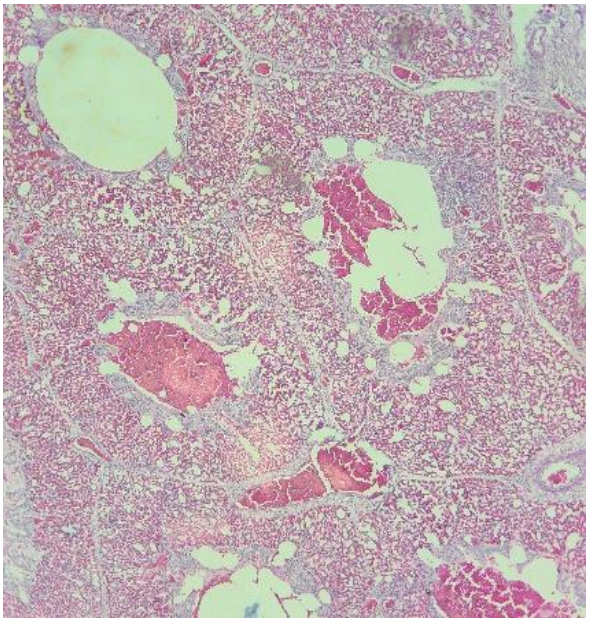

Plate 29: Section of a lung with diffuse severe congestion of capillaries, focal parabronchiolar heamorrhages and alveolar emphysema in a bird from group II at 14th day (H & E × 100)

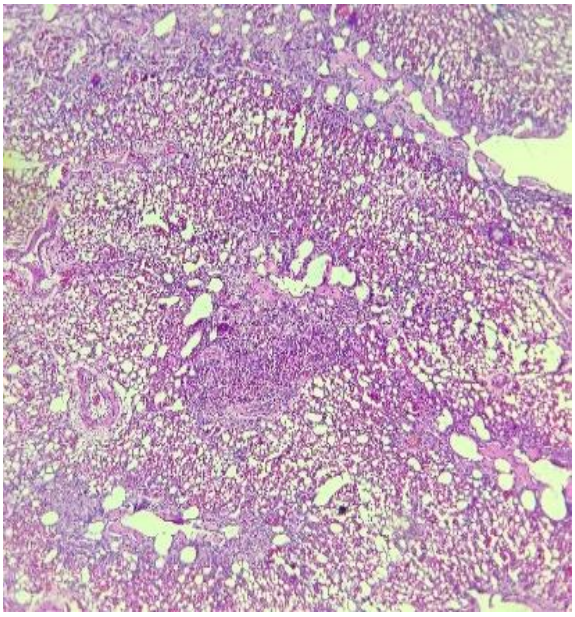

Plate 30: Bronchitis, bronchiolitis and focal pneumonic changes in lung section of a bird from group II at 28th day (H & E × 100)

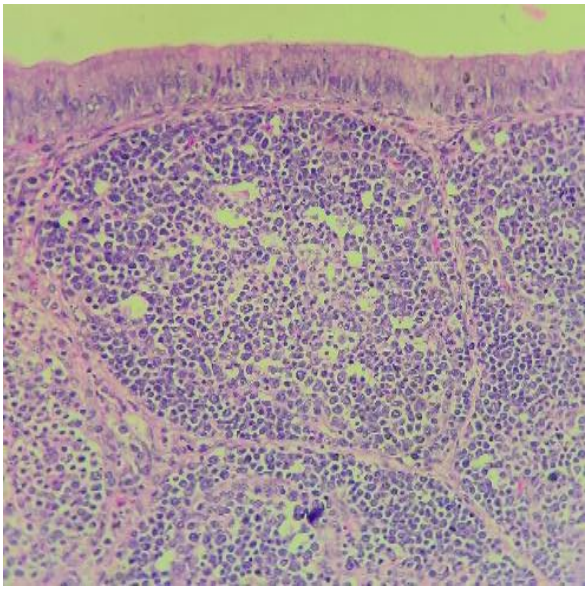

Plate 31: Vacuolations of varied size indicating depopulation of lymphocytes in bursal follicle of bird from group II at 14th day interval (H & E × 100)

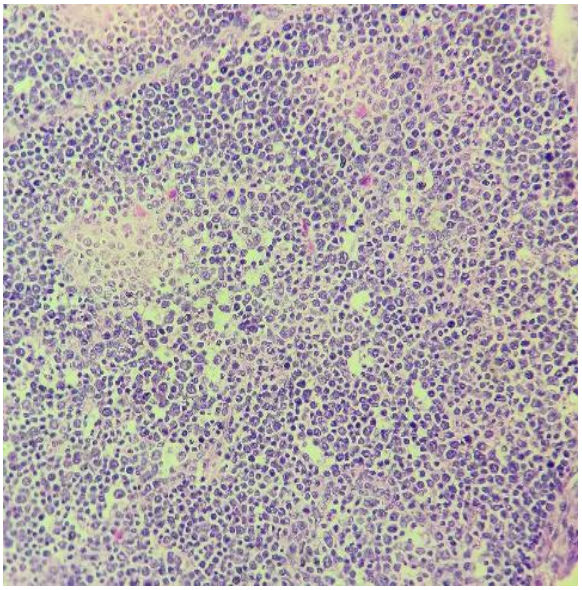

Plate 32: Focal areas of necrosis & vacuolation in bursal follicle of a bird from group II at 14th day interval (H & E × 400)

Group II (Profenofos Treatment group)

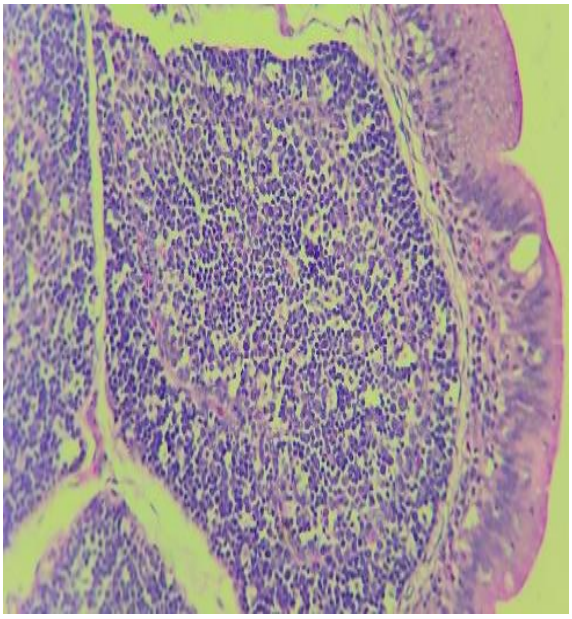

Plate 33: Microphotograph of Bursa with vacuolation in a bird from group II at 28th day (H & E × 100)

