AMELIORATIVE EFFECT OF COW URINE DISTILLATE ON ATRAZINE INDUCED TOXICITY IN BROILERS

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/13/040

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(INDIA)

2021

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"AMELIORATIVE EFFECT OF COW URINE DISTILLATE ON ATRAZINE INDUCED TOXICITY IN BROILERS"** or part thereof has not been submitted for any other degree or diploma of any University, not 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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PATIL ANJALI ARUN has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled **"AMELIORATIVE EFFECT OF COW URINE DISTILLATE ON ATRAZINE INDUCED TOXICITY IN BROILERS"** submitted by him is the result of research work is sufficient to warrant its presentation to the examination in the subject of **VETERINARY PATHOLOGY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

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

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This is to certify that the thesis entitled **"AMELIORATIVE EFFECT OF COW URINE DISTILLATE ON ATRAZINE INDUCED TOXICITY IN BROILERS"** submitted by **PATIL ANJALI ARUN** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY PATHOLOGY** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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ACKNOWLEDGEMENT

प्रथमवयसि दत्तं तोयमल्पं स्मरन्तः
शिरसि निहितभारा नारिकेलाः नराणाम् ।
सलिलममृतकल्पं दद्युरजस्रजीवनाजस्रं
न हि कृतमुपकारं साधवो विस्मरन्ति ।।

Remembering the small amount of water which it was given in its early age, the coconut trees carry nectar like water on their head throughout their life. [In the same manner] good and noble people do not forget a favour done to them.

Likewise, I take immense pleasure in expressing my appreciativeness for every person who has guided, encouraged, helped and supported me in life.

Gratitude is powerful trait, they say, which I express primely to my Advisor and Research Guide, ***Dr. P. R. Rathod****, Assistant Professor, Department of Veterinary Pathology, Post Graduate Institute of Veterinary and Animal Sciences, Akola. No words can justify his contribution not only to this thesis but my personality as a whole. I sincerely thank him for believing in me when I was vulnerable and under confident, and encouraging me every single day to explore my potential.*

I am much obliged to thank ***Dr. A. U. Bhikane,*** *Associate Dean, Post Graduate Institute of Veterinary and Animal Sciences, Akola for providing all the necessary facilities to undertake and polish off my research, and for lending financial support. I am extremely thankful to* ***Dr. R. S. Ingole****, Assistant professor and Head, Department of Pathology, PGIVAS, Akola for his words of motivation and encouragement which kept me sustained during the journey of this work.*

Heartfelt benediction towards the members of my advisory committee ***Dr. S. W. Hajare****, Assistant Professor and Head Department of Pharmacology and Toxicology, PGIVAS Akola for their guidance, unstinted interest, incessant help, extensive discussions and valuable guidance during my research work.* ***Dr. M. A. Gole****, Assistant Professor, Department of Poultry Science for their expert guidance and whole hearted support that made me comfortable during my course work and research.* ***Dr. Mohini V. Khodake****, Assistant Professor, Department of Veterinary Biochemistry for valuable and important suggestions during the journey.*

I consider myself the luckiest to be among seniors like ***Dr. Abhilash, Dr. Sheel and Dr. Revati*** *who proved to be my mentors as well as friends, and without whom these two years would have been really monochromatic and unexciting. I have learnt umpteen attributes from them not only regarding Veterinary Pathology but also of life, in general. I have always found them by*

my side in the moments of dilemma and bemusement. I am deeply obliged to my beloved colleagues, ***Dr. Nilesh, Dr. Punam*** *and* ***Dr. Krupan*** *who have been a source of constant inspiration and joy. Apart from providing a great company also taught me collegiate professionalism.* ***Dr. Kale*** *and* ***Dr. Chopde*** *are the seniors from Veterinary Pathology Deparment, PGIVAS, Akola whom I owe mere thanks for being source of timely support, corrective criticism and positive reinforcement.*

Friends have always been an inseparable part of my life. I'd not thank them but rather express my eternal love for ***Pankaj, Rashika, Pradhnya, Pranali, Gayatri, Atul, Megha, Khushhal, Pooja, Sujata, Rujuta, Shailaja, Sapna, Shweta*** *and* ***Dhanshree*** *who have illuminated the avenue of my life by their existence. I want to thank* ***Shubham*** *for listening to me crib about everything and being a part of my Master's journey. I must specially mention my roommate* ***Ayanika*** *who tolerated me and my outlandish demeanor without any complaints. Dear juniors* ***Prajakta, Prerna, Bharti, Kabeul, Anand and Pankaj*** *proved to be very cooperative in my coursework. The active co-operation and sincere help from* ***Bhaskar kaka*** *without which the thesis work could never be completed can never be forgotten.*

And last but not the least, I wish to acknowledge the people having supreme paramountcy in my life. My parents ***Archana & Dr. Arun Patil,*** *elder brother* ***Ajinkya*** *are the basis of my very existence. The morals inculcated by my parents have made me the person I am today. They have always sufficed me with constant support to my every decision ever, encouragement to set high goals and the confidence to achieve them. Their unadulterated love and innumerable sacrifices cannot be requited no matter what I do. I owe everything to them and consider myself very fortuitous to be born as their daughter.*

It is difficult for me to mention the name of everyone in this piece of paper. I would like to take this opportunity to extend my deepest sense of gratitude and words of appreciation towards those, who have dedicated their today for my tomorrow.

You cannot teach a man anything; you can only help him discover it in himself. - Galileo

Place: Akola **(Patil Anjali Arun)**

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LIST OF ABBREVIATIONS

Abbreviation	Full form
%	- Per cent
/	- Per
@	- At the rate of
<	- Less than
µg	- Microgram
µ	- Micron
^{0}C	- Degree celcius
ALT	- Alanine aminotransferase
AST	- Asparate aminotransferase
ATR	- Atrazine
b. wt.	- Body weight
BUN	- Blood urea nitrogen
cm^{-1}	- Per centimetre
CPCSEA	- Committee for the Purpose of Control and Supervision of Experiments on Animals
CRD	- Completely Randomized Design
CRT	- Creatinine
Cumm	- Cubic millimetre
CUD	- Cow urine distillate
DLC	- Differential leucocyte count
ELISA	- Enzyme-linked immunosorbent assay
EPA	- Environmental Protection Agency
ESR	- Erythrocyte sedimentation rate
et al.	- Et alia (and others)
etc.	- Etcetera
eV	- Electron volt
Fig.	- Figure
fL	- Feltometer
FTIR	- Fourier Transformed Infrared Spectroscopy
GCUD	- Gir cow urine distillate
Gm	- Gram
gm/dL	- Gram per decilitre
H&E	- Hematoxyline and Eosin
H_2SO_4	- Sulphuric acid
Hb	- Haemoglobin
HRP	- Horse Radish Peroxidase
hrs	- Hours
i.e.	- That is
IAEC	- Institutional Animal Ethical Committee
IM	- Intramuscular
IP	- Intra peritoneal
IU/L	- International Unit per litre
IV	- Intravenous
Kg	- Kilogram
Kv	- Kilovolts

Abbreviation	Full form
LC_{50}	- Lethal concentration
LD_{50}	- Lethal dose
LDH	- Lactate dehydrogenase
LPO	- lipid peroxidation
LYC	- lycopene
M. Wt.	- Molecular weight
MCH	- Mean corpuscular haemoglobin
MCHC	- Mean corpuscular haemoglobin concentration
MCV	- Mean corpuscular volume
meV	- Million Electron Volts
Mg	- Milligram
mg/dL	- Milligram per decilitre
mg/kg	- Milligram per kilogram
mg/L	- Milligram per litre
ml	- Milli litre
mM	- milli molar
mV	- milli-Volts
NaCl	- Sodium chloride
NS	- Non significant
NSAID	- Non-steroidal Anti-inflammatory Drugs
NTA	- Nanoparticle Tracking Analyser
OD	- Optical density
PCV	- Packed cell volume
Pg	- Picogram
PGE_2	- Prostaglandin E2
PO	- Per orally
ppm	- Part per million
RBC	- Red blood corpuscles
Reg. No.	- Registration Number
rpm	- Revolutions per minute
SC	- Subcutaneously
SGOT	- Serum glutamate oxalatacetate transaminase
SGPT	- Serum glutamate pyruvate transaminase
SOD	- Superoxide dismutase
TEC	- Total erythrocyte count
TEM	- Transmission electron microscopy
TLC	- Total leucocyte count
TP	- Total protein
U/L	- Unit per litre
UV-Vis	- Ultraviolet–visible
viz.	- Namely
WBC	- White blood corpuscles
WHO	- World health organization
wt.	- Weight

CHAPTER I

INTRODUCTION

In worldwide, from the last few decades pesticide and agrochemical component became an important part of agricultural system. In a rapidly growing human population with their increasing demands for the food production ultimately raises pressures on the intensive use of pesticide and fertilizers for more production. These indiscriminate uses of pesticide may contaminate the environment. However, worldwide surveys, documented about the contamination and impact of agrochemical residues in soils, terrestrial and aquatic ecosystem which include the coastal marine system and their harmful effects on human and nonhuman biota also (Carvalho, 2017).

In India manufacturing of pesticide started in the year 1952. From the year 1958, more than 5000 metric tonnes of pesticides had manufactured while, in the mid years of 1990 these manufacturing has been increased up to 85,000 metric tonnes with registration of 145 pesticides. Majority of the pesticide produced were insecticides. In Asia, India is one of the major pesticides producing country with an annual production of 90,000 tonnes. In world, India stands at 12^{th} position for the manufacturing of pesticides (Sharma *et al*., 2019). Presently, approximately 2 million tonnes of pesticides had been utilized throughout the globe, which were contributes as herbicides (47.5%), insecticides (29.5%), fungicides (17.5%) and miscellaneous pesticides (5.5%) (Sharma *et al.,* 2019). Due to the indiscriminate uses of pesticide dispersion of pesticide residues in the soil may leads to contaminate environment and causes mass killings of nonhuman biota such as bees, birds, amphibians, fish, and small mammals as reported by Carvalho, (2017). Herbicide is an agent, usually chemical drug used for killing or inhibiting the growth of unwanted herbs such as residential or agricultural weeds and invasive species. It reduces soil erosion, cost of farming and save energy with increase in crop production (Pacanoski, 2007).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine, ATR) belongs to the group of chlorotriazine herbicide. It is selective triazine herbicide. The herbicide atrazine can be used to control weed in corn, sorghum, sugarcane, pineapple, Christmas tree and other agricultural crops (Severi-aguiar and Silva-Zacarin, 2011). It can also used as a non selective herbicide on non-cropped industrial lands and on fallow land (Extoxnet, 1993). This triazine herbicide primarily used on corn crop (Solomon *et al*., 1996 and Hayes *et al.*, 2011).

Atrazine is frequently detected in ground water and surface water, as a result of its mobility in soil (Elbaz *et al*., 2019). United states Environmental Protection Agency (EPA) has been listed atrazine as a Restricted Use Pesticide (RUP) due to its persistence in water and various adverse health effects on humans. It remains present in the soil degrades with half-lives of a few weeks to several months. It may migrate out from deeper soil to surface runoff to lakes, streams and river. The half life of atrazine is generally >200 days on surface waters (ATSDR, 2003) while soils half life ranging from 20- 146 and 58- 547 days for aerobic and anaerobic respectively (Enoch *et al*., 2007). It has high liposolubility. It can enter into the bodies of animal through water, feed, through grazing or may be due to feed chain. In addition to this if toxicant gets enter into the body, it may induce physiological changes without showing any clinical intoxication signs (Curic *et al*., 1999). Herbicide atrazine classified as a class III toxicant on the basis of toxicity in the scale of I to IV (I being the most toxic) by the Environment protection agency (Singh *et al*., 2017).

Atrazine act as an inhibitor of photosynthesis and persist in soil for 7 to 18 months. The duration of presence of residue in soil depends upon soil properties, soil environment and weather condition (Burnside *et al.*, 1971). Due to indiscriminate use of pesticide there are possibility of health hazard to various farm animals, wild animals, poultry and human being. Atrazine primarily target an endocrine system in animals and humans as a result atrazine alters the natural hormonal system (Saquib *et al.*, 2014). It leads to impairment of various organ systems such as cardiovascular system, excretory

system by causing nephrotoxicity. Atrazine mainly damages the liver and leads to weaken the immune system (Zhang *et al.*, 2019). It has been also recorded limbed deformities, abnormal sexual changes and also affects the ecosystem causes decline the population of frogs and amphibians (Saquib *et al.*, 2014).

In Indian Vedas, cow with scientific name *Bos indicus* is appraised as a valuable and holy animal. Cow is considered as representative of natural beneficence and worshiped as a mother of mankind (Sharma *et al*., 2020**).** In Hindu belief sacredness of cow is crucial and central element. For early nomads the cow was an important requisite member of family. As agriculture was the main occupation of the migrants, cow provide them milk and its product. Also fulfils the necessities of life such as fuel and manure for farm (Mala and Venkatalakshmi, 2015).

Panchagavya is amalgamation of five ingredients namely cow urine, dung, milk, curd and ghee. Cow urine is one of the ingredients believed to have therapeutic value (Jarald *et al*., 2008). It is reported that cow urine has a spiritual cleansing effect. Cow excretory product *i.e.* urine has been delineated as water of life or Amrita" (beverages of immortality) or nectar of God. (Gulhane *et al*., 2017). Cow urine popularly called as *Gowmutra* and renowed for its anti-ageing, antiglycemic and weight loss advantages (Maji *et al*., 2016). Gowmutra is capable of removing all the imbalances within the body, therefore maintaining the physical general health of the body (Randhawa and Sharma, 2015). The main component of cow urine is water, about 95% water present in cow urine. Other than water cow urine consist of 2.5% urea and remaining 2.5% it consist of salt, minerals, enzymes and hormones (Gulhane *et al.*, 2017). Cow urine distillate consist of water, aurium hydroxide, urea, uric acid, creatinine, lactose, sodium, nitrogen, iron, calcium, potassium, manganese, sulphur, carbolic acid salts, hippuric acid other vitamins (A, B, C, D, E) minerals, enzymes (Mondal and Palbag 2018).

United States has been granted the patents for the medicinal properties of cow urine such as bioenhancer, antifungal, antibiotic and anticancer agents for the Patents No. 6896907 and 6410059 reported by Gulhane *et al.* (2017).

Cow urine was given as an alternative to antibiotic they observed the excellent immunomodulatory properties and also increases the egg production and egg quality of laying birds (Chauhan, 2018).

Therefore, the present study was designed to evaluate the efficacy of the cow urine distillate against the toxicity induced by atrazine in broilers with the following objective:-

1. To study the pathology of atrazine induced toxicity in broiler birds.
2. To study the protective effect of cow urine distillate against atrazine induced toxicity in broilers.

CHAPTER II

REVIEW OF LITERATURE

Nowaday, agricultural environment is contaminated continuously with various persistent organic pollutants, which are widely applied in agriculture products like herbicides. Atrazine (ATR) is one of the most intensively used herbicides around the world for removing grassy weed or broadleaf. It has been estimated that a large proportion of ATR remains in the environment after application, causing residual amount exceeding the permissible limit in surface water, rainwater, ground water and agricultural soils (Zhang *et al*., 2019).

Cow's urine has been mentioned in ancient holy text like *Charaka- Samhita, Sushruta Samhita, Atharva Veda, Bhavaprakash, Raj Nighantu, Amritasagaretc*. Cow's urine has been entitled as "*Sanjibani*" and "*Amrita*" in Ayurveda as people who daily drink cow's urine are said to live healthy and salubrious life, clear from diseases. *Gomutra* is capable of curing various diseases such as Diabetes, arthritis, cancer, thyroid, asthama, heart attack, eczema, constipation, migraine, ulcer, acidity, prostate, renal colic, gynaecological problems, blockage in arteries and several other diseases (Mondal *et al*., 2018).

Considering the above facts, the present study was carried out to investigate the protective effect of cow urine distillate against the atrazine induced toxicity in broilers.