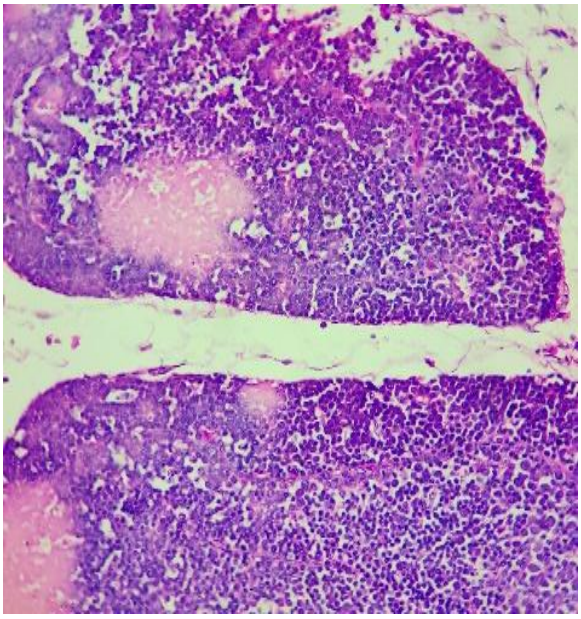

Plate 34: Focal areas of necrosis in bursal follicles of a bird from group II at 28th day interval (H & E × 100)

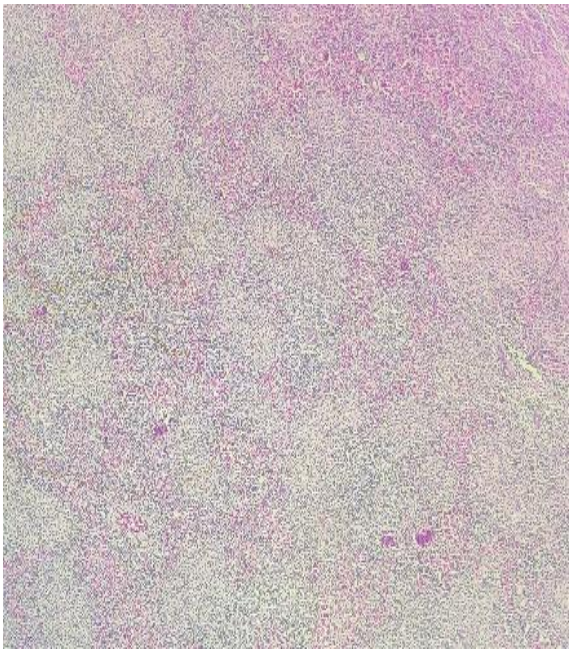

Plate 35: Note diffuse congestion of splenic capillaries in a bird from group II at 14th day (H & E × 40)

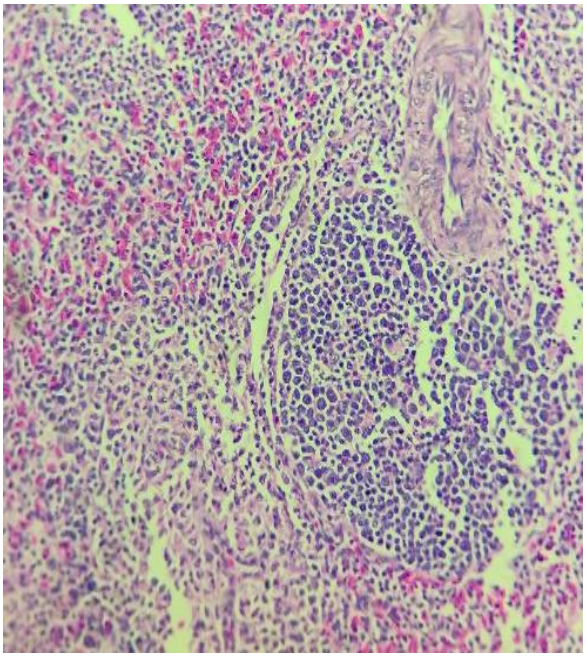

Plate 36: Mild depopulation of lymphocytes around central splenic artery in a malpighian corpuscle of spleen in a bird of group II at 28th day (H & E × 400)

Histo-pathological alterations:

Histo-architecture of lung sections of birds sacrificed from group I & III at 14th and 28th day of experiment appeared near to normal.

Group II (Profenofos Treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At these intervals of study, there was mild, diffuse congestion and hemorrhages and focal pneumonic patches over lungs of birds in group II (Plate 28). Our findings were in accordance with Kafle *et al.,* (2018), Kammon *et al*, (2011), who reported the OP pesticides toxicity in birds.

Histo-pathological alterations:

14th day of experiment:

At this stage of sacrifice the lung sections showed diffuse severe congestion of pulmonary and parabronchial capillaries and parabronchiolar heamorrhages (Plate 29).

28th day of experiment:

At 28th day of experiment, lung sections showed diffuse areas of alveolar emphysema with formation of bullae. Also, mild para-bronchiolar and intra-alveolar areas faintly stained edema fluid was noticed in some sections of lungs. Focal areas of bronchitis, bronchiolitis and pneumonia were also noticed at this stage (Plate 30).

Findings in our study were found in accordance with the observations of Kafle *et al.,* (2018[a]) and Kammon *et al*., (2011). Kafle *et al.,* (2018[b]) noted that pulmonary edema was a common microscopic lesion in most organophosphate pesticides toxicated animals.

Group IV (Profenofos + *T. pupurea* treatment group):

Gross pathological alterations:

14th and 28th day of experiment:

At 14th and 28th day of experiment the mild hyperemic lesions were observed in lung sections of birds in group IV as compared to toxin control group (group II).

Histo-pathological alterations:

14th day and 28th day of experiment:

At 14th and 28th day of experiment sections of lungs showed diffuse congestion of pulmonary capillaries and intrabronchiolar heamorrhages. Overall, there was mild to moderate improvement in histoarchitecture of lungs in this treatment group (Plate 44).

4.5.2. (G) Bursa:

Gross pathological alterations:

At **0th, 14th and 28th day** of experiment, the gross pathological examination of bursa of sacrificed birds of all groups did not show any appreciable gross pathological changes.

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Histo-pathological alterations:

Histo-architecture of bursal sections of birds sacrificed from group I & III at 14th and 28th day of experiment appeared near to normal.

Group IV (Profenofos + *T. purpurea* treatment group)

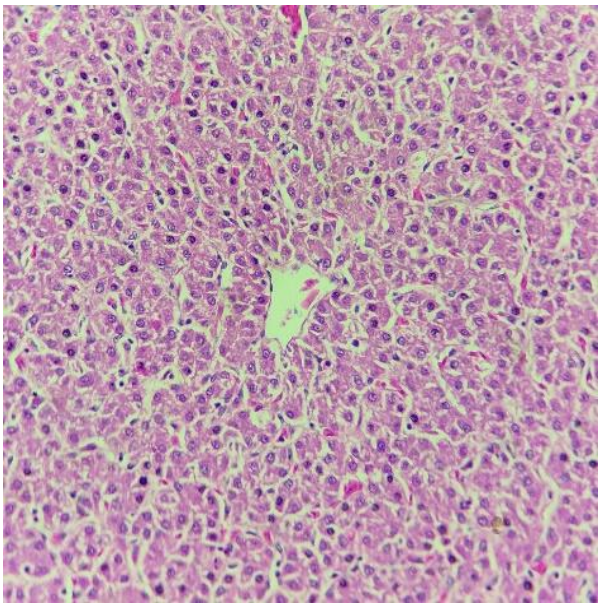

Plate 37: The compact histo-architecture of liver parenchyma and hepatic vein in bird of group IV at 14th day of interval (H & E × 100)

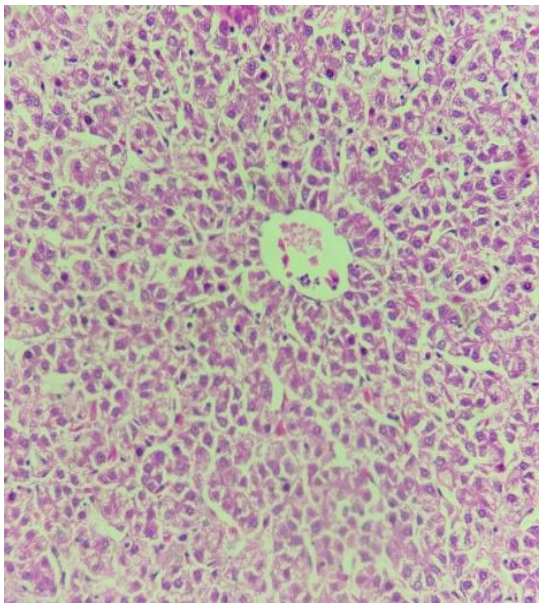

Plate 38: Note more compact histo-architecture of liver parenchyma of bird in group IV at 28th day interval of study (H & E × 400)

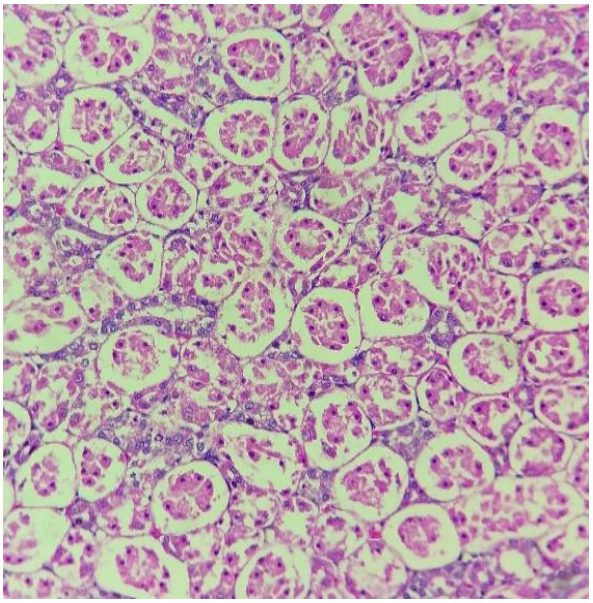

Plate 39: Note cellular swelling with early necrotic changes in tubular epithelial cells in kidney of bird from group IV at 14th day interval (H & E × 400)

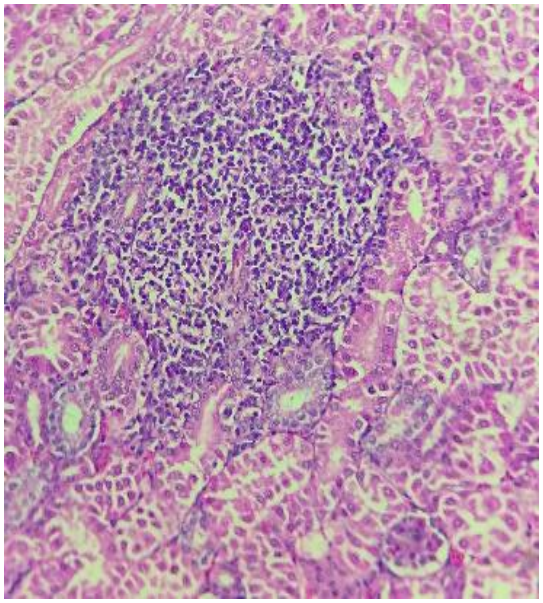

Plate 40: Note the lymphocytic aggregations in renal parenchyma of bird of group IV at 28th day (H & E × 400)

Group II (Profenofos Treatment group):

Histo-pathological alterations:

14th day of experiment:

At this stage of sacrifice, the histopathological examination of bursal tissue revealed that the mucosa was intact and in the lymphoid follicle of bursa vacuoles of varied size were evident indicating depopulation of lymphocytes (Plate 31). Also, repeated focal areas of necrosis were evident in lymphoid follicles in it at this stage of sacrifice (Plate 32).

28th day of experiment:

At this stage of sacrifice, the bursal mucosa was more or less intact (except denudation at places) however there were vacuolations in the bursal mucosa. In the bursal follicles the follicular structure was less intact, however there were marked vacuolations (depopulation of leukocytes) in the individual follicles.