The literatures scanned related to present study are grouped under following heads :

2.1 Physical and chemical properties of atrazine

2.2 General performance and clinical observation

2.3 Haematological investigation

2.4 Biochemical investigation

2.5 Gross and histopathological observations

2.1 Physical and chemical properties of atrazine

Atrazine (2-chloro-4- ethylamino-6-isopropylamino-1,3,5-triazine) is a member of s-triazine group of herbicides. It is a chorotriazine herbicide (EL-Shenawy *et al*., 2011). Atrazine is selective herbicide commonly used on many crops for the control of annual grasses and broadleaf weeds in Croatian agriculture. Residues of atrazine are found in some crops, in soil, environmental water and drinking water samples. Atrazine possesses high liposolubility and can enter the bodies of animal through water, feed and feed chain or through grazing. Furthermore, it may induce physiological changes without any visible clinical intoxication. Residues of atrazine and its metabolites after per oral administration of low doses were found in various tissues of chickens, egg layers, turkeys and rats (Curic *et al*., 1999).

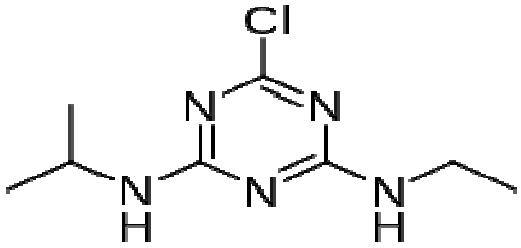
2.1.1 Atrazine: pharmacology, metabolism and pharmacokinetics in animals

The overall pathway for metabolism of atrazine (other closely related chlorotriazine herbicides) is consistent across mammalian species, although quantitative differences exist due to species variations in the kinetics of individual steps. Adams *et al.* (1990) studied metabolism of three triazine herbicide (atrazine, simazine and terbutryn) in rat, mouse, goat, sheep, pig, rabbit and chicken by using in vitro hepatic microsomal system. They observed that principle phase I metabolites were 4- or 6- monodealkylated-s-triazine and phase I reaction were cytochrome P-450 mediated. They further noted that there were species specific variation in the rats of metabolism and in the ratios of primary metabolites, but no sex related differences were noted. Phase II products were glutathione conjugates of the parent and of the two mono de-alkylated metabolites. Fenton's reaction generated hydroxyl radicals, gaves dealkylated 2- chloro-s-triazine, supporting a possible role of active oxygen radicals in cytochrome p-450 mediated reactions. Metabolites isolated from tissues, excreta, milk and eggs are primarily pathway end-products. With the exception of the liver and kidney, all tissue concentrations of metabolites were lower than blood levels. All observations indicated that atrazine is

rapidly metabolised in the liver, then excreted from the body with no significant accumulation and without metabolism in tissues.

2.1.2 Physical and Chemical Properties of atrazine

The following are the physiochemical properties of atrazine :

IUPAC name	6-chloro-N^2-ethyl-N^4-(propan-2-yl)-1,3,5-triazine-2,4-diamine
Other names	Atrazine 1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine 2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine 6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
Chemical formula	$C_8H_{14}ClN_5$
Molar mass	215.69 g·mol^{-1}
Appearance	colourless solid
Melting point	175 °C (347 °F)
Boiling point	200 °C (392 °F)
Chemical structure	

2.1.3 Chemistry and biochemistry of atrazine

Atrazine is prepared from cyanuric chloride, which is treated sequentially with ethylamine and isopropyl amine. Like other triazine herbicides, atrazine functions by binding to the plastoquinone binding protein in photosystem II, which animal's lacks. Death of plant takes place as results of starvation and oxidative damage caused by breakdown in the electron transport process. Oxidative damage is accelerated due to high light intensity recorded by (Arnold *et al*., 2001). Atrazine affects primarily

endocrine system in humans and animals. Studies recorded that atrazine is an endocrine disruptor that can cause hormone imbalance. Atrazine has been found to act as an agonist of the G protein-coupled estrogen receptor 1 (Eric *et al*., 2014). Atrazine has been shown to covalently bind to (chemically react with) a large number of mammalian proteins (Dooley *et al*., 2008).

2.2 General Performance and clinical observation

2.2.1 Atrazine

Santa *et al*. (1987) studied hepatotoxicity induced by the herbicide atrazine in the Wistar albino rat. In present study four groups of 10 male adult Wistar albino rats weighing 240-280 g were dosed by gavage with sub acute doses of atrazine@100, 200 and 400 mg/kg body weight/day for a period of 14 days and 600 mg/kg body weight/day for a period of 7 days. Significant decreased in the body weight was recorded in all atrazine treated rats at 14 days of treatment groups. The atrazine was given @ 400 mg/kg body weight for 14 days revealed more weight loss as compared with the 7day atrazine treatment group @ 600 mg/kg body weight.

Wilhelms *et al*. (2005) investigated effect of atrazine on sexual maturation in female Japanese quail induced by photo stimulation or exogenous gonadotropin. Atrazine was administered @ 0, 1, 10, 100 and 1,000 ppm in the diet of the female quail undergoing photo periodically induced sexual maturation. Japanese quail exhibits clinical signs of severe toxicity with decrease in growth and feed intake was noted at high dietary concentration (1,000 ppm) of atrazine.

Juliani *et al*. (2008) studied the effects of atrazine on female Wistar rats: The study was divided in two blocks: sub acute and sub chronic treatment. Each block was constituted by: a control group (n = 5) and an experimental group (n=5). In sub acute treatment, the females received distilled water (control group) and atrazine @ 400 mg/kg (experimental group) for a period of 14 days where as in sub chronic treatment group females

received distilled water (control group) and atrazine @ 0.75 mg/kg (experimental group) for a period of 30 days. All the rats were dosed daily by oral gavages route. Significant decrease in the food consumption and body weight (18.6%) were observed in the rats receiving atrazine @ 400 mg/kg body weight. Non significant decrease in the food consumption was recorded in rats treated with atrazine @ 0.75 mg/kg of body weight while; significant increase (8.5%) in the body weight was recorded during the period of 30 days. Sub acute group showed clinical signs of fatigue, weakness, slight trembling in their hind legs and presence of a strong odour in their urine were noted while sub chronic treatment group also revealed fatigue and weakness in rats.

Kanth (2008) revealed the toxicopathology of atrazine in Wistar rats. For the present experiment seventy two Wistar rats were procured and rats were randomly divided into four different groups with 6 male and 12 female in each group. Group A, B and C were treated with graded dose of atrazine @ 30, 60 and 120 mg/kg body weight given by oral gavage route every day for a period of sixty consequent days respectively and group D served as control group. Rats from B and C group revealed dullness, lethargy, reduced feed intake, while C group showed one mortality in the last week of experiment. Significant decrease in the dose dependant variation was noted in the body weights of group C rats followed by B and A group.

Ramesh *et al*. (2009) investigated effect of atrazine on blood parameters of common carp Cyprinus carpio (*Actinopterygii:Cypriniformes*). Cyprinus carpio fish exposed to acute toxicity of atrazine @ 18.5 ppm for a period of 24 hrs. The fish exhibited clinical signs of increased opercular movement, mucous secretion, jerky movement, floating on the sides, hypersensitivity showing violent erratic and fast swimming were noted during entire experimental period

Hussain *et al.* (2010) carried study on pathological and genotoxic effects of atrazine in male Japanese quail (*Coturnix japonica*). In present study 96 mature male Japanese quail were procured and randomly kept in eight groups (A-H) i.e. 12 birds in each group. Atrazine was administered orally at 0, 10, 25, 50, 100, 250 and 500 mg/kg body weight to all experimental groups except group B by oral route daily for a period of 45 days. The mitomycin C was given @ 2 mg/kg body weight to the birds of group B which served as a positive control. Significant decrease in feed intake and body weight were observed in all the atrazine treated groups in Japanese quail.

Chand (2011) studied effect of cow urine ark in atrazine induced toxicity in broilers. Total thirty six one week old age broiler chicks were used for the experiment. Birds divided into six groups each group containing six birds (n= 6). Group I served as control, Group II treated with cow urine ark @ 1ml/bird/day by oral route. Group III and V treated with atrazine @ 150 and 300 mg/kg body weight respectively while group IV treated with atrazine @ 150mg/kg body weight orally along with cow urine ark @ 1ml/bird/day oral route. Group VI administered atrazine @ 300 mg/kg of body weight by oral route along with cow urine ark @ 1ml/bird/day orally. Birds of group V exhibited the clinical signs of dullness, ruffled feathers, depression, listlessness and reduced feed intake while some birds exhibited diarrhoea. Atrazine treated group with or without cow urine ark revealed a significant reduction ($P \leq 0.05$) in the body weight as compared to control group in broiler birds.

Pereira *et al*. (2012) recorded cytotoxic and genotoxic effects of atrazine in Wistar rats. Twenty five adult male Wistar rats having age of 2 months were randomly divided into three groups. Group I served as negative control group (n =10) received only filtered water, group II served as positive control group (n= 5) received water and group III received one of dose of methyl methanesulfonate (MMS) on the day of euthanasia and group IV

(n=10) treated with an aqueous solution of atrazine. Rats received atrazine @ the dose of 400 mg/kg body weight/day given by oral gavage route for a period of 14 days. Atrazine treated rat's revealed clinical signs of hair bristling, whitish eyes, paw tremor and locomotion difficulties while marked reduction in body weight were observed during the entire experimental period.

Blahova *et al*. (2014) evaluated haematobiochemical and histopathological responses and recovery ability of Common Carp (*Cyprinus carpio* L.) after acute exposure to atrazine herbicide. Eighty common carp were procured and divided into five groups. Group I served as control, group II, III, IV and V receiving atrazine @ of 5, 15, 20 and 30 mg/L in water was given for 96 hours respectively. The control and atrazine treated group at 5 and 15 mg/L were used 20 fishes in each group whereas, other atrazine treated groups @ 20 and 30 mg/L were selected only 10 fish in each group. Atrazine treated group revealed dose dependent variation in the clinical signs of abnormal behaviour such as reduced reflexes, erratic swimming, loss of equilibrium and accelerated respiration were reported in common carp. Mortality was reported as 60% in the highest concentration of atrazine treated group (30mg/L) within 96 hours of the experimental study period.

Nazar *et al*. (2016) investigated effect of fenugreek seed extract on some haematological and biochemical parameters in atrazine treated male rats. Twenty eight adult male rats weighing around (253-339) gm were randomly selected and equally divided into four groups, each group comprising of 7 males in each. Group I (control) received control diet and corn oil, group II received control diet combine with atrazine @150mg/kg body weight by oral route, group III rats received boiled aqueous extract of 2.5% of fenugreek seeds in the diet with atrazine given @ 150 mg/kg body weight by oral route and group IV received of 5% fenugreek seeds combine with atrazine @ 150 mg/kg body weight by oral route. The treatment was given for the twenty eight days. Significantly decrease in the body weights and food intake was recorded in the atrazine alone treated group while non significant

difference were noted in atrazine given in combination with fenugreek extract (2.5%) group and atrazine treated in combination with fenugreek extract group (5%) in rats at the end of 2 and 3 weeks of age.

Abarikwu *et al*. (2017) reported that the effects of co-exposure to atrazine and ethanol (EtoH) on the oxidative damage of kidney and liver in Wistar rats. Thirty male Wistar rats were procured and randomly assigned into five groups, each group containing six (n=6) animals. Group I was served as a control, group II treated with ethanol (EtoH) @ 5 g/kg for a period of 21 days by oral gavage route while III, IV and V groups were administered with different doses of atrazine @ 50,100,300 mg/kg body weight along with EtoH for 21 days respectively. Significant decreased relative organ weights kidney (11.58%) and liver (26.7%) and body weights of rats were recorded in group V as compared with the control group.

Elbaz *et al*. (2019) studied atrazine induced toxicity in goat leads to alleviated spermatozoa to some extent by polyphenol enriched feed. During the In-vivo study, young male goats were fed for 6 months with standard ration (control; n=5) and standard ration supplemented with atrazine @ 15 mg/kg body weight daily (n=3). The reduction in growth rate leads to lower in the body weights of male goats were reported in atrazine given @ 15 mg/kg body weight groups.

2.2.2 Cow urine distillate

Garg *et al*. (2005) evaluated effect of distilled cow urine on the nutrient utilization by white leghorn layers. One hundred day old birds were selected for the present study and divided into two groups of 50 each. One group was kept as control and second treatment group was given cow urine @ 1 ml per bird. Significant increase in the feed intake while decreased in the feed conversion ratio and feed efficiency ratio was noted in the cow urine treated group at 15 day intervals of study period in white leghorn layers.

Nirmala (2010) studied safety evaluation of cow urine in Wistar albino rats. Cow urine was administered orally @ 5, 10, 15, 20 and 0.25, 0.5, 0.75mL/kg body weight in acute and chronic toxicity studies respectively as compared with distilled water treated animals for a period of 90 days. Noted non significant differences in the body weight in the entire cow urine treated group when compared with control group.

Jojo (2010) observed that the immunomodulatory effect of cow urine distillate (CUD) on humoral and cell mediated immune parameters in broiler chicks. Eighty day old Vencobb chicks were procured and were randomly divided in four groups (n= 20). Group 1 received CUD and vaccinated with NDV vaccine (LaSota strain), group 2 received Levamisole @ 10 mg/kg body weight and vaccinated, group 3 received normal feed and vaccinated with NDV vaccine while group 4 was kept served as control (untreated and unvaccinated). Birds of three groups (Group 1, 2 & 3) received scheduled IBD and booster NDV vaccine (R2B strain) on $14^{th}$and 28^{th}day. Significant increase ($P<0.01$) in body weight was noted in CUD treated groups of rats as compared with the control groups at the end of 42^{nd} days of experiment.

Chand (2011) reported mild degree of clinical signs and significant increase in body weights in cow urine distillate treated group as compared with the atrazine treated group in broiler birds.

Sachdev *et al*. (2012) evaluated antidiabetic, antioxidant activity of gomutra ark in Wistar albino rats. For the acute toxicity study 30 male Wistar rats were divided into five groups (n= 6) while single dose of gomutra ark was given in various doses @ 2, 4, 8, 16, and 32 mL/kg by oral route. The clinical signs noted were tremors, clonic convulsions, tonic extensions, catatonia, spasticity, opisthotonus, ataxia, sedation, ptosis, respirationin acute toxicity study. In chronic toxicity study rats were divided into two groups having six rats in each group. First group served as control

received laboratory food and *ad-libitum* water. The second group was given gomutra ark 1mL/kg twice daily. No signs of toxicity were seen in chronic toxicity study.

Kadagi *et al*. (2012) determined hypoglycemic activity of cow urine distillate in streptozotocin induced diabetic rats. The Wistar albino rats were divided into five groups (n=10). Group I served as control, group II served as diabetic control, group III served as a diabetic positive control, group IV and V were administered cow urine distillate @ 0.5 and 1 mL/kg body weight respectively. Diabetes was induced in rats by injecting streptozotocin @ 45mg/kg by intraperitoneal route. Significantly ($P<0.001$) increased body weights was recorded in cow urine distillate treated group as compared to the diabetic control on day 14^{th}, $21^{st}$and 28^{th} days of the experiment.

Pancha (2015) studied immunomodulatory effect of cow urine distillate in healthy and cyclophosphamide induced immunosuppression in Swiss Albino male mice. The mice were divided into eight groups (I, II, III, IV, V, VI, VII and VIII) each group containing six animals. Group I administered with normal saline and served as control. Group II was given cyclophosphamide @ 60mg/kg body weight, group III, IV and V were administered with cow urine distillate @ 2, 4 and 6mL/kg body weight, while group VI, VII and VIII were administered cyclophosphamide @ 60 mg/kg body weight along with cow urine distillate @ 2, 4 and 6mL/kg respectively given for a period of 28 days. No significant difference in the body weights was noted in cow urine distillate treated group and cyclohosphamide treated group as compared to control.

Padmapriya and Devi (2016) evaluated the effect of different concentration of Gir cow urine in growth and biochemical changes to fresh water fish *Cirrhinus mrigala* Fingerlings (Hamilton). Cow urine was given at different dosage of 0.001%, 0.01%,0.1%,1%,2%,4%,5%,10%,20%,25%, 50%

and 100% concentration while one group served as a control. The control and cow urine treated groups were sacrificed at 30th days and the growth was analyzed. The maximum growth rate of 0.00532 gm/day was observed in the *Cirrhinus mrigala* fingerlings when treated with 0.1% of Cow urine when compared with control group.