Our findings were in accordance with Shahzad *et al.,* (2013), who noted in their study that the histopathological damage to lymphoid organs (bursa) might be due to the modulation of nervous system consequences to altered production of lymphocytes, phosphorylation and oxidative damage. The histopathological alterations in spleen sections might be due to the immunosuppression in these lymphoid organs (Ahmad *et al.,* 2015), which was supported by the observations of altered leucocytes count in this study.

Group IV (Profenofos + *T. pupurea* treatment group):

Histo-pathological alterations:

14th day and 28th day of experiment:

Overall bursal section of birds at this sacrifice revealed little improvement in the histoarchitecture, however focal areas of mild depopulation of lymphocytes from few follicles was still evident. Also, the observations of improved leucocyte count and heamatological parameters observed in group IV supports the

histological findings of bursal section in this group as compared to findings in birds of group II (Plate 45 & 46).

4.5.2. (H) Spleen:

Gross pathological alterations:

At **0th, 14th and 28th day** of experiment, the gross pathological examination of spleen of sacrificed birds of all groups did not showed any appreciable gross pathological changes

Group I (Healthy control group) and Group III (*Tephrosia purpurea* treatment group):

Histo-pathological alterations:

Histo-architecture of spleen sections of birds sacrificed from group I & III at 14th and 28th day of experiment appeared near to normal.

Group II (Profenofos Treatment group):

Histo-pathological alterations:

14th day of experiment:

Histopathological alterations in the spleen sections of the birds at 14th day of study did not reveal any significant changes, except for mild to moderate congestion of sinusoidal capillaries and mild depopulation of lymphocytes around central splenic artery in a malpighian corpuscles (Plate 35).

28th day of experiment:

At 28th day of experiment the spleen sections revealed the moderate depopulation of lymphocytes from malpighian corpuscles of spleen. In one section of spleen there were multiple small areas of necrotic changes were noticed. Which was the only additional change seen on this interval of study, when compared to the changes noticed at 14th day (Plate 36).

Group IV (Profenofos + *T. pupurea* treatment group)

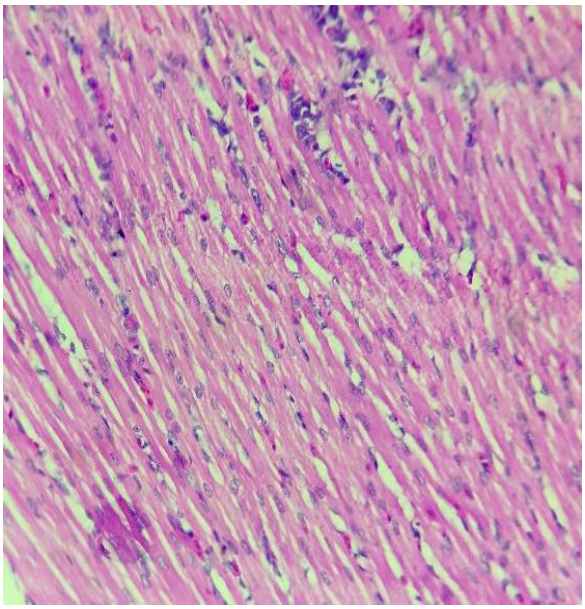

Plate 41: Section of heart revealed improved histo-architecture of myocardial muscles of a bird from group IV at 28th day of interval (H & E × 100)

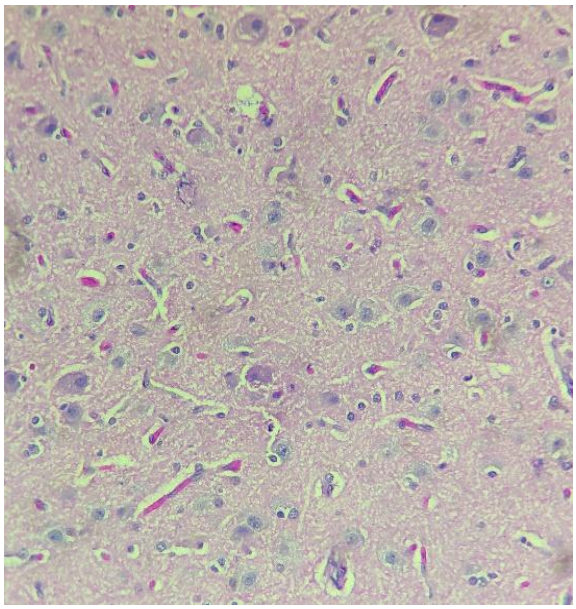

Plate 42: Congestion of microcapillaries and neuronal degenerations in brain section of a bird from group IV at 28th day (H & E × 400)

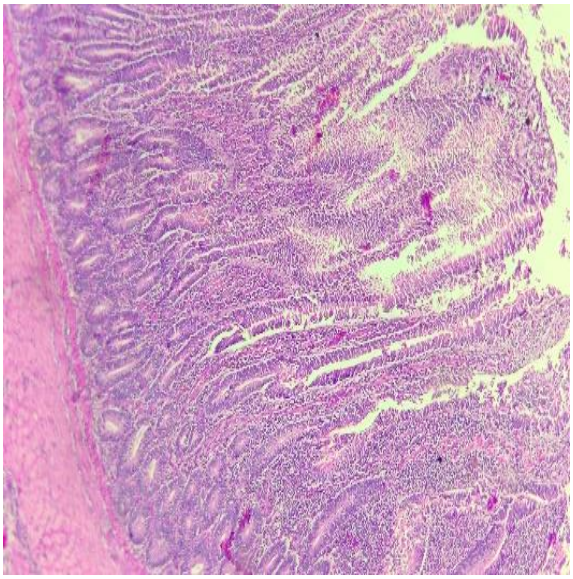

Plate 43: Sections of intestine revealed improved histo-architecture in a bird of group IV at 28th day of experiment (H & E × 40)

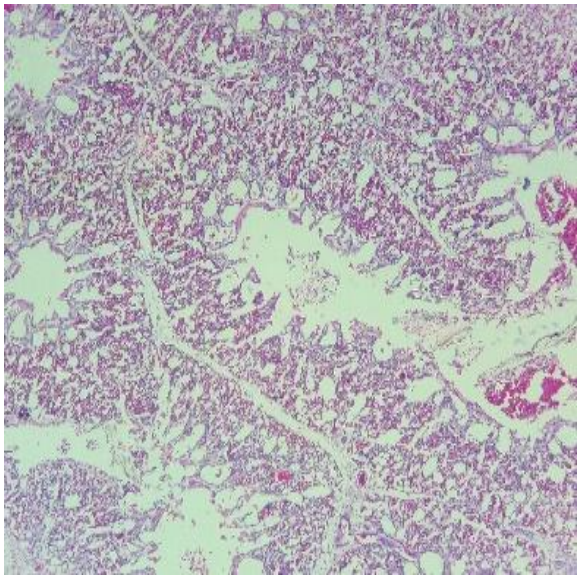

Plate 44: Focal congestion of pulmonary capillaries & heamorrhages in a bird from group IV at 14th day (H & E × 100)