Fefar (2016) conducted study on clinico-pathological and immunomodulatory effects of cow urine distillate and its synergistic action with aqueous poly herbal extract containing *Ocimum sanctum* (Tulsi), *Tinospora cordifolia* (Guduchi) and *Withania somnifera* (Ashwagandha) in commercial broiler chicks. The CUD served @ 1 mL/kg body weight alone or its combination with aqueous polyherbal extract @ 2mL/kg body weight for 35 days against the intermediate IBD vaccine given at 13th days and cyclophosphamide @ 150 mg/kg of body weight at 15th days of experiment. No observable clinical signs of abnormalities were noticed in CUD and aqueous polyherbal extract. Significant ($P<0.05$) increased in weekly body weight and FCR levels were recorded in CUD group at the end of 28 days of the experiment.The birds treated with cow urine distillate does not revealed any clinical sign whereas, significant ($P<0.05$) increase in the means of body weights were reported in group IV (cow urine distillate) and V (cow urine distillate + poly herbal extract) as compared to group I. Significant difference in the feed conversion ratio was recorded in the cow urine distillate treated group (IV) and cow urine distillate along with poly herbal extract treated group (V) as compared to control group I at 28th and 35thday of experiment.

Tadavi *et al*. (2017) studied clinical and haematological changes induced by chlorpyrifos and its amelioration by cow urine distillate in broilers. Sixty day old broiler chicks were divided into four groups comprising of 15 birds in each. Group I served as control, group II was treated with chlorpyrifos @ 50 ppm in feed, group III was treated with cow urine distillate @ 10 mL/litre of drinking water and group IV was treated with chlorpyrifos @ 50 ppm and cow urine distillate @ 10 mL/litre of drinking water for a period

of 28 days. They recorded clinical signs of closed eyes, dropped neck and wings, unable to stand and diarrhoea was observed from 2nd week of post intoxication of chlopyrifos group, however signs were more or less were present in the cow urine distillate treated groups. Similarly higher values of feed consumption and FCR were recorded in group III as compared to control.

2.3 Haematological investigation

2.3.1 Atrazine

Ramesh *et al*. (2009) determined the effect of atrazine on blood parameters of common carp Cyprinuscarpio (*Actinopterygii: Cypriniformes*). Cyprinuscarpio fish exposed to acute toxicity of atrazine @ 18.5 ppm for a period of 24 hrs. Numerical decreased in the red blood cells (RBCs), hemoglobin levels while increase in the white blood cells (WBCs) count were recorded in acute concentration of atrazine treated group as compared with the control in *C. Carpio.*

Saquib (2009) recorded haematological alteration in atrazine induced toxicity in broilers. One hundred twenty day old broiler chicks were randomly divided into four groups comprising 30 birds in each group. The atrazine was given @ 375, 250, 125 mg/kg body weight in IInd, IIIrd and IVth group of the experimental birds for the study period of 28 days respectively. Significant decrease in the mean levels of Hb, PCV and TEC were recorded in atrazine treated @ 250mg/kg body weight of birds. Macrocytic hypochromic anaemia and leucocytopenia recorded in group IV while microcytic hypochromic anaemia and leucocytosis were noted in IInd and IIIrd group as compared with control. Significant heterophilia was recorded in IIIrd and IVth group and monocytosis were noted in IInd, IIIrd and IVth group.

Hussain *et al*. (2010) observed significant decrease in the total erythrocyte counts, hemoglobin and hematocrit values while, leucocyte count revealed significant decrease in the high dose of atrazine treated group from 50 - 500 mg/kg body weight (E- H) when compared to control group.

Chand (2011) recorded significant decrease in the Hb, PCV, TEC values and significant increase in TLC values whereas, differential leucocyte count reported lymphocytopenia , heterophilia and monocytosis in atrazine treated group in broiler chicks.

EL-Shenawy *et al*. (2011) studied mitigating effect of ginger against oxidative stress induced by atrazine herbicides in mice. Male mice weighing around 30gm – 35gm were divided into four groups. Group I treated as a control, group II treated with 120 mg ginger extract/kg intraperitonially (0.1 ml/mice) on each alternative day for a period of 14 days, group III mice given atrazine @ dose level of 78.25 mg/kg body weight by intraperitonial route for 14 days and group IV mice were given ginger extract 120 mg/kg along with atrazine 78.25mg/kg body weight for 14 days. Significant decrease ($P < 0.01$) in Hb, PCV, TEC, MCV, MCH and MCHC levels was observed in atrazine treated group as compared to control. Significant increase in the percentage of lymphocytes and monocytes were recorded in the atrazine group as compared to control.

Ezenwaji *et al*. (2012) investigated haematological alteration induced by atrazine in Albino rats. A thirty six (36) male Albino rats were used for the present study and divided into four groups of nine rats each. Group I served as the control while groups II, III and IV were treated with atrazine @ 150, 200 and 300 mg/kg body weight by oral route for a period of 28 days. Significant decrease in the mean values of Hb, PCV and RBC were reported at 21stand 28th day in all atrazine treated group while similar finding was noted in total WBC counts throughout the study in all atrazine treated group. However, group IV revealed significant increase ($P<0.05$) in

lymphocyte and significant decrease (P<0.05) in neutrophil count at 28thday in atrazine treated group when compared with control.

Blahova *et al*. (2014) reported significant (P<0.05) decrease in haemoglobin, haematocrit, leukocyte, lymphocyte levels while significant increase in monocytes, neutrophil, granulocytes, metamyelocytes and myelocytes count in atrazine treated group when compared with control in common carp.

Michael (2018) investigated hematobiochemical profile and histological alteration of atrazine in *Clarias gariepinus*. Three hundred healthy fishes were procured and divided into six group, each group containing fifty fishes. Group I served as a control while other five groups were treated with different concentration of atrazine @ dose of 0.10, 0.15, 0.20, 0.25 and 0.30 mL/liter of water. Significant decrease (P<0.05) in the mean values of PCV, Hb, WBC, MCHC, MCV and MCH were recorded in all the atrazine treated groups in fish.

2.3.2 Cow urine distillate

Chauhan *et al*. (2001) investigated the immunomodulatory effect of Kamdhenu ark in mice. Study was conducted in 20 mice and divided into 2 groups. Groups I was kept as control while group II mice were given 1 mL Kamdhenu ark in water for a period of 90 days. Results indicated that there was numerical increase in B- and T- lymphocyte blastogenesis.

Kumar *et al.* (2005) reported immunomodulatory effect of cow urine on lymphocyte proliferation in developing stages of chicks. Sixty chicks were randomly divided into two groups (n=30). The group I (control) was served as control and group II treated with distilled cow urine @ 10 mL/liter through drinking water for 28 days. Lymphocyte proliferation activity was noted maximum during first two weeks of development. Increase in the T- and B-cell blastogenesis was noticed by 1.81% and 2.21% respectively in cow urine treated group as compared with control.

Nirmala (2010) recorded non significant alteration were noted in Hb, PCV, TEC, MCV, MCH, MCHC and DLC levels in the cow urine treated group as compared with control group in Wistar albino rats.

Chand (2011) observed significant increase in the Hb, PCV, TEC values while significant increase in TLC count were reported in cow urine ark treated groups (groups IV and V) when compared with control in broiler birds.

Verma *et al*. (2011) studied immunomodulatory effect of cow urine in Albino rats. For the present experiment thirty six Albino rats were divided into six groups each comprised of six rats. Group I served as control, group II was administered with 500mg/kg^{-1} body weight of whole freeze dried cow urine whereas group III to VI were administered with 500mg/kg^{-1} body weight ethy acetate, methanol, acetone and aqueous fractions of cow urine respectively. The red blood cell and white blood cell count was significantly increased by 8%, 7%, 6% and 13%, 12% and 8% in freeze dried cow urine, its methanol and aqueous fractions treated group respectively. Haemoglobin levels revealed significant increase in entire treatment group except control group.

Kadagi *et al*. (2012) recorded non significant difference in the levels of Hb, PCV, TEC and TLC in the cow urine distillate treated group in rats.

Joshi *et al*. (2012) studied immunomodulatory effect of cow urine distillate in rabbits. Total of fifteen rabbits were selected and divided in three groups. Group I was kept as control, rabbits of group II treated with 5mL gir cow urine distillate (GCUD) orally daily, group III rabbits were given GCUD along with 2.5% citric acid @ 5mL each by oral route daily for 60 days. Significant increase in the levels of total leucocyte count (29% and 49%), absolute lymphocyte count (24.50% and 41%) and absolute neutrophil

count (32% and 49%) were recorded in II and III groups respectively in rabbits.

Sanganal *et al*. (2012) studied effect of cross bred cow urine in rats during sub acute study. Acute and sub acute oral toxicity studies of Holstein Friesian cross bred cow urine was conducted to determine the median lethal dose for both male and female rats. In the repeated dose 28 day sub acute oral toxicity study the blood and serum samples were analyzed on day 0, 14 and 28 for estimating different hematological parameters They revealed significant ($P<0.01$) decrease in the MCHC and erythrocyte count and significant ($P<0.01$) increase in neutrophil and MCV counts in both the sexs of rats in cow urine treated group at 28 days.

Joshi and Chauhan (2013) investigated anticancerous effect of *Taxusbacaata* and distilled Badri cow urine in mice. Ninety seven mice were selected and divide them into 11 groups. The mice of 9 test groups were given different extracts of *Taxusbaccata* alone and in combination with CUD (2ml/day/mice), daily orally from day 1 for 6 months. Total of eleven groups were control (9 mice), DEN (diethyl nitrosamine) treated negative control (8 mice), CUD (without DEN, 8 mice), A (aqueous extract of leaves, 8 mice), B (ethanolic extractof leaves, 8 mice), G (methanolic extract of leaves, 8 mice), H (ether extract of bark, 8 mice), CUD (cow urine distillate with DEN, 8 mice), CUD + A (8 mice), CUD + B (8 mice), CUD + G (8 mice), CUD + H (8 mice). They revealed significant increase in values of leucocyte count (TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) in mice treated with either CUD alone and in combination with *Taxusbaccata* extracts group. The study revealed that the values of total leucocyte count (TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) were significantly increased in the treated groups of mice either by CUD alone and in combination with *Taxusbaccata* extracts. At 180th day, it was found that there was an increase in body weight, Hemoglobin content (Hb), total erythrocyte count (TEC), total leucocyte count

(TLC), absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) levels in CUD +A treated group as 23%, 23.99%, 41%, 40%, 40.31%, and 40.13%, respectively.

Panicker *et al*. (2013) studied the effect of cow urine ark and Aloe vera extract on haematological parameters in white leghorn chicken. Fourty birds were divided into four groups containing 10 birds in each group. Group A served as a control, group B received cow urine ark @ 1mL, group C given Aloe Vera @ 1mL and cow urine ark along with Aloe Vera were given @ 0.5mL by oral route daily for a period of 90 days respectively. Significant increase in Hb, TEC, TLC and PCV were reported while haematopoiesis activity was increased in the birds treated with cow urine ark and Aloe Vera extract group

Naseema *et al*. (2014) investigated immunomodulatory effect of Vechur cow urine in cyclophosphamide induced immunosuppressed Swiss albino mice. The cow urine distillate was administered by oral route @ 10.8mL/kg body weight for a period of 19 days. Significant ($P<0.05$) increase in the levels of total leucocyte count and lymphocyte count were recorded in the Vechur cow urine treated group.

Pancha (2015) revealed significant increase in the mean levels of TLC values in the cow urine distillate treated group alone and in combination with cyclophosphamide in mice at 28 days of experiment.

Bhatele *et al*. (2016) investigated beneficial and curative effect of cow urine distillate in modulating toxic effect of mercury in chickens. Twenty four day old broiler chicks were procured, reared in battery brooders for a week and divided into four groups (n=6). The group I birds served as control, group II birds were administered with cow urine distillate @ 1mL/bird/day and group III birds received mercuric chloride @ 50 ppm mixed with feed whereas, group IV birds received mercuric chloride @ 50 ppm in feed along with cow urine distillate @ 1mL/bird/day for a period of 45 days.

The birds given mercuric chloride showed significant decrease in the level of haemoglobin, packed cell volume, total erythrocyte count and erythrocytic indices. However Group IV birds received cow urine distillate along with mercuric chloride did not show any toxic effects of the mercuric chloride and the haematological parameters remained near normal range.

Tadavi *et al*. (2017) revealed significant increased in the mean values of TEC, TLC and lymphocyte count whereas, significant decreased in MCV, MCH levels and non significant differ in the mean levels of Hb, PCV, MCHC, heterophil, monocyte, eosinophil and basophil count were observed in CUD treated group when compared with the chlropyrifos treated group.

Oladele *et al*. (2017) investigated activity of photo activated cow urine distillate in adult male Wistar rats. Twenty Wistar rats were randomly assigned into five groups (n= 4) comprising in 4 rats each. Group A served as a control while group B, C, D and E given CUD @ 1, 2, 3 and 4mL respectively. Significant increase in the haematocrit, RBC (except group D), WBC count (except group E) and percentage of lymphocytes count was recorded in all CUD treated group as compared with control.

Praveena *et al*. (2019) reported haematological variables of *Oreochromis mossambicus* against *Aeromonas hydrophila* infection by using dissimilar types of gaumutra distillate. Fishes were exposed to different cow urine distillate (CUD) namely, T1 (Gir calf), T2 (Gir cow), T3 (Gir Bull calf), T4 (Gir Bull) at 0.1 % concentration for seven days while group C served as control. *O. mossambicus* injected with heat-killed *A. hydrophila* (1×10^{-1} cells) during post stimulation phase. Significant difference in the mean values of Hb, RBCs, WBCs, MCH, MCV, MCHC and TLC were noted at various concentration of cow urine distillate group as compared with the control group.

2.4 Biochemical investigation

2.4.1 Atrazine

Santa *et al*. (1987) observed significant increase in the levels of serum ALT (alanine amino transferase) and SAP (alkaline phosphatase) in atrazine treated group given @ 600mg/kg body weight for 7 days in rats.

Kanth (2008) recorded dose dependent significant increase in AST, ALT, BUN and creatinine value in all atrazine treated group as compared to control groups in rats.

Chand (2011) noted significant decrease in serum total protein, albumin, globulin and calcium level in atrazine treated groups in broiler birds.

EL-Shenawy *et al*. (2011) observed significant decrease in value of total protein and total lipid in atrazine treated group as compared to control and ginger treated groups in mice.

AL-Attabi (2012) investigated protective role of clomiphene citrate (CC) against atrazine (ATR) toxicity in adult male rats. Thirty adult male rats were used and equally divided into five groups. Group I was served as control, group II was given atrazine @ 50mg/kg body weight, and group III, IV and V were given atrazine @ 50mg/kg body weight in combination with different doses of clomiphene citrate @ 0.5, 0.6and 0.7mg/kg daily for 30 days. Atrazine alone treated group reveals significant elevation in levels of the serum total cholesterol, triglycerides, AST, ALT and creatinine as compared with control in rats.

Hussain *et al*. (2012) recorded significant ($P< 0.05$) decrease in serum total protein, serum albumin and serum testosterone values while significant increase in serum ALT and AST concentration were recorded at 45 day in all atrazine treated groups in Japanese quail.

Pereira *et al*. (2012) recorded non significant difference in the serum ALT levels in the atrazine treated group when compared with the control in Wistar rats.

Chand *et al*. (2013) investigated effect of atrazine on various organ system in broilers. The present study was carried out on eighteen healthy one week old White Leghorn broilers chicks and they were divided into three groups with six birds in each group. Group I was the control. In group II and III, atrazine was given orally at 150 mg/kg body weight and 300 mg/kg body weight, respectively. The significant increase ($P<0.05$) in the mean levels of ALT, AST, blood urea nitrogen and creatinine values were recorded in atrazine treated birds.

Blahova *et al*. (2014) recored significant ($P< 0.05$) increase in the values of serum ALT, AST, ALP and LDH in all atrazine treated group when compared with control.

Liu *et al*. (2014) determined effect of atrazine induces oxidative damage in kidney of Wistar rats. A four weeks age female Wistar rats were selected for the present study and were treated with atrazine @ 0, 5, 25 and 125 mg/kg body weight for 28 days. Significant increased ($P<0.05$) serum levels of BUN (blood urea nitrogen) and creatinine were recorded in atrazine treated rats given @ 125 mg/kg body weight treated group as compared with control.

Nazar *et al*. (2016) noted significant increase in total cholesterol values in atrazine combine with 5% fenugreek group whereas non significant increase in triglycerides and low density lipoprotein (LDL) were recorded in atrazine treated group.

Michael (2018) reported significant increase ($P<0.05$) in the activities of enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogense (LDH) in all atrazine treated groups as compared with control.

2.4.2 Cow urine distillate

Gururaja *et al*. (2009) studied the ameliorative effect of CUD against carbon tetrachloride-induced hepatotoxicity in rats. Thirty six male Albino Wistar rats were randomly assigned for the present study and divided into six groups (n=6). Group I served as control, group II received single dose of CCl_4/olive oil(1:1@5mL/kg) given by intraperitoneal route, group III received silymarin @ 100mg/kg by oral route once daily followed by a single dose of CCl_4/olive oil (1:1, 5 mL/kg, intraperitonially) while groups IV, V and VI received cow urine distillate @ dose of 2.7, 5.4and 10.8 mL/kg body weight by oral route followed by single dose of CCl4 /olive oil (1:1, 5 mL/kg, intraperitonially) on 7^{th}day. Decreased in the levels of AST, ALT, ALP, GGT and total bilirubin were recorded in CUD treated group as compared to carbon tetrachloride toxicity group.

Nirmala (2010) recorded non significant alteration in the serum ALT, AST, total protein, serum albumin, serum globulin and creatinine values in CUD treated group as compared to control group in Albino rats.

Jojo (2010) observed significant increase in the values of serum protein, albumin and globulin in CUD treatment groups when compared to control group.

Chand (2011) recorded significant decrease in AST, ALT, blood urea nitrogen and creatinine values and significant increase in serum protein and calcium level were recorded in the CUD treated group in broiler birds.

Mishra and Gupta (2011) studied effect of noni and cow urine ark on serobiochemical parameters in Albino rats. Twenty four Albino rats of either sex were used and were divided into four groups having 6 rats in each group. Group I was treated as control, group II rats given aqueous extract of Noni @ 1000mg/rat/day, group III rats receiving alcoholic extract of Noni @ 1000mg/rat/day for 90 days and group IV rats were treated with cow urine ark

@ dose rate of 1ml/rat/day in drinking water. They showed significant increase in the levels of total protein, albumin and globulin in all treatment groups as compared to respective control groups.

Sanganal *et al*. (2011) conducted an acute toxicity study to determine the median lethal dose for both male and female rats separately. Five groups of male and female rats, each consisting of six rats were used for estimating LD50 value. They noted significant ($P<0.01$) increase in the levels of ALT, AST, ALP, creatinine, BUN, bilirubin and non significant difference in total protein in cow urine (Holstein Friesian cross bred) treated group in rats.

Panicker *et al*. (2013) observed significant decrease in the AST, ALT, GGT values of liver enzymes whereas increase in albumin and protein levels were reported in CUD treated group when compared with control.

Shukla *et al*. (2013) investigated anti-urolithiatic effect of cow urine ark (medicinal distilled cow urine) against ethylene glycol (EG) induced renal calculi in rats. Thirty six male Wistar rats were randomly divided into 6 equal groups. Group I rats served as control, group II served as EG control, group III and IV (preventive groups) received cow urine ark @ 1 and 2mL/kg by oral route respectively while group V and VI received 1 and 2mL/kg in combination with cow urine ark and ethylene glycol by oral route respectively for a period of 28^{th}days. Significant decrease in the levels of serum creatinine, blood urea and calcium oxalate were reported in cow urine ark treated group when compared to urolithiatic treated group at 28^{th} day.

Chawda *et al*. (2014) assessed the Lipid-lowering activity of cow urine ark (CUA) in guinea pigs fed with a high cholesterol diet. Thirty guinea pigs weighing around 520 – 860 gm of either sex were divided into five groups comprised of six guinea pigs in each groups. Group I served as control, group II served with high fat diet with distilled water, group III treated with high fat diet in combination with lower dose of CUA @ 0.8mL/kg, group IV

served high fat diet with higher dose of CUA @ 1.6 mL/kg and group V served with high fat diet in combine with rosuvastatin @ 1.5mg/kg body weight for a period of 60 days. Significant decrease ($P< 0.05$) in the values of serum triglycerides, total serum cholesterol, very low density lipoprotein cholesterol, AST, ALP and LDH level were recorded in cow urine ark treated group given @ 0.8 and 1.6 ml/kg body weight in guinea pigs.

Pancha (2015) noted significant increase in total protein and albumin level in cow urine distillate given in combination with cyclophosphamide treated group as compared to cyclophosphamide alone treated group in mice.

Bhatele *et al*. (2016) recorded significant decrease in levels of serum blood urea nitrogen, creatinine, glucose and significant increase in level of serum proteins were recorded in CUD treated group as compared with the mercuric chloride treated group.

2.5 Gross and Histopathological investigation

2.5.1 Atrazine

Santa *et al*. (1987) noted proliferation, degeneration of smooth endoplasmic reticulum, lipid accumulation, mitochondria malformation and alteration in bile canaliculi were observed in the hepatocytes of atrazine treated group while similar changes with less magnitude were observed in the liver of atrazine given @ 200 and 400 mg/kg body weight of rats.

Curic *et al*. (1999) assessed morphological changes in gilts induced with low-dose of atrazine. Experiment was performed on twenty gilts (10 experimental and 10 control) of 6-7 months of age having body mass of 80-100 kg. The gilts (n = 10) received atrazine @ dose 2mg/kg body mass in the feed during 19^{th}days of the oestrus cycle. Grossly, atrazine treated gilts was showed cystic ovarian degeneration whereas, histopathological lesions revealed parenchymatous degeneration, infiltration of lymphocytes in interstitial tissue of liver and kidneys, sub-acute glomerulitis with atrophy of

single glomeruli, desquamation epithelial cells of some tubules presence of proteinaceous casts in lumen of kidney, degeneration of muscular fibres in the myocardium, multiple follicular cysts, persistence of corpus luteum present in ovaries, lymphoid depletion in lymphoid follicles of lymph nodes and spleen was observed in atrazine treated group.

Kanth (2008) reported histopathological lesion of liver showing bile duct hyperplasia, infiltration of lymphocyte and macrophages, necrosis of hepatocytes in liver, kidney showed focal tubular necrosis, shrinkage of glomeruli tuft, presence of granular cast in tubular epithelium. Vacuolar changes in brain matrix. Intestine revealed goblet cell hyperplasia with ulcer formation due to denudation of epithelium in the atrazine treated group.

Saquib (2009) recorded enlargement of gall bladder and hepatomegaly in liver, while, heart showed epicardial congestion, cardiac dilatation and hydopericardium. Lungs were congestion and consolidation in all atrazine treated group. Microscopic finding of liver showed Kuffer cell hyperplasia, proliferation of reticular fibers in all atrazine treated groups except regeneration of hepatocytes in 3rd group. Kidney showed atrophy, shrinkage of glomerular tuft, hypercellularity with degeneration, disintegration of glomerular tufts, degeneration of epithelial lining of PCT and DCT in 2ndgroup. Sub acute interstitial nephritis, infiltration of lymphocytes and macrophages along with fibroplasia, glomerular degeneration, proliferation and fusion of endothelial and epithelial cells with few lymphocytes with or without marked lobulation in glomerular tuft was also reported in rest of the atrazine treated group. Heart revealed the separation of myofibers with or without hyalinization and variable degree of infiltration of lymphocytes, epicarditis in some cases, degeneration and hyalinization of purkinje fibers were noted in 2nd and 3rd group. Metaplastic changes in the wall of aorta were observed in higher dose of atrazine treated group. Lung revealed severe degeneration of epithelium lining of tertiary bronchi, marked proliferation of connective tissue with thickening of intertubular septa, consolidation of lung and pneumonitis in some cases were observed. Bursa revealed massive

degeneration in lymphoid cells with increase in reticular fibers and degeneration of epithelial lining in all atrazine treated group.

Chand (2011) observed congestion, epicardial hemorrhages, misshapen hearts and small size testes in V group whereas mild congestion was noted in III group as compared with control. Heart showed swelling of muscle fibers, separation of muscle bundle, congestion and degeneration of myocardium in higher toxic group of atrazine in III and V groups. Degeneration of seminiferous tubules with detachment of basement membrane of seminiferous tubules was seen in atrazine alone treated groups.

Pereira *et al*. (2012) revealed histological alterations of dilatation of spaces of Disse containing sinusoids, vacuolization of the cytoplasm, leakage of cytosol, karyolysis and nuclear pyknosis in liver of animals treated with atrazine group.

Chand *et al*. (2013) reported the enlargement, swollen kidney along with hemorrhages and necrotic foci. Dilation of ureters also noticed in group II. Microscopic examination of kidney from group II and III (Atrazine @ 150 and 300 mg/ kg body weight) revealed degeneration of proximal convulated tubules and distal convulated tubule along with hemorrhages and decreased Bowman's capsule space. Enlargement, swollen, rounded borders, congested and hemorrhagic was noted grossly in liver of group III whereas, microscopic finding revealed were mild congestion of central vein, swelling of hepatocytes and increase in sinusoidal space in liver from II and III toxic group.

Blahova *et al*. (2014) noted moderate to marked dystrophic change, hydropic to vacuolar degeneration, dilatation of capillaries and hyperaemia in liver. Severe multifocal lamellar teleangiectasis noted due to rupture of the retaining pillar cells and dilation of the lamellar capillary with pooling of the blood with formation of thrombi in gill samples of fish.

Liu *et al*. (2014) noted histopathological lesions of swelling of epithelial cells in juxtamedullary renal tubules in kidney of rats.

Abarikwu *et al*. (2017) recorded mild periportal congestion with cellular infiltration especially mononuclear cells and congestion in the liver of the EtoH + 50 mg/kg atrazine animals and EtoH + 100 mg/kg atrazine treated group. They observed mild portal and central venous congestion in the liver of the EtoH + 300 mg/kg atrazine exposed groups. Whereas, kidney showed mild renal cortical congestion in the EtoH + 50 mg/kg atrazine exposed group. Moreover, kidney from EtoH +100 mg/kg atrazine exposed groups reported periglomerular interstitium, infiltration and mild congestion of the renal cortex. Degeneration and presence of proteinaceous casts in the lumen of the tubules of kidney was reported in the EtoH + 300 mg/kg atrazine group.

Li *et al*. (2017) studied Lycopene (LYC) mitigates atrazine (ATR) induced cardiac inflammation via blocking the NF-jB pathway and NO production. For the experiment, male mice were divided into six groups each group containing ten (n= 10) mice. Group I served as control, group II received LYC @ 5mg/kg body weight, group III received ATR @ 50mg/kg body weight, group IV received ATR @ 200mg/kg body weight and group V received ATR @ 50 mg/kg and LYC @ 5 mg/kg body weight while group VI received ATR @ 200mg/kg body weight and LYC @ 5 mg/kg body weight for 21 days. Myocardial fiber irregular arrangement, cellular swelling and severe inflammatory reaction with mononuclear cell infiltration was recorded in heart from ATR treated group.

Michael (2018) grossly observed severe eroded gill mucosa in the gills of fish exposed to sub lethal concentrations of atrazine treated group. Histological lesions of the gill revealed sloughing off, thickening of lamella while liver recorded vacuolar degeneration, swelling of hepatocytes and necrosis in fish exposed to atrazine treated group.

Zhang *et al.* (2019) investigated the atrazine induces nephrotoxicity in Japanese quail (Coturnix C. coturnix). Two hundred male Japanese quails were divided into four equal groups and treated with 0, 50, 250 and 500 mg/kg ATR by oral route for 45 days. Kidneys of 250 and 500

mg/kg treated quail showed disordered arrangement of renal tubular epithelial cells and dilated Bowman's capsule space with partially atrophied glomerulus. Width of Bowman's capsule space was significantly increased as compared to control. The wall of renal tubules was composed of swollen, unclear columnar epithelial cells without oval or rounded basal nuclei. Renal tubular epithelial cell swelling and endoplasmic reticular degeneration alteration was noted in the kidney of group III and IV.

2.5.2 Cow urine distillate

Asma *et al*. (2006) evaluated antagonisitic effect of kamdhenu ark and its bioenhancing role against cadmium toxicity in mice (*Mus musculus*). For the experiment twenty five male mice were divided into five groups, each group contain five mice. Group I served as control, while mice of groups II, III, IV and V received a single dose of cadmium chloride (0.005mg/0.01ml intraperitoneal route) along with this, group III, IV and V received $ZnSO_4$ (100 ppm) kamdhenu ark only and kamdhenu ark + $ZnSO_4$ respectively for 60 days. On microscopic examination liver exposed to single injection of cadmium chloride up to 60 days showed severe necrotic changes. While, animals exposed with a single injection of cadmium chloride and 100 ppm of Zn up to 60 days showed less cellular damage in hepatocytes. However, the animal exposed with a single injection of cadmium chloride and 100 ppm of kamdhenu ark up to 60 days showed recovery in hepatocytes characterized by normal cellular features with prominent nuclei. Similar effects were also noticed when the animals exposed to single injection of cadmium chloride, Zn and kamdhenu ark up to 60 days revealing normalcy of hepatocytes with no cellular disintegration in comparison to cadmium chloride treated only.

Gururaja *et al*. (2009) observed the lesion of mild degree of fatty change, necrosis and lymphocyte infiltration in section of liver of rats treated with cow urine distillate as comparable to the normal control and sylimarin treated groups.

Chand (2011) observed misshapen along with congestion and epicardial hemorrhages in heart in group V and mild congestion in group III. Group V exhibited the smaller testes as compared to control. Microscopically in atrazine treated groups (III and V) showed swelling of muscle fibers, separation of muscle bundle and congestion in heart. Degeneration of myocardium in higher toxic group was also noticed in atrazine alone treated groups. Degeneration of seminiferous tubules was seen along with detachment of basement membrane of seminiferous tubules. Grossly the magnitude and severity of the lesion were less in atrazine along with cow urine ark treated group as compared to atrazine treated group only. Microscopically effect of low dose of oral atrazine was restored by cow urine ark to major extent. At higher doses of atrazine ameliorative effect of cow urine ark was noticed.

Kadagi *et al*. (2012) noted partial restoration towards normal cellular population and size of islet cells in the pancreas of rats receiving CUD along with streptozotocin. However, pancreas showed regeneration and proliferation of β-cells in rats of group IV and V (cow urine distillate treated).

Shukla *et al*. (2013) revealed marked reduction in number of crystal of urates deposition in kidney in rats receiving cow urine distillate group.

Chawda *et al*. (2014) noted histological lesions showed less fatty change, ballooning degeneration with repaired and regeneration in liver while alteration in the kidney revealed normal in cow urine ark treated group.

Mishra (2014) studied the ameliorative effect of cow and goat urine distillates against acephate toxicity in Wistar rats. One hundred and ninety two rats were randomly divided into twelve groups for subacute toxicity comprised of sixteen rats in each group (8 males and 8 females). Group I was served as control, group II and III were treated with cow and goat urine distillate @ 1mL/100g body weight by oral route respectively. Group IV, V and VI were treated with acephate @ 60, 90 and 135mg/kg body weight by oral route respectively. Group VII, VIII and XI were administered with acephate@60, 90 and 135 mg/kg body along with cow urine distillate @

1mL/100 kg body weight respectively and group X, XI and XII administered acephate @ 60, 90 and 135mg/kg body weight along with goat urine distillate @ 1mL/100 g body weight for 28 days respectively. Grossly, liver and brain showed mild congestion, kidney showed minimal and focal haemorrhages. On histopathology, liver showed focal to multifocal congestion, dilatation of sinusoidal spaces, dilatation of central vein, fatty changes, cytoplasmic rarefication and mononuclear cell infiltration. Kidneys revealed mild to moderate congestion, vacuolar, hydropic, cystic degeneration, haemorrhagic cystic degeneration, coagulative necrotic changes, hyaline cast in lumen of exposed tubules and mononuclear cell infiltration. There was lymphocyte depletion, minimal to mild congestion in spleen. Neurotoxicity was characterized by vacuolar degeneration and congestion in brain. Heart showed congestion, minimal to mild haemorrhages and fragmentation. On microscopic examination in cow and goat urine distillate treated group rats, slight to moderation improvement was found in rats of groups IX and XII while in rats of group VII, VIII, X and XI moderate degree of improvement was recorded.

Pancha (2015) observed normal histoarchitecture of spleen in CUD treated group. The sections of spleen from CUD treated group showed normal structure of white and red pulps as it was observed in normal control sections. The lymphoid follicles contain predominant small dense lymphocytes and reticular cells. In cyclophosphamide and cow urine treated group histopathological alterations and marked improvement in splenic tissue were noticed distinction between white and red pulp was observed. In spleen the white pulp showed increase in number of lymphocyte, while thymus sections revealed marked distinction between cortical and medullary zones. Hassall's corpuscles seen in medullary zones with moderate lymphocyte population in CUD treated group as compared to cyclophosphamide treated group.