Group IV (Profenofos + *T. pupurea* treatment group)

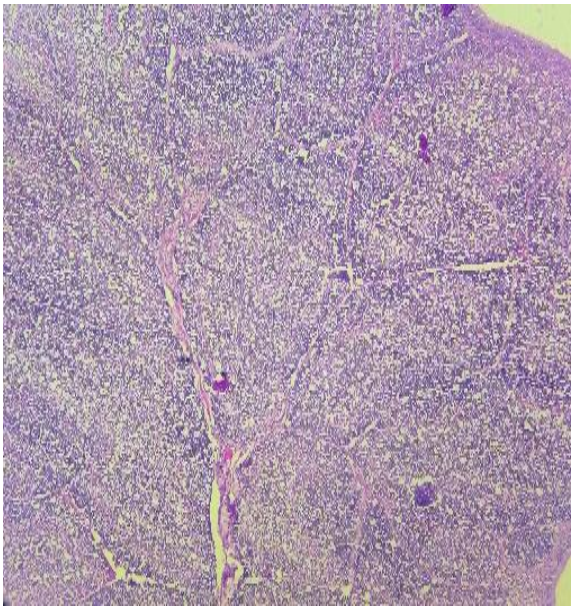

Plate 45: Section of Bursa with compact structure and less depopulation of group IV birds at 14th day of study (H & E × 40)

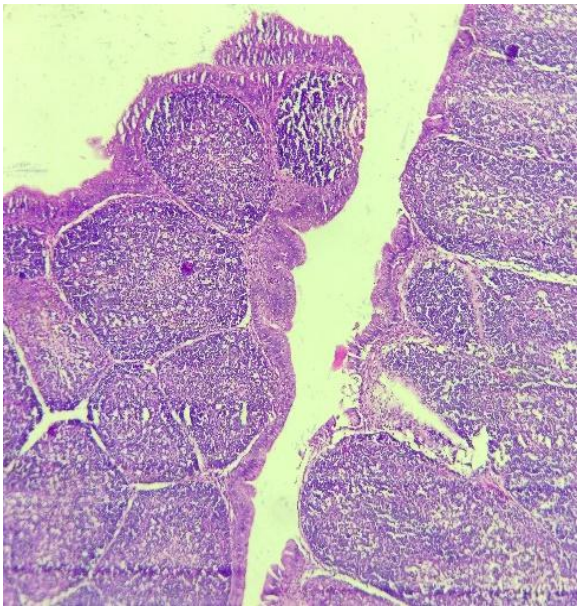

Plate 46: More or less intact bursal epithelium & mild depopulation of lymphocytes from follicles in a bird from group IV at 28th day (H & E × 40)

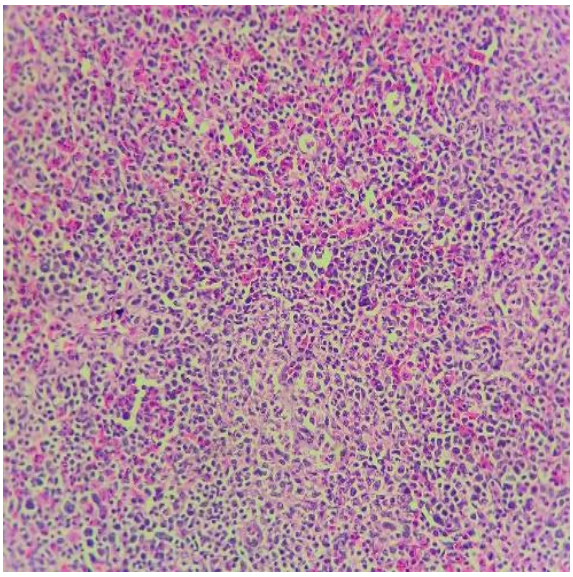

Plate 47: Diffuse congestion of splenic capillaries in a bird from group IV at 14th day (H & E × 100)

Our findings were similar with Ahmad *et al.,* (2015), Khudair *et al.,* (2017) and Shahzad *et al.,* (2013). In their studies on OP pesticide toxicity stated that histopathological damage to lymphoid organs (spleen) might be due to the modulation of nervous system (AChE inhibition) consequences to altered production of lymphocytes, phosphorylation and oxidative damage. The histopathological alterations in spleen sections might be due to the immunosuppression in these lymphoid organ (Ahmad *et al.,* 2015).

Group IV (Profenofos + *T. purpurea* treatment group):

Histo-pathological alterations:

14th day and 28th day of experiment:

Histo-architecture of spleen of birds of this group revealed compact population of lymphocytes in the malphigian corpuscles, however the congestion of sinusoidal capillaries was mild to moderate in nature, indicating little improvement in histoarchitecture of spleen after addition of *T,purpurea* in profenofos toxicated birds (Plate 47). Also, the observations of improved heamatological and biochemical parameters of birds in group IV were supports the histological findings of bursal section in this group as compared to birds in group II.

4.5.2. (I) Testis and ovary:

Gross and Histo-pathological alterations:

Histoarchitecture of testis of birds at 14th and 28th day of sacrifice did not reveal any significant alterations in birds of all groups this could be due to sacrifice carried out at early age of reproductive maturity in this study.

4.6 Phytochemical properties and organoleptic properties of *Tephrosia purpurea* leaves powder:

Organoleptic properties and Proximate analysis:

The Organoleptic properties and proximate analysis of *Tephrosia purpurea* leaves powder are given in Table 4.23 & 4.24.

Table 4.23: Organoleptic properties of *Tephrosia purpurea* leaves powder

Sr. No.	Characteristic Organoleptic Properties	Observation
1	Form	Powder (couarse in nature)
2	Colour	Dull green
3	Odour	Faint characteristics
4	Taste	Slight bitter

Table 4.24: Proximate analysis of *Tephrosia purpurea* leaves powder.