Tiwari *et al*. (2016) studied ameliorative effect of CUD against imidacloprid toxicity in white leghorn cockerels. Day old fourty chicks were randomly divided into five groups each group comprised of eight cockerels. Group I was kept as control, group II and III were given imidacloprid@1/10th

and 1/20th of LD_{50} respectively and whereas group IV and V were given imidacloprid @1/10thand 1/20th of LD_{50} dose in combination with cow urine distillate @1 mL/bird/day, respectively for a period of sixty days continuously by oral route. The histopathological lesion of liver revealed degeneration, necrosis of hepatocytes and distortion of hepatic cord with haemorrhages, congestion of central vein whereas kidney showed degeneration of tubular epithelium, glomerular tuft, interstitial haemorrhages in group II and III while more or less severe intensity histopathological lesion reported in IV and V groups.

Sharma and Jain (2018) evaluated ameliorative effect of cow urine distillate against carbendazim induced toxicity in Albino rats. For the present study total of thirty six healthy two months old male Albino rats weighing around 140-150g were selected for the experiment. The rats were randomly divided into six groups, each group containing six rats. Group I served as control, group II given cow urine distillate (CUD), group III received Carbendazim @ 400mg/kg body weight by oral route, group IV received Carbendazim@400 mg/kg body weight along with CUD and group V received Carbendazim @600 mg/kg body weight by oral route while group VI received Carbendazim @ 600 mg/kg body weight along with CUD for a period of 28 days. Histopathological examination of carbendazim exposed rats showed congestion, sinusoidal dilatation, degenerative changes with vacuolation in liver, degeneration of convoluted tubules, reduction in the Bowman's space in kidney, degenerative changes in seminiferous tubules were noticed. Magnitude and severity of microscopic lesions were less pronounced in rats which received carbendazim along with cow urine distillate.

CHAPTER III

MATERIALS AND METHODS

The present study entitled, "Ameliorative effect of cow urine distillate on atrazine induced toxicity in broilers" was carried out at Department of Veterinary Pathology and Poultry Research Station, Post Graduate Institute of Veterinary and Animal Sciences, Akola. The experiment was designed to evaluate the toxicopathology of atrazine and its amelioration with cow urine distillate in broiler birds.

Eighty, a day old broiler chicks (n=80) were procured from M/s. Amruta Hatcheries Private Limited, Amravati and were acclimatized for a period of one week. These chicks were randomly divided into four equal groups comprised of twenty birds (n=20) in each group. The diet was provided to birds as per BIS 2007. From the second week onwards T1, T2, T3 and T4 group were given respective dietary treatments up to fourth week of age. However at the beginning of fifth week respective dietary treatment was withdraw and all groups were fed with normal control diet for a period of one week. The details of treatment for each group were as under:

Table 3.1. Details of experiment treatment for various groups

Group	Treatment		Period	Withdrawal period
	Atrazine (mg/kg of feed)	Cow Urine Distillate (ml/ lit once a daily)		
Control (I)	-	-	2-4 week	7 days
II	@ 250 mg/kg of feed	-	2-4 week	7 days
III	-	@ 10 ml/liter of drinking water	2-4 week	7 days
IV	@ 250mg/ kg of feed	@ 10 ml/liter of drinking water	2-4 week	7 days

3.1 IAEC approval

The Institutional Animal Ethical Committee (IAEC) of Post Graduate Institute of Veterinary and Animal Sciences, Akola (Reg. No. 312/G0/ReBi/S/2000/CPCSEA) approved the experimental protocol. The experimental protocol met the national guidelines as per the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, forest and climate changes, Government of India.

3.2 Procurement of atrazine and cow urine distillate:

3.2.1 Atrazine

Technical grade atrazine (Plate 3.1) was procured from Maharashtra Insecticide Pvt. Ltd. Midc Phase 3 and 4, MIDC, Akola Maharashtra 444104.

3.2.2 Cow urine distillate

Preparation of Cow urine distillate

Collection of Sample: Desi cow urine sample was collected from cow farm (Gorakshan/Private Owners, Akola) using sterile container and stored for further use as cow urine distillate (CUD). Preparation: Cow urine was then distilled at 100°C using distillation apparatus (Plate 3.2). The single distilled cow urine then acidified by lowering the pH below 2.0 with the addition of 85% Orthophosphoric acid. The single distilled cow urine was again distilled at 100°C using a distillation apparatus to remove ammonia. The distillate was stored in sterile glass flask at refrigerator (4°C) (Plate 3.3).

3.3 Procurement of Feed

The commercial feed was procured from Godrej Agrotech Ltd. Deepak Square, Akola, Maharashtra as per BIS (2007) guidelines.

Plate 3.1. Technical grade Atrazine (97%) used during experiment

Plate 3.2. Photograph showing distillation unit

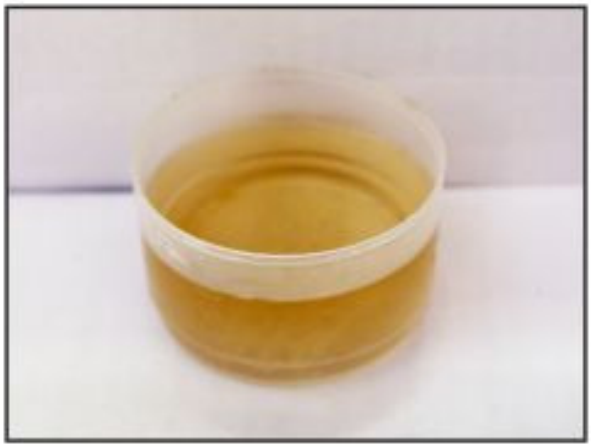


Plate 3.3. Prepared cow urine distillate

3.4 Medication and vaccination

Immediately after arrival, chicks were provided with antistress medications like electrolyte powder, vitamin mixture through drinking water. The experimental chicks were vaccinated for Ranikhet disease and Infectious Bursal Disease as per standard vaccination protocol. The details of the vaccination presented in Table 3.2.

Table 3.2. Details of the vaccination of the experimental birds

Sr. No.	Disease	Vaccine	Age	Route
1	Ranikhet disease	Lasota	7th Day	Intraocular
2	IBD	Gumboro	14th Day	Intraocular
3	IBD Booster	Gumboro	21th Day	Drinking water
4	Ranikhet disease Booster	Lasota	28th Day	Drinking water

All the birds were maintained under hygienic conditions. Feed and water was provided with *ad-libitum* and were kept under observation throughout the experimental period for 35 days. Before start of the experiment, initial body weight of individual bird from each group was recorded. Following parameters were studied during experimental period of 4th week and at 5th week (7th day PWP) of experiment (i.e. 2nd to 5th week age).

Parameters studied :

3.5 General performance

3.6 Haematological observations

3.7 Biochemical observations

3.8 Gross pathological observations

3.9 Histopathological investigations

3.10 Statistical analysis

3.5 General Performance

The general performance of birds during experiment was evaluated on the basis of clinical observations, average weekly feed

consumption, average weekly body weight and average weekly body weight gain as follows.

3.5.1 Clinical observations

Birds were observed for general condition, clinical sign and symptoms throughout the experimental period. One mortality was reported in group T2 and necropsy examination has been conducted at that time.

3.5.2 Feed consumption (g) and Feed Conversion Ratio (FCR)

During the experimental period average weekly feed consumption was recorded for each group during 1st to 5th week of age of birds and FCR was calculated by using following formula:

$$FCR = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

3.5.3 Average weekly body weights (g)

Initial body weight of birds i.e. at 0th day and after completion of 1st week of age was recorded followed by weekly body weights at the end of 2nd, 3rd, 4th and 5th week age for control and treatment group birds.

3.5.4 Average weekly body weight gain (g)

The average weekly body weight gain during 1st to 5th week age of individual bird was calculated as a difference in body weight attained at the end of week and start of that particular week.

3.6 Hematological observations

Six birds from each group were randomly selected for the collection of blood samples. Collection of blood samples were done at the end of experiment i.e. at 4th week and at 5th week (7 day PWP). Two ml of blood was collected aseptically from jugular vein by using a disposal syringe and then drawn into two vials. For haematological observations blood was collected in EDTA (Ethylene diamine tetraacetic acid) anticoagulant vial. For biochemical estimation blood clot activator vials were used. Thin blood

smears were prepared for differential leukocyte count. Hematological parameter studied included Hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Total leucocytes count (TLC) and Differential leucocytes count (DLC) were estimated on the day of collection of blood as per the standard methods described as under.

3.6.1 Hemoglobin (g/dL)

Hemoglobin (Hb) was estimated by Sahli's hemometer as per the standard method described by Benjamin (2001) and values were expressed in g/dL.

3.6.2 Packed cell volume (%)

Packed cell volume (PCV) was estimated using microhematocrit method described by Pierson (2000).

3.6.3 Total erythrocyte count (x 106 /cumm)

Total erythrocyte count (TEC) was carried out by hemocytometer and standard blood diluting pipette as this method described by Nambiar (1960) by using Natt and Harrick diluting fluid for both RBC and WBC counts.

The diluting fluid was prepared as per Natt and Harrick (1952) containing the following chemicals.

Natt and Harrick diluents

NaCl	- 3.88 g
Na_2SO4	- 2.50 g
Na_2 HPO4 H2O	- 2.91 g
KH_2 PO4	- 0.25 g
Formalin (37%)	- 7.50 ml
Methyl Violet	- 0.10 ml
Distilled water	- 100 ml

3.6.4 Erythrocyte indices

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as per standard equations mentioned by Benjamin, (2001).

a) Mean corpuscular volume (fL)

Mean corpuscular volume (MCV) was calculated by using following formula.

$$\text{MCV (fL)} = \frac{\text{PCV (\%) x 10}}{\text{RBC counts in million / cumm}}$$

b) Mean corpuscular hemoglobin (pg)

Mean corpuscular hemoglobin (MCH) was calculated by using following formula.

$$\text{MCH (pg)} = \frac{\text{Hb (g/dl) x 10}}{\text{RBC counts in million /cumm}}$$

c) Mean corpuscular hemoglobin concentration (g/dL)

Mean corpuscular hemoglobin concentration (MCHC) was calculated by using following formula.

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dl) x 100}}{\text{PCV (\%)}}$$

3.6.5 Total Leucocytes Counts (TLC) (x 103 cumm)

Total leucocyte count was estimated by using Natt and Harrick diluting fluid with improved Neubaure's chamber and method described by Natt and Herrick (1952).

3.6.6 Differential leucocyte count (DLC)

Differential Leucocyte count (DLC) was carried from the thin blood smear prepared from fresh blood at the time of blood collection. The smear was air dried and stained by Leishman's stain as described by Benjamin (2001). A total of 100 leucocytes were counted under oil immersion (100x) and were expressed in percentage.

3.7 Biochemical observations

At the end of experiment (at 4^{th} week and at 5^{th} week (7th day PWP) withdrawal period) six birds from each group were selected for blood collection, serum was separated and stored at -20^{0}C until further use. Biochemical parameters included estimation of serum Total Protein, Albumin, Globulin, BUN, Creatinine, ALT and AST using Diagnostic kits supplied by AGD Biomedicals (P) Ltd. Andheri (E) Mumbai India, by using autoanalyzer (Make AGD Biomedical Model No.AGD2020) as per standard methods as under.

3.7.1 Serum Total protein (g/dl)

Serum total protein level in each group was estimated by Biuret method (Vatzidis, 1977).

3.7.2 Serum Albumin (g/dl)

Serum albumin levels was estimated by Bromocresol Green method (Gustaffson, 1978).

3.7.3 Serum Globulin (g/dl)

Serum globulin levels were estimated as a difference between total protein and albumin for each group.

3.7.4 Serum Creatinine (mg/dl)

Serum creatinine level was estimated by modified Jaffe`s method described by Bartels *et al*. (1972).

3.7.5 Serum Blood Urea Nitrogen (mg/dL)

Serum blood urea nitrogen was estimated by using the standard method mentioned in the biochemical AGD kits and values expressed in mg/dL.

3.7.6 Serum alanine transaminase (ALT / SGPT) (IU/L)

Serum alanine transaminase (ALT) level was estimated as per UV Kinetic method-liquid (Acta, 1976).

3.7.7 Serum asparate aminotransferase (AST/ SGOT) (IU/L)

Serum asparate aminotransferase (AST) level was estimated as per UV Kinetic method-liquid (Acta, 1976).

3.8 Gross pathological examination

At the end of 4^{th} and 5^{th} week (7 day PWP) six birds from each group were randomly selected and sacrificed. A detailed necropsy examination was carried out and gross pathological lesions observed on various visceral organs were recorded.

3.9 Histopathological observations

After detailed necropsy examination tissues of heart, lung, liver, kidney, thymus, brain, sciatic nerve, bursa of fabricius and spleen were collected in 10% neutral buffer formalin solution. After fixation, tissues were processed using alcohol (Dehydration), xylene (Clearing) and impregnation in paraffin wax followed by embedding as per routine method. Block was ready for sectioning and sections of 4 to 6 µ were cut in rotary microtome and stained with H & E stain as per the method described by Luna (1968) for recording histopathological observations.

3.10 Statistical Analysis

The data obtained during present investigations was analyzed by applying Completely Randomized Design (CRD) as described by Snedecor and Cochran (1989).

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation entitled with "Ameliorative Effect of Cow Urine Distillate on Atrazine Induced Toxicity in Broilers" was carried in Department of Veterinary Pathology, PGIVAS, Akola. In the present investigation the objective was to elucidate pathological studies on the effect of atrazine on broilers and its amelioration with cow urine distillate. The trial was conducted on eighty day old broilers at Poultry Research Centre, Post graduate Institute of Veterinary and Animal Science, Akola during period of 11th November 2020 to 16th December 2020. The experimental trial was approved by Institutional Animal Ethical Committee, PGIVAS, Akola. The trial was conducted as per the guidelines given by CPCSEA, New Delhi.

Total eighty day old broiler chicks were procured from M/s. Amruta Hatcheries Pvt. Ltd., Amravati. On seventh day of housing birds were randomly divided into four groups, containing twenty birds in each group. The birds were given *ad-libitum* commercial feed and clean water free from pesticide. From eighth day of age, the birds were given dietary treatment of atrazine @ 250 mg/kg through feed from 2nd to 4th week. Cow urine distillate was given @ 10mL/lit of drinking water from 2nd week to 4th week of experiment.

All the birds were closely observed throughout the experimental period i.e. five weeks, for any clinical signs of toxicity and mortality. Body weight, body weight gain, feed consumption and feed conversion ratio was recorded weekly. At the end of 4th and 5th weeks of experiments six birds from each group were sacrificed to study hematobiochemical, gross and histopathological changes. The data obtained from the various parameters was statistically analysed and the results were presented along with discussion.

4.1 General performance

General performance of the birds was determined on the basis of clinical signs of toxicity exhibited by the bird, body weight, body weight gain, feed consumption and feed conversion ratio (FCR) on weekly during the experimental period upto 5th week of experiment.

4.1.1 Clinical observation

The first week of experiment was kept as acclimatization period, birds did not showed any untoward clinical sign, symptoms and all the birds were appeared healthy.

Throughout the experiment trial all the birds from the control group did not showed any untoward clinical signs (Plate 4.1). The birds from the T2 group fed with atrazine @ 250 mg/kg of feed given from 8th to 28th day of experiment exhibited clinical signs from 10th day of experiment. Birds exhibited clinical signs of dullness, depressed, inactive and closed eyes in second week (Plate 4.2). At 3rd week of experiment, birds revealed lower feed intake while some birds showed paralysis (Plate 4.3). Birds from T2 group showed pasty faeces and severity of the symptoms increases day by day. One mortality in T2 group was recorded at the end of 3rd week of experiment.

Wilhelms *et al*. (2005) reported the reduced feed intake in atrazine treated group in female Japanese quail while Kanth (2008) also recorded mortality and similar clinical signs in atrazine treated group in Wistar rats. Chand (2011) reported the clinical signs of dullness, reduced feed intake, ruffled feathers and diarrhoea in atrazine fed birds @ 300mg/kg bodyweight. Blahova *et al.* (2014) reported the 60% mortality in highest atrazine treated group in common carp.

Birds of T3 and T4 group did not show any abnormal clinical signs and symptoms. The present finding was in accordance with the finding recorded by Chand (2011) and Tadavi *et al.* (2017) in broiler birds in cow urine distillate alone treated group as well in pesticide and cow urine distillate combine treated group.

Plate 4.1. Birds from T1 and T3 group showing normal behaviour throughout experiment

Plate 4.2. Birds from T2 group treated with atrazine exhibited nervous symptoms like dullness, depression and closed eyes at 2nd week of age

Plate 4.3. Bird from T2 group showing paralysis at 3rd week of age

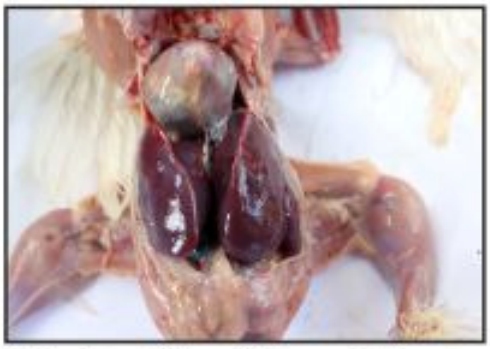


Plate 4.4. Liver (T2 group) showing enlargement with round borders and dark discolouration at 4th week of age

Plate 4.5. Kidney (T2 group) showing swelling, congestion and hemorrhages with distended ureter at 4th week of age

Plate 4.6. Heart (T2 group) showing epicardial hemorrhages at 4th week of age

During the withdrawal period (5th week) no clinical signs were observed in all treatment and control group. Literature scanned did not show any information related to withdrawal of atrazine toxicity from the feed in respect to clinical signs in broilers as well as in other species.

4.1.2 Average weekly body weight (gm)

Weekly body weights of birds were recorded from the 0th day to 5th week. The average weekly body weight (g) of birds are mentioned in Table 4.1 and respective graph represented in Fig. 4.1.

Average weekly body weights recorded at 0th day and 1st week of experiment showed non significant difference between control and treatment groups. A significant ($P<0.01$) decrease in body weight in T2 group at 2nd, 3rd and 4th week of experiment was observed as compared to control and T3 groups. The mean values were recorded as 411.85 ± 9.74 g, 817.42 ± 15.63 g and 1274.63 ± 25.69 g in 2nd, 3rd and 4th week of experiment respectively.

T3 (cow urine distillate @10 ml /lit of water) group showed significant increased in the average weekly body weight as compared to group T2 and T4 at 2nd , 3rd and 4th week of experiment. Recorded results were 436.7 ± 7.34 g, 926. 95 ± 14.87 g and 1488.95 ± 25.77 g in 2nd, 3rd and 4th week respectively. Improvement in the average weekly body weight was observes in T4 group treated with atrazine @ 250mg/kg of feed along with cow urine distillate @10 ml /lit of water as compared with (T2) atrazine treated group. This indicates the protective effect of cow urine distillate.

Similar results of significant decreased in body weight were observed by Wilhelms *et al*. (2005), Hussain *et al*. (2010) in Japanese quail, Chand (2011) in broilers, Santa *et al*. (1987), Juliani *et al*. (2008), Kanth (2008), Pereira *et al*. (2012), Nazar *et al*. (2016), Abarikwu *et al*. (2017) in Wistar rats, Elbaz *et al*. (2019) in goats during atrazine toxicity.

Results suggested that low dose of atrazine has minute negative effect on growth but reveals significant difference at higher concentration of atrazine treated group.

Table 4.1. Mean weekly body weight (g) per bird in different groups during experimental period from 0^{th} day to 5^{th} week

Groups	0 day	1^{st} Week	2^{nd} Week	3^{rd} Week	4^{th} Week	5^{th} Week	Pooled mean
T1	47.40 ± 0.08	195.05 ± 3.45	464.35 ± 7.39^{a}	932.1 ± 24.44^{a}	1522.15 ± 28.97^{a}	2217.93 ± 29.95^{a}	826.95 ± 68.56
T2	47 ± 0.79	195.75 ± 3.83	411.85 ± 9.74^{c}	817.42 ± 15.63^{b}	1274.63 ± 25.69^{b}	2033.92 ± 26.92^{b}	714.25 ± 60.99
T3	47.10 ± 0.89	195.2 ± 4.77	436.7 ± 7.34^{b}	926.95 ± 14.87^{a}	1488.95 ± 25.77^{a}	2202.64 ± 66.39^{a}	813.46 ± 68.15
T4	47.30 ± 0.72	196.1 ± 5.39	414.5 ± 9.82^{bc}	821.5 ± 20.08 ^{b}	1325.4 ± 37.55^{b}	2042.86 ± 71.38^{b}	742.95 ± 62.37
CD (0.05)	-	-	24.38	53.81	83.93	148.18	-
P value	NS	NS	**	**	**	*	NS

Mean values with common alphabet as superscript do not differ significantly

Significance levels : NS= Non Significant, *P≤ 0.05 and ** P≤ 0.01

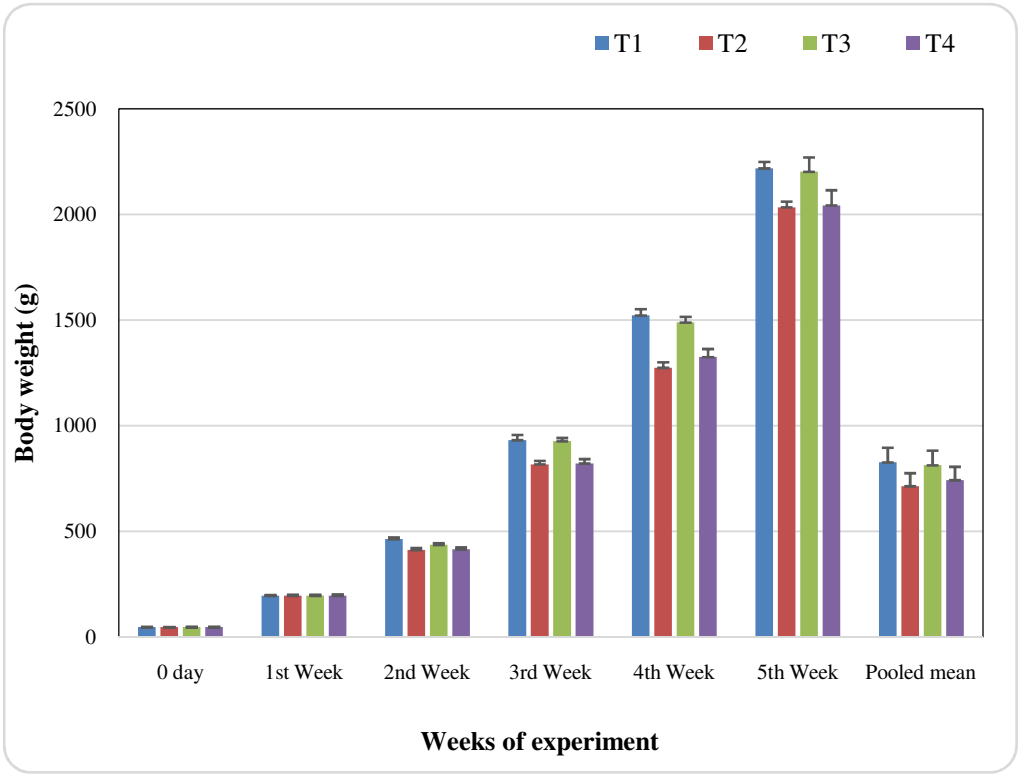


Fig. 4.1. Average weekly body weight (g) in different groups during experimental period from 0th day to 5th week

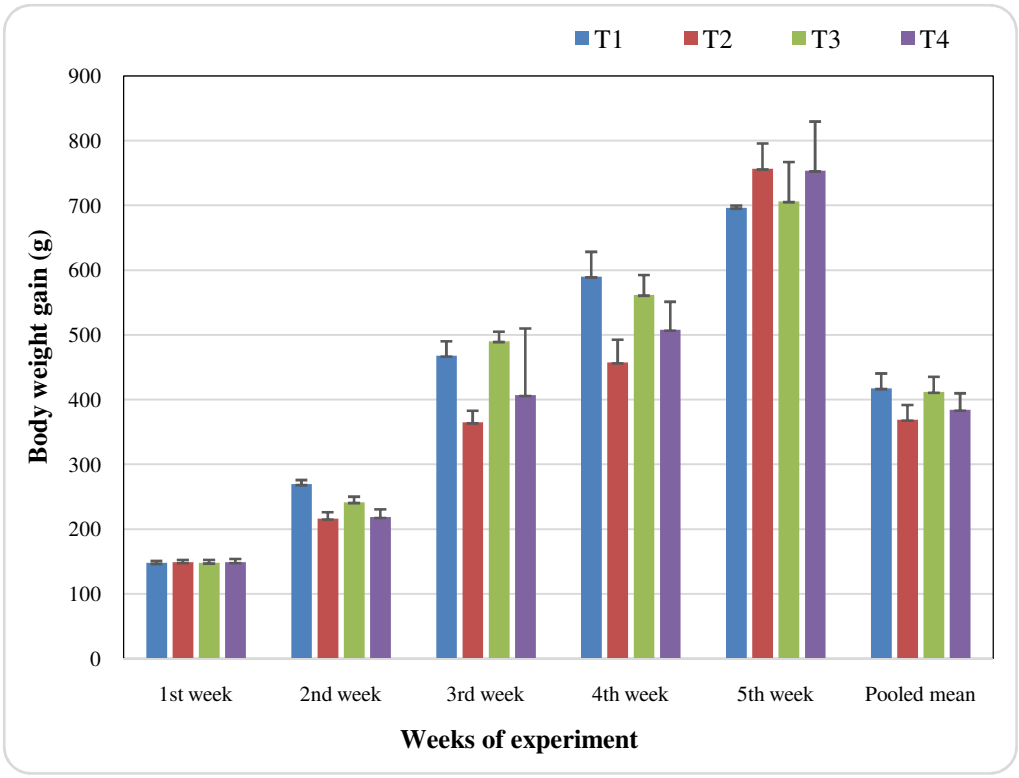


Fig. 4.2. Average weekly body weight gain (g) in different groups during experimental period 1st to 5th week

Decreased in body weight in atrazine toxicity might be partially attributed to energy restriction due to decreased feed intake and it may also be due to feed hatred (Wilhelms *et al*., 2005, Hussain *et al*., 2011).

Analogous results were also reported by Chand (2011) and Tadavi *et al.* (2017) in broilers in cow urine distillate group as an ameliorative agent against the different pesticide toxicity. Kadagi *et al*. (2012) also reported the significant increase in body weight in rats in combine group treated with cow urine distillate and streptozotocin.

Cow urine distillate has the antioxidant property hence, it act as a bioenhancer (Banga *et al*., 2005) and Chauhan *et al*. 2009 stated that cow urine enhances the immune competence leads to improve general health of individual.

At 5^{th} week of age (withdrawal period) significant difference was observed between the control and treatment groups. The T1 group showed significant difference from T2 and T4 group but non significant with the T3 group. Similarly T2 group differ significantly from T1 and T3 group but non significant difference was observed in T4 group. A non significant difference was recorded in average pooled mean values in control and other treatment groups. The average pooled mean values from different treatment groups were recorded as 826.95 ± 68.56, 714.25 ± 60.99, 813.46 ± 68.15 and 742.95 ± 62.37 in T1, T2, T3 and T4 respectively.

Literature scanned but did not find any related work done in broilers or any other species about the withdrawal period of atrazine toxicity.

4.1.3 Average weekly body weight gain (g)

Average weekly body weight gain from 1^{st} week to 5^{th} week of experiment in control and treatment groups are depicted in Table 4.2 and graph presented in Fig. 4.2.

Table 4.2. Mean weekly body weight gain (g) per bird in different groups during experimental period from 1st to 5th week

Groups	1st week	2nd week	3rd week	4th week	5th week	Pooled mean
T1	147.65 ± 3.48	269.3 ± 6.75[a]	467.75 ± 22.73[a]	590.05 ± 38.69	696.42 ± 39.80	417.50 ± 23.21
T2	148.75 ± 4.02	216.1 ± 10.29[b]	364.7 ± 18.51[b]	457.21 ± 35.68	756.76 ± 39.46	368.97 ± 23.18
T3	148.1 ± 4.57	241.5 ± 8.98[b]	490.25 ± 14.92[a]	562 ± 30.99	706.28 ± 61.13	411.97 ± 23.75
T4	148.8 ± 5.44	218.4 ± 12.51[b]	407 ± 23.08[b]	507.94 ± 43.61	753.71 ± 76.30	384.19 ± 25.89
CD (0.05)	-	27.76	56.33	_	_	_
P value	NS	**	**	NS	NS	NS

Mean values with common alphabet as superscript do not differ significantly

Significance levels : NS= Non Significant, *P≤ 0.05 and ** P≤ 0.01

Significant difference in the mean values of weekly body weight gain in the control and treatment groups were observed at 2nd and 3rd weeks of the experiment. Non significant difference was observed at 4th and 5th week.

Significant decreased in body weight gain was recorded in atrazine intoxicated T2 group as compared to T1 and T3 group. Significant increase in body weight gain was observed in T3 group. Improvement in average mean body weight was noted in T4 group when compared with T2 group at 3rd and 4th week of age. These results indicate the protective role of cow urine distillate against the atrazine induced toxicity.

Similar findings about of weekly body weight gain was also recorded by Wilhelms *et al.* (2005) and Hussain *et al*. (2010) in Japanese Quail administered with atrazine @ 250 and 500 mg/kg body weight.

At the end of 5th week (withdrawal period) numerical increase in body weight gain was observed in T2 group. Non significant difference in the average mean values of weekly body weight gain were noted at all the treatment and control group at the end of 5th week of age.

The pooled mean values of average weekly body weight gain were noted as 417.50 ± 23.21g, 368.97 ± 23.18g, 411.97 ± 23.75g and 384.19 ± 25.89 in T1, T2, T3 and T4 respectively. Non significant difference in body weight gain were recorded in all the treatment and control group.

4.1.4 Average weekly feed consumption (g)

Average weekly feed consumption (g) from 1st to 5th week is mentioned in Table 4.3 and graph represented in Fig. 4.3.

Table 4.3. Average weekly feed consumption (g) during experimental period

Groups	1st week	2nd week	3rd week	4th week	5th week	Pooled mean
T1	3781	8515	14586	19978	15803	12533.60 ± 2863.56
T2	3419	6754	12559	16536	16957	11245 ± 2688.37
T3	3017	7180	15814	16702	18202	12183 ± 2998.79
T4	3271	9252	12902	17234	15726	11677 ± 2509.98
P value	NS					

NS= Non Significant.

Numerical decrease in the average feed consumption was recorded in T2 group as compared with rest of the group at 2nd, 3rd and 4th weeks of experiment. Feed consumption was noted better in T4 group as compared to T2 group in 2nd to 4th week of experiment indicating beneficial effect of the cow urine distillate.

Whereas, the average pooled mean feed consumption were recorded non significant difference and are noted as 12533.60 ± 2863.56g, 11245 ± 2688.37g, 12183 ± 2998.79 and 11677g ± 2509.98g in T1, T2, T3 and T4 group respectively at the end of 5th weeks of experiment (7th days of PWP).

The similar finding was noted by Hussain *et al*. (2010) in Japanese Quail, Nazar *et al.* (2016) in male rats and Juliani *et al*. (2008) in female Wistar rats in atrazine treated groups. Reduction in the feed intake was supported by alteration in average body weight and average body weight gain.

Similar results of non significant differ in the feed consumption were noted by Tadavi *et al*. (2017) in cow urine treated group against chlorpyrifos toxicity indicating the beneficial effect of cow urine distillate by improving better feed consumption in broilers.