Sr. No.	Proximate Principle	Result
1	Moisture	8.15 %
2	Ether Extract (EE)	2.76 %
3	Total Ash	8.42 %
4	Crude Protein (CP)	21.41 %
5	Crude Fiber (CF)	23.7 %
6	Nitrogen Free Extract (NFE)	35.56 %

This findings were supported by Mbomi *et al*., (2011).

The phytochemical properties of aqueous extract of *Tephrosia purpurea* leaves powder are given in Table no. 4.25

Table 4.25: Phytochemical analysis of aqueous extract of *Tephrosia purpurea* leaves powder

Sr. no.	Phytochemical constituents	Aqueous extract of *Tephrosia purpurea*
1	Tannins	**+**
2	Saponins	**+**
3	Flavonoids	**+**
4	Steroids	**-**
5	Terpenoids	**+**
6	Glycosides	**+**
7	Alkaloids	**-**
8	Phenolic compounds	**+**
9	Anthraquinones	**-**
10	Carbohydrate	**+**

The aqueous extract of *Tephrosia purpurea* plant leaves powder showed presence of Tannins, Terpenoids, Saponins, Phenolic compounds, Flavonoids, Glycosides, and Carbohydrate compound, however, there was absence of steroids, alkaloids and anthraquinones. Our findings were in accordance with Kumar *et al.,* (2019) and Gopalakrishnan *et al.,* (2009).

Summary and Conclusions

CHAPTER-5

SUMMARY AND CONCLUSIONS

The present experiment was planned to study the sub-acute oral toxicity of Profonofos in Gramapriya birds through oral gavage for 28 experimental days. Also, hepatoprotective effects of *Tephrosia purpurea* were assessed in toxicated birds for 28 experimental days.

A total of 100 healthy day old 'Gramapriya' chicks were procured and sheltered in animal house of College of Veterinary and Animal Sciences, Parbhani. All the chicks were vaccinated with Marek's disease vaccine on the day of hatching at hatchery and maintained till the end of trial. Birds were randomly allotted to the control and treatment groups and were kept in four separate pens. Considering the age groups, birds were given *ad libitum* nutritionally balanced Gavaran feed i.e. starter feed and finisher feed. Also, offered ample quantity of fresh drinking water. In accordance with poultry scientific vaccination schedule, birds were vaccinated against different poultry diseases.

Birds were acclimatized to animal house for 7 days of period. The study was carried out for a period of 28 experimental days in 100 Gramapriya birds. They were uniformly distributed into four groups of 25 birds in each, as represented in Table 1. The group I served as healthy control and was given standard feed and water ad libitum for 28 days. The birds of groups II were intoxicated daily with a solution of Profenofos @ 1.6 mg/kg body weight through oral gavage. The birds of group III were treated on plant control and fed with a *T. purpurea* leaves powder @ 0.1 % of feed daily for 28 days. The group IV was treated with Profenofos @ 1.6 mg/kg body weight daily through oral gavage + *T. purpurea* leaves powder @ 0.1 % of feed daily. At different time intervals (0^{th}, 14^{th} and 28^{th} day of experiment) the blood was collected and processed for heamatological and biochemical studies. Also, the Gross & histopathological observations in the selected vital organs were recorded at 14^{th} and 28^{th} day after sacrification of birds.

The parameters studied during experiment were growth parameter, haematological, biochemical estimations, organ weights and different pathological parameters in birds of all groups. The growth parameter (Feed consumption, weight gain and FCR) was recorded weekly from 0 day of experiment. Whereas, at 0^{th}, 14^{th} and 28^{th} day of experiment the haematological parameters (Hb, PCV, TEC, TLC, DLC and BCT) and biochemical parameters (serum Glucose, serum TP, serum albumin, serum globulin, serum AST, serum ALT, serum ALP, BUN, uric acid and serum AChE) were recorded from six slaughtered birds of each group. At these same intervals of study the pathological studies (Mean organ weights, gross and histopathological examinations of tisuues) were also examined in birds from each group in experiment. Clinical signs and mortality were thoroughly observed throughout the experimental period in all groups.

Experimental birds in healthy control group **(group I)** were given standard feed *ad libitum* and birds of **Group III** were fed with a *T. purpurea* leaves powder @ 0.1 % of feed daily for 28 days. The birds in group I and III were observed physiologically active and remained healthy throughout the biological study period. There were no considerable clinical signs, symptoms and mortality observed in birds of Group I and III, throughout the experimental period. All the studied parameters were found within normal physiological limits.

Birds of toxicity control group **(Group II)**, were intoxicated with a solution of Profenofos @ 1.6 mg/kg bwt. through oral gavage, daily for period of 28 days of experimentation. The birds of group II showed mild clinical symptoms, in which sluggishness, dullness, depression, decreased water and feed intake, reduced appetite, hurdling and ruffled feather were observed during last weeks of experimental trial. The weekly feed consumption and body weight gain were markedly decreased 2^{nd} week onwards of experiment, however, the mean FCR were considerably elevated at 2^{nd}, 4^{th} and 5^{th} week of experiment. On 14^{th} and 28^{th} day of experiment, there were significant reduction in heamatological parameters such as Hb (g/dl); PCV (%); TEC (10^6/cmm), TLC (10^3/cmm) and lymphocytes (%), however, the blood clotting time and heterophil (%) counts showed considerable elevation at 14^{th} and 28^{th} day of experiment. The biochemical

parameters of this group showed the variation throughout the experimental period. There were significant increased levels of serum glucose; liver health indicators (Mean AST, ALT and ALP levels), kidney health indicators (Mean SUA, mean BUN levels) of birds in group II throughout the experiment, However, mean levels of STP, albumin, globulin and the AChE (OP pesticide toxicity indicator) showed significantly decreased levels due to profenofos toxicity. Mean absolute weights of liver and kidney of birds of group II showed significantly decreased. At 14^{th} and 28^{th} days of experiment pathological examination of slaughtered birds showed focal areas of congestion and necrosis on liver and intestinal tissues. On 14^{th} and 28^{th} day of experiment, histopathological examination the sections of liver, kidney, spleen, bursa and intestine of birds of group II, showed significant heamorrhagic, degenerative and necrotic changes. Dilated portal vein, fibrous connective tissue proliferation, hyperplasia of bile duct and newly formed bile duct (Liver sections); diffuse degenerative changes, multifocal necrotic changes and hyperplasia in tubular epithelial cells (Kidney sectios); congestion of microcapillaries, moderate gliosis (Brain sections) were observed at 14^{th} and 28^{th} day of experimental slaughter.