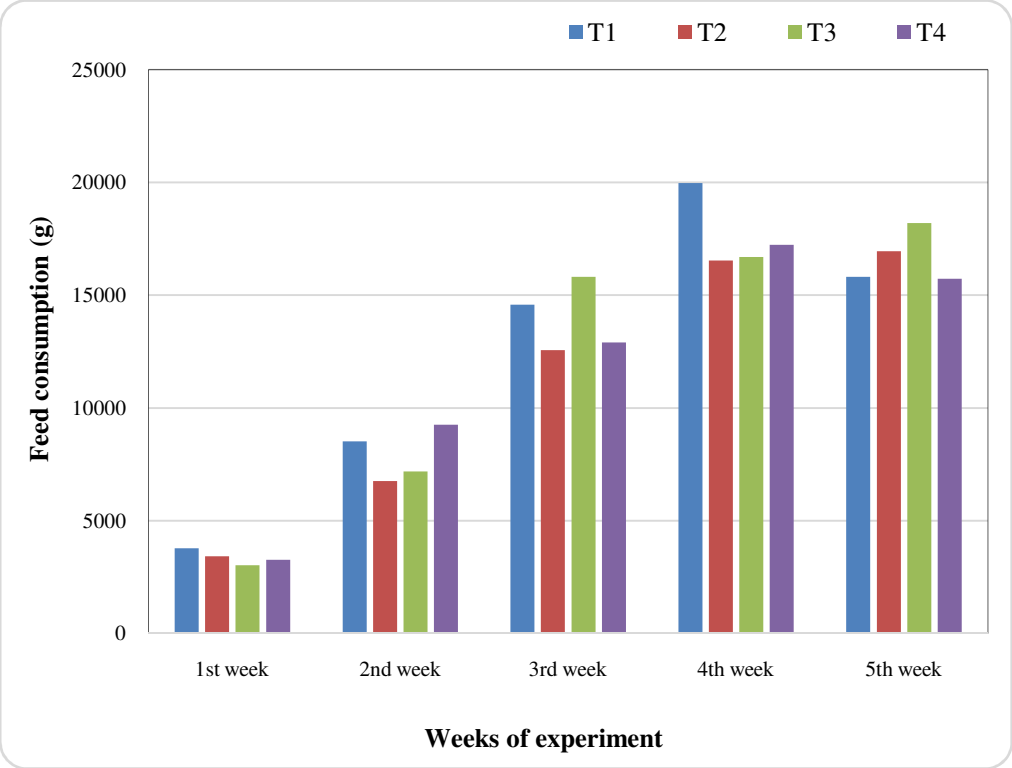


Fig. 4.3. Average weekly feed consumption (g) in different treatment groups during experimental period from 1st to 5th week

Fig. 4.4. Average weekly feed conversion ratio in different groups during experimental period from 1st to 5th week

At the end of 5th week of experiment (7th days PWP) average weekly feed consumption data recorded as 15803g, 16957g, 18202g and 15726g in T1, T2, T3 and T4 group respectively and showed non significant difference in all the group. A non significant difference was recorded in average pooled mean values from different treatment values. The recorded pooled mean values were 12533.60 ± 2863.56, 11245 ± 2688.37, 12183 ± 2998.79 and 11677 ± 2509.98 in T1, T2, T3 and T4 group respectively.

4.1.5 Feed Conversion Ratio

The calculated feed conversion ratio (FCR) in different groups during experimental period from 1st week to 5th week recorded in Table 4.4 and graph depicted in Fig. 4.4.

Table 4.4. Feed Conversion Ratio (FCR) in different groups during experimental period from 1st to 5th week

Groups	1st week	2nd week	3rd week	4th week	5th week	Pooled mean (1st to 5th week)
T1	1.28	1.58	1.56	1.69	1.62	1.45 ± 0.10
T2	1.15	1.56	1.72	1.90	1.72	1.61 ± 0.13
T3	1.02	1.49	1.61	1.49	1.84	1.49 ± 0.13
T4	1.10	2.12	1.59	1.71	1.49	1.60 ± 0.17
P value	NS					

NS= Non Significant.

The average pooled mean values of feed conversion ration were found to be non significant differ and the values recorded as 1.45 ± 0.10, 1.61 ± 0.13, 1.49 ± 0.13 and 1.60 ±0.17 in T1, T2, T3 and T4 group respectively. The numerical increase in the feed conversion ratio was noted in T2 group throughout the experiment as compared to treatment and control group.

The present finding was corroborated with the finding of Tadavi *et al*. (2017) in which improvement in values of feed conversion ratio was noted when supplemented with cow urine distillate in pesticide toxicity.

4.2 Hematological investigations/ observations

Six birds from each group were slaughtered at the end of 4th and 5th weeks of experiment and blood samples were collected from those six birds. Samples were analysed for haematology. The mean values of all haematological parameters related to erythrocyte (Hb, PCV, TEC, MCV, MCH, MCHC) are mentioned in Table 4.5 and mean values related to leucocyte mentioned in Table 4.6 and respective graphs depicted in Fig 4.5 and Fig. 4.6.

4.2.1 Hemoblobin (g/dL)

The mean values of haemoglobin (Hb) in control and treatment groups from 4th and 5th week were mentioned in Table 4.5 and graph represented in Fig 4.5 (A).

At the end of 4th week of experiment, significant differences in the mean haemoglobin concentration was observed in control and treatment groups. The haemoglobin concentration significantly decreased in T2 group (atrazine treated) when compared to T1, T3 and T4 group. Significant improvement in T4 (atrazine along with cow urine distillate treated) group was noted as compared to T2 group (atrazine treatment). The mean values of haemoglobin in T1 control (11.18 ± 0.08) and T3 cow urine distillate treated group (10.68 ± 0.20) showed non significant difference.

The present observation of decreased in the mean values of haemoglobin in atrazine toxicity group was in accordance with the finding of Chand (2011), Saquib *et al*. (2014) in broiler birds. Hussain *et al*. (2010) in Japanese quail, Ramesh *et al.* (2009) and Blahova *et al*. (2014) in common carp, El- Shenawy *et al*. (2011) in mice, Ezenwaji *et al*. (2012) in Albino rats and Michael (2018) in *Clarias gariepinus.*

Decrease in haemoglobin concentration in T2 group may be caused due to anaemia as a result of drop in erythropoiesis. Atrazine causes the renal damage which probably resulted in decreased erythropoietin

production from kidney and eventually leads to decreased in erythropoiesis synthesis (Chand, 2011).

In the present study T4 group showed significant increase in the values of haemoglobin concentration as compared to T2 group at the end of 4th week of experiment. Improvement in the values of haemoglobin indicates beneficial effect of cow urine distillate against the atrazine toxicity in broilers.

A similar result was observed by Bhatele *et al*. (2016) in cow urine distillate treated group against the mercuric chloride toxicity in chickens. Chand, (2011) and Tadavi *et al.* (2017) noted the similar results in cow urine distillate against the different pesticide toxicity in broilers.

A non significant alteration in haemoglobin concentration recorded by Nirmala, (2010), Kadagi *et al*. (2012) in Albino rats in cow urine treated groups. Significant increase in haemoglobin level were recorded by Verma *et al*. (2011) in Albino rats, Joshi and Chauhan (2013) in mice and Panicker *et al.* (2013)in white leghorn chicken in cow urine treated groups.

In the present study ameliorative effect of cow urine distillate against toxicity on haemoglobin concentration may be due to the presence of iron in traces and other haematinic component were found in cow urine (Bapu, 2001, Panicker *et al*., 2013).

At 5th week of experiment (7 days post withdrawal period) the mean values of haemoglobin were recorded as 13.96 ± 0.23, 8.68 ± 0.19, 13.15 ± 0.42 and 9.78 ± 0.15 in T1, T2, T3 and T4 groups respectively. Significant increased in the mean values of haemoglobin concentration in the T1, T3 and T4 groups as compared with the T2 group were observed at 5th weeks of experiment (7th days of PWP). The T2 and T4 group showed significant improvement in the haemoglobin levels due to restoration in the haemoglobin values towards normal levels might be due to removal of the toxic compound from the feed.

Literature scanned but no reports were found regarding the withdrawal period of atrazine in broilers or in another species.

4.2.2 Packed Cell Volume (PCV) %

Haematological values related to packed cell volume at end of 4th and 5th week of experiment mentioned in Table 4.5 and related graph represented in Fig. 4.5 (B).

In the present study, at the end of 4th week of experiment, significant decrease in mean values of packed cell volume (PCV) was observed in T2 group as compared to control and T3 groups. Non significant difference in PCV values were recorded in T1 and T3 group. The T4 group showed significant decreased in the value of PCV as compared to T1 and T3 but numerical increase was recorded as compared to T2 group. Improvement in the haematocrit level indicates the mitigating effect of cow urine distillate on atrazine toxicity.

Hussain *et al*. (2010) in Japanese quail, Chand (2011) and Saquib *et al.* (2014) found the similar result of reduction in haematocrit level in broilers in atrazine toxicity whereas, Ramesh *et al*. (2009) and Blahova *et al*. (2014) noted similar results in Common Carp (*Cyprinus carpio*). EL-Shenawy *et al*. (2011) and Ezenwaji *et al*. (2012) also found reduction in PCV level in mice and Albino rats respectively. Michael (2018) in *Clarias gariepinus* recorded decreased PCV value.

A non significant alteration in packed cell volume recorded by Nirmala, (2010), Kadagi *et al*. (2012) in Albino rats in cow urine treated groups.

In T4 group haematocrit level was significantly decreased as compare to T1 and T3 group. But numerically higher values were recorded in T2 group. These indicate the improvement in haematocrit level in cow urine distillate treated group. The improvement in haematocrit values might be due to presence of traces of iron or erythropoietin present in the cow urine distillate.

Table 4.5. Hematological values related to erythrocytes in different groups at the end of 4^{th} and 5^{th} week (n=6)

Groups	Hb (gm/dL)		PCV (%)		TEC (10^6/ mm^3)		MCV (fL)		MCH (pg)		MCHC (g/dl)	
	4^{th} week	5^{th} week	4^{th} week	5^{th} week	4^{th} week	5^{th} week	4^{th} week	5^{th} week	4^{th} week	5^{th} week	4^{th} week	5^{th} week
T1	11.18 ± 0.08^{a}	13.96 ± 0.23^{a}	33.54 ± 0.66^{a}	40.83 ± 0.95^{a}	6.01 ± 0.13^{a}	6.07 ± 0.14^{a}	57.29 ± 1.88^{b}	67.46 ± 2.70	18.65 ± 0.45^{b}	23.04 ± 0.72^{a}	32.61 ± 0.46	34.26± 0.92^{a}
T2	7.91 ± 0.26^{c}	8.68 ± 0.19^{d}	23.74 ± 1.03^{b}	26 ± 0.90^{c}	3.38 ± 0.15^{c}	4.05 ± 0.08^{c}	71.47 ± 3.43^{a}	64.19 ± 1.94	23.58 ± 1.01^{a}	21.46 ± 0.56^{ab}	33.04 ± 0.53	33.48 ± 0.62 ^{a}
T3	10.68 ± 0.20^{a}	13.15 ± 0.42^{b}	32.05 ± 0.55^{a}	39 ± 1.44^{a}	5.74 ± 0.18^{a}	6.07 ± 0.09^{a}	57.05 ± 1.62^{b}	64.17 ± 1.77	18.67 ± 0.62^{b}	21.65 ± 0.50^{a}	32.71 ± 0.27	33.76 ± 0.27^{a}
T4	8.61 ± 0.18^{b}	9.78 ± 0.15^{c}	25.84 ± 0.54^{b}	32 ± 0.86^{b}	4.68 ± 0.15^{b}	4.96 ± 0.14^{b}	57.70 ± 2.62^{b}	64.92 ± 3.64	19.20 ± 0.81^{b}	19.76 ± 0.57^{b}	33.30 ± 0.30	30.72 ± 1.17^{b}
P value	**	**	**	**	**	**	**	-	**	**	-	*
CD (0.05)	0.58	0.79	2.15	3.13	0.46	0.34	7.32	NS	2.21	1.76	NS	2.41

Value indicate mean ± S. E. mean values with common alphabate as superscript does not differ significantly.

Significance levels **P≤ 0.01, *P≤ 0.05, NS= non significant.

In 5th week of experiment (7th days of PWP) the significant decreased (P< 0.01) in the mean haematocrit level was observed in T2 and T4 group as compared with the T1 and T3 group respectively while non significant difference in haematocrit values were recorded between T2, T4 and T1, T3 group.

Literature scanned but no reports were recorded related to withdrawal of atrazine in broilers or any other species.

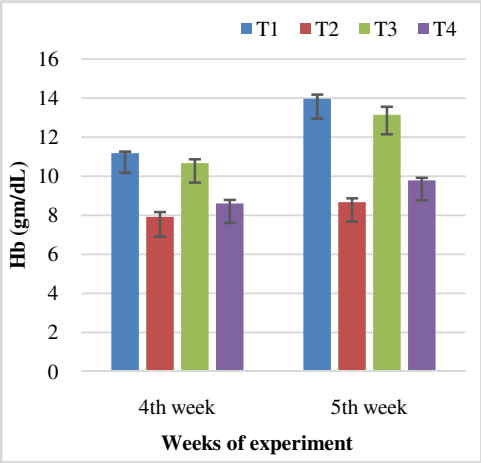
4.2.3 Total Erythrocyte Count (TEC) 10^6 /mm^3)

Total erythrocyte count at 4th week and 5th week of experiment noted in Table 4.5 and graph of the same represented in Fig. 4.5 (C).

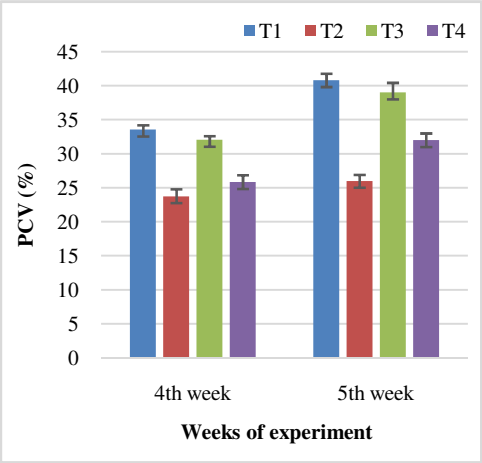
In the present investigation at the end of 4th week of experiment, the mean values of TEC in T2 group showed significant decreased (3.38 ± 0.15) as compared to T1 (6.01 ± 0.13), T3 (5.74 ± 0.18) and T4 (4.68 ± 0.15) groups. In case of T4 group reveals significant lower value in TEC count as compared to T1 and T3 group but significant increased value was recorded when compared with T2 group at the end of 4th week of experiment. T1 and T3 group showed non significant difference in TEC value.

Ramesh *et al*. (2009) recorded the decreased in TEC values in Common Carp *(Cyprinus carpio),* Ezenwaji *et al*. (2012) noted similar result in Albino rats. Chand (2011) in broiler birds, Hussain *et al*. (2012) in Japanese quail and Saquib *et al*. (2014) in broilers found reduction in total erythrocyte count in atrazine treated group.

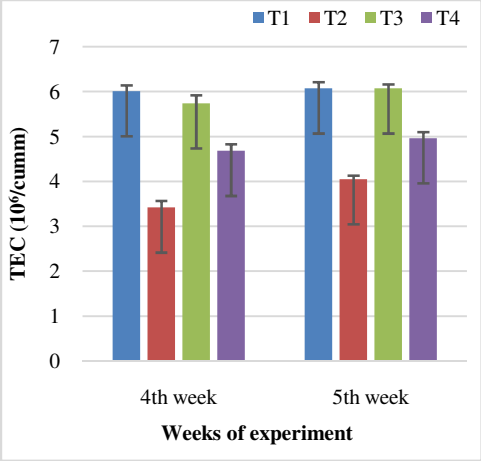
In avian animals, erythropoiesis takes place in the vascular sinuses of the bone marrow. Myelotoxic effects, as well as the production of mitotic indices and impaired heme biosynthesis in bone marrow, may cause a decrease in erythrocytes and haemoglobin concentration. This could result in a decrease in the number of TECs in the atrazine-affected population. (Hussain *et al*., 2011).