The birds of **group IV** were treated as treatment group, in which Profenofos @ 1.6 mg/kg body weight daily through oral gavage and for hepatoprotective ameliorations due to feeding of *T. purpurea* leaves powder @ 0.1 % of feed daily for 28 experimental days. The altered growth parameters (Weekly feed consumption and FCR) due to profenofos toxicity in group II, were partially improved at 2^{nd}, 3^{rd} and 4^{th} week of experiment. However, the haematological parameters viz. TEC and TLC counts showed mild improved values on 14^{th} and 28^{th} day of experiment. Moreover, the Hb (g/dl); PCV (%); lymphocytes (%) and heterophil (%) in DLC and blood clotting time (sec.) were not much improved as compared to toxin control group (Group II). Biochemical parameters of birds of group IV showed mild to moderate improvement as compared to values of birds in group II. The levels of growth indicators (Mean serum glucose and STP); liver health indicators (Mean AST and ALT levels), kidney health indicators (Mean SUA and mean BUN levels) of birds in group IV showed partial improvement as compared to values of group II birds. On 14^{th} and

28th day of experiment, there were no considerable gross changes in organs of group IV slaughtered birds. Microscopically the liver, kidney and heart showed hemorrhagic and degenerative changes same as that was observed in birds of group II birds. Whereas, necrotic changes were reduced in the sections of liver, kidney and intestine of birds in group IV as compared to group II birds.

CONCLUSIONS:

The results obtained from present study, following conclusions can be drawn:

1) When Profenofos was fed @ 1.6 mg/kg bwt. daily through oral gavage for 28 days in Gramapriya birds, subacute toxicity was induced in experimental birds with prominent alterations in growth, haematological, biochemical parameters and gross and histopathological alterations in vital organs.

2) Feeding of *T. purpurea* to birds of group III (plant control) was found non-toxic and showed at par levels of growth, haematological and biochemical parameters and gross histopathological observations in vital organs as compared to birds in healthy control group.

3) The birds in treatment group (Profenofos @ 1.6 mg/kg bwt. + *T. purpurea* leaves powder @ 0.1 % of feed daily for 28 experimental days) showed mild to moderate improvement in growth, haematological, biochemical parameters and gross histopathological alterations in vital organs as compared to birds of a healthy control group. It was observed that the plant treatment was more of hepatoprotective as evidenced by heamatobiochemical and pathological studies.

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Vitae

VITAE

The author Dr. Harshal Jaypal Shete was born on 31 August 1995 at Ashta Taluka- Walwa, District – Sangli, Maharashtra state. He passed his 10th examination (2011) from Mahatma Gandhi Secondary and Higher-secondary School, Ashta and 12th (2013) from Yashwantrao Chavan Institute of Science, Satara (Maharashtra).

Later, he cracked National Eligibility Entrance Test (NEET) - 2013 and got admitted in Krantisinh Nana Patil College of Veterinary and Animal Sciences, Shirwal in the year 2013 and successfully completed it with first division and C.G.P.A. - 7.203 in the year 2018. During his graduation he represented MAFSU University in All India Inter University Championship 2015-16 in Malkhamb during 07th January to 11th January, 2016 held at Punjabi University, Patiala. Also, he presented a clinical case report in 1st Clinical Case Conference on "Domestic, Pet and Wild Animals Practice" held on 20th January, 2017.

After completion of UG, author got admission in College of Veterinary and Animal Sciences, Parbhani for Post-graduation in Department of Veterinary Pathology. During his post-graduation he has published abstracts in 3rd Clinical Case Conference on "Diagnostic and Therapeutic Challenges in Farm and Companion animals held on 23rd January 2019; XXXVI Annual Conference of Indian Association of Veterinary Pathologists, held at College of Veterinary Sciences and Animal Husbandry, Aizwal, Mizoram, during 6-8 Nov 2019 and XXXVII Annual Conference of Indian Association of Veterinary Pathologists, held at College of Veterinary and Animal Sciences, Nagpur during 26th to 29th of Dec 2020.

Under the guidance of his guide and department of teachers till date he has published two articles in national/ international journal of repute.

Thesis Abstract

APPENDIX-G

THESIS ABSTRACT

a)	Title of the thesis	:	"SUBACUTE ORAL TOXICITY OF PROFENOFOS AND HEPATOPROTECTIVE EFFECTS OF *Tephrosia purpurea* IN GRAMAPRIYA BIRDS"
b)	Full name of student	:	Shete Harshal Jaypal
c)	Name and address of Major Advisor	:	Dr. S. D. Moregaonkar, Professor and Head, Department of Veterinary Pathology, Collège of Veterinary and Animal Sciences, MAFSU, Parbhani-431 402 (M.S.)
d)	Degree to be awarded	:	M. V. Sc.
e)	Year of award of degree	:	2021
f)	Major subject	:	Veterinary Pathology
g)	Total number of pages in the thesis	:	129
h)	Number of words in the abstract	:	295
i)	Signature of Student	:	
j)	Signature, Name and address of forwarding authority (HOD/SH).	:	Dr. S. D. Moregaonkar Professor and Head Dept. Veterinary Pathology, COVAS, Parbhani

ABSTRACT

The present experiment was planned to study the sub-acute oral toxicity of profonofos in Gramapriya birds and hepatoprotective effects of *T. purpurea* in toxicated birds. 100 healthy day old 'Gramapriya' chicks randomly distributed into four treatment groups, having 25 chicks each up to 28 days. The birds of control group I was given standard feed and *ad libitum* of water; groups II were intoxicated daily with a solution of Profenofos @ 1.6 mg/kg body weight through oral gavage; group III were fed with a *T. purpurea* leaves powder @ 0.1 % of feed and group IV was treated with Profenofos @ 1.6 mg/kg body weight daily through oral gavage + *T. purpurea* leaves powder @ 0.1 % of feed daily.

Growth parameters (Feed consumption, weight gain and FCR); haematological studies (Hb, PCV, TEC, TLC, DLC and BCT); biochemical estimations (serum Glucose, serum TP, serum albumin, serum globulin, serum AST, serum ALT, serum ALP, BUN, uric acid and serum AChE); organ weights and different pathological parameters in birds of all groups examined at 0^{th} 14^{th} and 28^{th} day of study.

All parameters of birds from **Group I and III** showed non-significant changes throughout the experiment.

Growth parameters, of birds in **group II** were significantly reduced from 2^{nd} week of experiment. Whereas, the heterophil, leucocyte, BCT, glucose, AST, ALT, ALP, uric acid and BUN values were increased from 14^{th} day onwards. Hb, PCV, TEC, lymphocyte, STP, albumin and AChE were reduced from 14^{th} days onwards. Histopathological evaluation showed considerable microscopic lesions in liver, kidney, intestine and brain tissues of birds in group II from 14^{th} day of experiment.

Efficacy of *T. purpurea* leaves powder in **group IV** birds revealed the partial improvement in BCT, AST, ALT, SUA and BUN in profenofos toxicated birds. Histopathological alterations in liver, kidney, intestine, heart, and spleen were moderately improved after addition of *T. purpurea* leaf powder in the diet of toxicated birds.

प्रबंध सारांश

प्रबंधाचे शीर्षक	:	"उन्हाळी या वनस्पतीचा ग्रामप्रिया पक्ष्यांमध्ये प्रोफेनोफॉस निर्मित विषविकृतींवर यकृत पोषक उपयुक्ततेचा अभ्यास"
विद्यार्थ्याचे नाव	:	शेटे हर्षल जयपाल
मार्गदर्शक	:	**डॉ एस. डी. मोरेगांवकर** प्राध्यापक व प्रमुख, पशुविकृतीशास्त्र विभाग, पशुवैद्यकीय व पशुविज्ञान महाविद्यालय, परभणी
पदवी प्रदान करण्याचे वर्ष	:	२०२१
प्रदान करण्यात येणारी पदवी	:	एम. व्ही. एस. सी.
मुख्य विषय	:	पशुविकृतीशास्त्र विभाग
प्रबंधातील एकूण पृष्ठे	:	१२९
सारांशातील एकूण शब्द	:	२९८
विद्यार्थ्याची स्वाक्षरी	:	
विभाग प्रमुखाची स्वाक्षरी	:	**डॉ एस. डी. मोरेगांवकर** प्राध्यापक व प्रमुख, पशुविकृतीशास्त्र विभाग, पशुवैद्यकीय व पशुविज्ञान महाविद्यालय, परभणी

सारांश

सदरील अभ्यासामध्ये २८ दिवसांच्या कालावधीसाठी प्रायोगिक ग्रामप्रिया पक्ष्यांमध्ये प्रोफेनोफॉस आणि उन्हाळी या वनस्पतींचे यकृत पोषक उपयुक्ततेवर परीक्षण करण्यात आले आहे. १०० ग्रामप्रीया जातीचे पक्षी प्रत्येक गटांमध्ये २५ पक्षी याप्रमाणे गट १, गट २, गट ३, आणि गट ४ इत्यादी चार गटांमध्ये विभागण्यात आले. गट १ मधील पक्ष्यांना सशक्त नियंत्रण मानले गेले तर गट २ मध्ये विषारी नियंत्रण (प्रोफेनोफॉस १.६ मि. ग्रॅम / कि. ग्रॅम वजन तोंडावाटे), गट ३ मध्ये उन्हाळी वनस्पती पूड नियंत्रण (उन्हाळी वनस्पतींच्या पानांची पुड अन्नाच्या ०.१ टक्का इतके) तसेच गट ४ मध्ये प्रोफेनोफॉस १.६ मि. ग्रॅम/ कि. ग्रॅम वजन व अन्नाच्या ०.१ टक्का इतके उन्हाळी वनस्पतीच्या पानांची पुड असे मिश्र स्वरूपात वापरण्यात आले व २८ दिवानसांपर्यंत अभ्यास करण्यात आला

हया प्रयोगामध्ये ० व्या, १४ व्या व २८व्या दिवशी पक्ष्यांच्या खाद्याचे प्रमाण, पक्ष्यांच्या वजनातील वाढ व खाद्य - वजन रूपांतरण प्रमाण, हिमोग्लोबिन, लालपेशी, पी. सी. व्ही. , रक्तगोठण कालावधी आणि डी. एल. सी. तसेच रक्तजलातील प्रथिने, शर्करा, ए. एस. टी., ए. एल. टी., ए. एल. पी., यूरिया नायट्रोजन, युरिक ऍसिड आणि असिटील कोलीन एस्टरेज यांची तपासणी करण्यात आली. याबरोबरच पक्ष्यांच्या अवयवांचे वजन व ग्रॉस (डोळ्याने) व सूक्ष्मदर्शक यंत्राद्वारे अवयवांमध्ये झालेल्या विकृतीबदलांचा अभ्यास करण्यात आला.

प्रयोगामध्ये अभ्यासलेल्या विविध घटकांमध्ये **गट १ व ३** यामधील पक्ष्यांनी कोणताच असा लक्षणीय बदल दाखवला नाही.

अभ्यासाच्या २ऱ्या आठवड्यापासून **गट २** मधील पक्ष्यांच्या वजनांमध्ये खाद्य खाण्याच्या प्रमाणामध्ये कमतरता दिसून आली. तसेच रक्तातील व रक्तजलातील घटकांपैकी हिमोग्लोबिन, लालपेशी, पी. सी. व्ही., प्रथिने आणि असिटील कोलीन एस्टरेज चे प्रमाण १४ व्या दिवसापासून घटलेले दिसून आले व रक्तगोठणी, हेटरोफिल, शर्करा, ए. एस. टी., ए. एल. टी., ए. एल. पी., यूरिया नायट्रोजन आणि युरिक ऍसिड यांसारख्या घटकांमध्ये वाढत्या वयाबरोबर वाढ दिसून आली. तसेच अवयवांच्या सूक्ष्मदर्शिकेमधून केलेलया परीक्षणामध्ये विविध अवयवांमध्ये परिणामकारक बदल दिसून आला.

उन्हाळी वनस्पतीच्या पानांची पुड वापरलेल्या **गट ४** मधील पक्ष्यांच्या रक्तातील (रक्तगोठण कालावधी) व रक्तजलातील (ए. एस. टी., ए. एल. टी.) घटकांमध्ये व अवयवांच्या परीक्षणामध्ये समाधानकारक अशी सुधारणा दिसून अली आहे. उन्हाळी हि वनस्पती यकृत पोषक म्हणून उपयोगी असल्याचे निरीक्षण नोंदवले गेले.