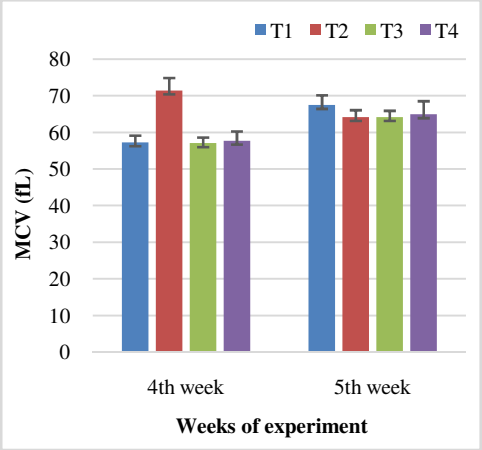
A) Mean values of Hb at the end of 4th and 5th week



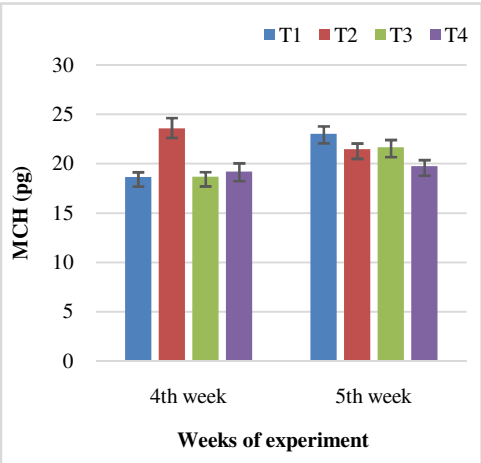
B) Mean values of PCV at the end of 4th and 5th week



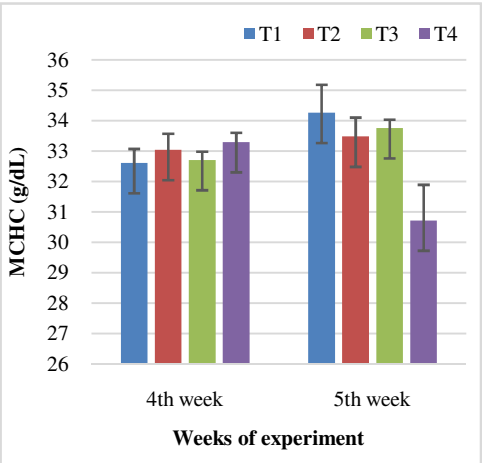
C) Mean values of TEC at the end of 4th and 5th week



D) Mean values of MCV at the end of 4th and 5th week



E) Mean values of MCH at the end of 4th and 5th week



F) Mean values of MCHC at the end of 4th and 5th week

Fig. 4.5. Hematological values related to erythrocytes in different groups at the 4th and 5th week of experiment (A to F)

In present experiment T4 group receiving cow urine distillate along with atrazine treated group showed significant higher values of total erythrocyte count, indicates the protective effect of cow urine distillate against the atrazine toxicity.

Higher values of total erythrocyte count in cow urine distillate group against other toxicity group were recorded by Chand (2011) and Tadavi *et al*. (2017) against the pesticide toxicity in broiler birds.

On the contrary Bhatele *et al*. (2016) reported the non significant difference in the values of erythrocyte count in chicks treated with CUD against mercury toxicity. A non significant alteration in total erythrocyte count was recorded by Nirmala, (2010), Kadagi *et al*. (2012) in Albino rats in cow urine treated groups. Joshi and Chauhan (2013) in mice and Panicker *et al*. (2013) in white leghorn chicken reported the significant increase total erythrocyte count in cow urine distillate treated groups.

Bapu (2001) stated that cow urine contain the erythropoietin and traces of iron which activates the bone marrow of long bone results in increases the erythropoiesis which leads to the higher values as compared to toxicity group.

At 5^{th} week of experiment (7^{th} PWP), s significant difference was recorded in total erythrocyte count in T2 group as compared with the T1, T3 and T4 group respectively. From the present finding it is suggested that for the complete withdrawal of atrazine from the body more time will required.

No reports were reported about the withdrawal period of atrazine in broilers or any other species.

4.2.4 Erythrocyte Indices

a) Mean corpuscular volume (MCV) (fL)

The mean values of MCV (fL) at the end of 4^{th} week and 5^{th} week of experiment are mentioned in Table 4.5 and graph depicted in Fig. 4.5 (D).

Significant increase in the mean values of MCV in T2 group as compared to T3, T4 and control group were noted at the end of 4th week of experiment. The mean values of MCV were recorded as 57.29 ± 1.88, 71.47 ± 3.43, 57.05 ± 1.62 and 57.70 ± 2.62 in T1, T2, T3 and T4 respectively. Non significant difference in the mean values of MCV were recorded in T1, T3 and T4 group at the end of 4th week of experiment.

Similar results of increase in MCV values in atrazine treated group was also recorded by Chand (2011) in broiler birds treated with atrazine @ 150 and 300 mg/kg body weight for 42 days.

At the end of 5th week (7th day PWP) non significant differences were observed between control and all the treatment groups. The mean values of MCV were found to be 67.46 ± 2.70, 64.19 ± 1.94, 64.17 ± 1.77 and 64.92 ± 3.64 in T1, T2, T3 and T4 group respetively.

Literature scanned but did not find any relatable data about withdrawal effect of atrazine in broilers or any other species.

b) Mean Corpuscular haemoglobin (MCH) (pg)

The mean MCH (pg) at the end of 4th week and 5th week of experiment are mentioned in Table 4.5 and graph depicted in Fig. 4.5 (E).

Significant increase in the mean values of MCH in T2 group as compared with T1, T3 and T4 group values whereas, non significant difference was noted in T1, T3 and T4 group at the end of 4th week of experiment. The mean values of MCH were 18.65 ± 0.45, 23.58 ± 1.01, 18.67 ± 0.62 and 19.20 ± 0.81 in T1, T2, T3 and T4 groups respectively.

Similar results of increase in MCH values in atrazine treated group was also recorded by Chand (2011) in broiler birds treated with atrazine @ 300 mg/kg body weight for 42 days

At the end of 5th week of experiment the mean values of MCH were recorded as 23.04 ± 0.72, 21.46 ± 0.56, 21.65 ± 0.50 and 19.76 ± 0.57 in T1, T2, T3 and T4 group respectively. Significant increase in T2 group as

compared with T1, T3 and T4 group and non significant difference in T1, T3 and T4 group was recorded at the end of 5th week of experiment.

Literature scanned but did not find any relatable data about withdrawal effect of atrazine in broilers or any other species.

c) Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dL)

The mean MCHC values at the end of 4th and 5th week of experiment were mentioned in table 4.5 and respective graph represented in Fig. 4.5 (F).

Non significant differences were observed in MCHC values in all the treatment and control group at the end of 4th week of experiment. The mean values of MCHC recorded as 32.61 ± 0.46, 33.04 ± 0.53, 32.71 ± 0.27 and 33.30 ± 0.30 in T1, T2, T3 and T4 group respectively.

Significant decrease in the values of MCHC was recorded in T4 group when compared with T1, T2 and T3 group. Whereas, non significant difference observed in T1, T2 and T3 group at the end of 5th week of experiment.

In the present study, at the end of 4th week of experiment the results observed in MCHC values were similar to Chand (2011) noted non significant changes in MCHC values in atrazine treated group broiler birds as compared to control group.

In atrazine treated birds increase in MCV and MCH indicates the macrocytic hyperchromic anaemia. This suggests the recovery phase in which bone marrows have to produce large number of erythrocyte. A significant reduction in haemoglobin, total erythrocyte count and packed cell volume levels was reported. The reason for reduction in these values were already discussed above and that might be the reason for variation in erythrocyte indices.

Literature scanned but could not find any report regarding withdrawal period of atrazine from the feed in broiler birds or any other species.

4.2.5 Total leucocyte count (TLC) ($10^3/mm^3$)

The mean values of TLC of control and other treatment groups were mentioned in Table 4.6 and respective graph represented in Fig. 4.6 (A)

At end of 4^{th} week of experiment, significant increase in the mean TLC values in T2 group as compared with the T1, T3 and T4 group were recorded. Whereas, T1 and T3 group showed the non significant difference. T4 group showed the decrease TLC value as compared to T2 group but increase value as compared to T1 and T2.

The present finding of significant increase in the TLC count in atrazine treated group also recorded by Ramesh *et al*. (2009) in Common Carp, Saquib (2009), Chand (2011) in broilers and Ezenwaji *et al*. (2012) in Albino rats in atrazine treated group.

Workers who confirmed leucocytosis believe leucocytes play a role in immunological function. The increase in these cells serves as a defensive response by increasing lymphocyte production and enhancing lymphocyte release from lymphoid tissue. According to Fink and Salibian (2005) the chemical leads to toxicity of pluripotential hematopoietic cells. This may result into a consequence of a depletion of circulating differential cells.

In T3 group non significant alteration in TLC was recorded. Similar result was also reported by Kadagi, *et al*. (2012) in cow urine treated rats. And contrary findings of increase in TLC count were reported by Joshi, *et al*. (2012) in rabbits, Joshi and Chauhan (2013) in mice, Panicker, *et al.* (2013) in white leghorn chickens and Naseema, *et al.* (2014) in Albino mice.

The present findings of T4 group reveals TLC values towards normal levels, were in accordance with the findings recorded by Chand (2011), Mishra (2014) and Pancha (2015) in CUD treatment against different toxicity. These effects might be due to bioenhancing property of cow urine distillate.

At the end of 5th week of experiment, the mean values of TLC count was recorded non significant difference among all the groups. The mean values of TLC were recorded as 12.92 ± 0.21, 13.11 ± 0.22, 12.85 ± 0.17 and 12.62 ± 0.16 in T1, T2, T3 and T4 group respectively.

No reports were recorded about the withdrawal period of atrazine in broiler birds or any other species.

4.2.6 Differential leucocyte count (DLC)

Absolute leucocyte count of different groups at the end of 4th week and 5th week of experiment mentioned in Table 4.6 and respective graph represented in Fig. 4.6 (B) to Fig. 4.6 (F).

a) Lymphocyte count (%)

The lymphocyte count of different groups at the end of 4th and 5th week were mentioned in Table 4.6 and graph represented in Fig. 4.6 (B).

Significant decrease in the mean percentage of lymphocyte was recorded in T2 (51.83 ± 1.45) atrazine treated group as compared to T1 control (65.17 ± 0.88), T3 cow urine distillate treated group (63.17 ± 2.13) and T4 atrazine along with cow urine treated group (58.50 ± 0.96). The T1 and T3 group showed non significant difference in lymphocyte count. Increase average mean values of lymphocyte count was observed in T4 group as compared with the T2 group while significant decrease mean value as compared with the T1 and T3 group at the end of 4th week of experiment.

Similar results were observed by Chand (2011) in broiler birds, Blahova *et al*. (2014) in Common Carp (*Cyprinus carpio*) and Saquib *et al.* (2014) in different dose of atrazine treated group in broiler birds.

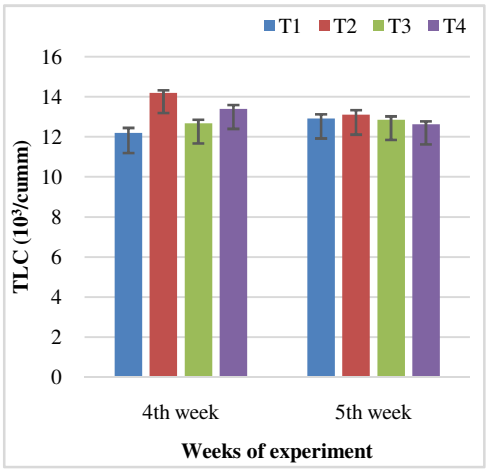
In the present study, atrazine produces stress condition in birds which further leads to secretion of corticosteroids from the adrenal gland results in lymphocytopenia.

Table 4.6. Hematological values related to leucocyte in different groups at the end of 4th and 5th week (n=6)

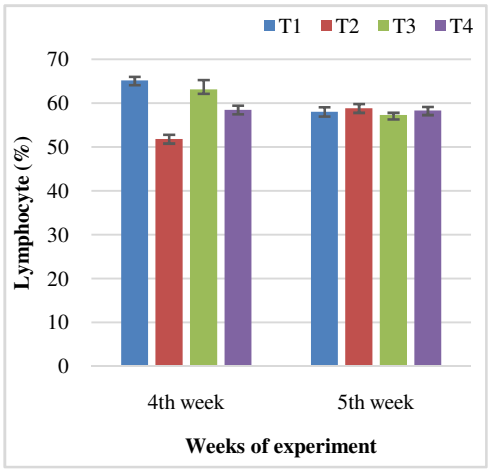
Groups	TLC (10^3/cumm)		Lymphocyte (%)		Heterophil (%)		Monocyte (%)		Eosinophil (%)		Basophil (%)	
	4th week	5th week	4th week	5th week	4th week	5th week	4th week	5th week	4th week	5th week	4th week	5th week
T1	12.19 ± 0.26^{c}	12.92 ± 0.21	65.17 ± 0.88^{a}	58 ± 1.13	35.33 ± 2.25^{bc}	38.83 ± 1.17	1.17 ± 0.22^{b}	1.5 ± 0.22	2.33 ± 0.42	1.17 ± 0.28	0.50 ± 0.22	0.5 ± 0.22
T2	14.19 ± 0.13^{a}	13.11 ± 0.22	51.83 ± 1.45^{c}	58.83 ± 0.48	41.17 ± 1.25^{a}	38.66 ± 0.71	5.17 ± 0.60^{a}	1.33 ± 0.33	1.67 ± 0.33	0.83 ± 0.37	0.17 ± 0.17	0.33 ± 0.21
T3	12.67 ± 0.18^{c}	12.85 ± 0.17	63.17 ± 2.13^{a}	57.33 ± 0.50	33 ± 1.92^{c}	39.66 ± 0.66	1.50 ± 0.34^{b}	1.5 ± 0.22	1.33 ± 0.21	1.00 ± 0.33	0.33 ± 0.21	0.5 ± 0.22
T4	13.40 ± 0.19^{b}	12.62 ± 0.16	58.50 ± 0.96^{b}	58.33 ± 0.85	38.67 ± 1.18^{ab}	39.5 ± 0.67	2.17 ± 0.31^{b}	1.16 ± 0.30	1.00 ± 0.37	0.67 ± 0.19	0.33 ± 0.21	0.33 ± 0.21
P value	**	-	**	-	*	-	*	-	-	-	-	-
CD (0.05)	0.57	NS	4.24	NS	5.04	NS	1.20	NS	NS	NS	NS	NS

Value indicate mean ± S. E. mean values with common alphabet as superscript does not differ significantly.

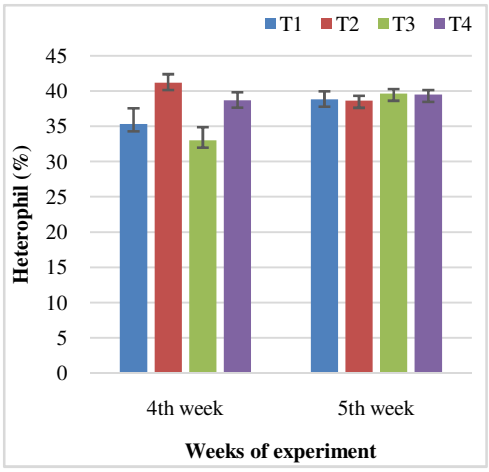
Significance levels **P≤ 0.01, *P≤ 0.05, NS= non significant



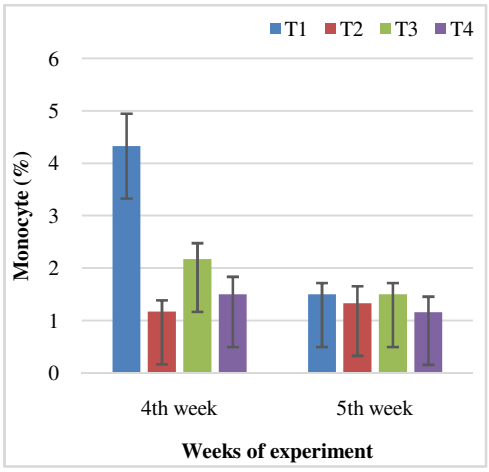
A) Mean values of TLC at the end of 4th and 5th week



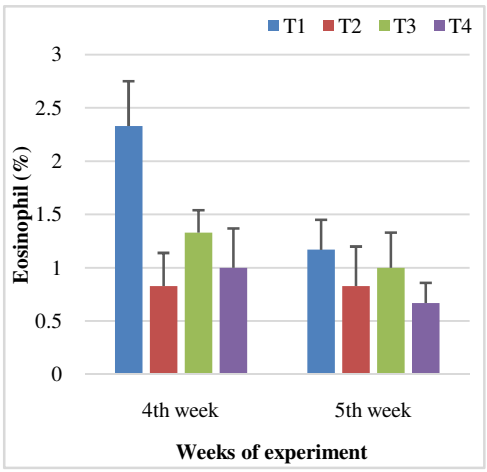
B) Lymphocyte (%) at the end of 4th and 5th week



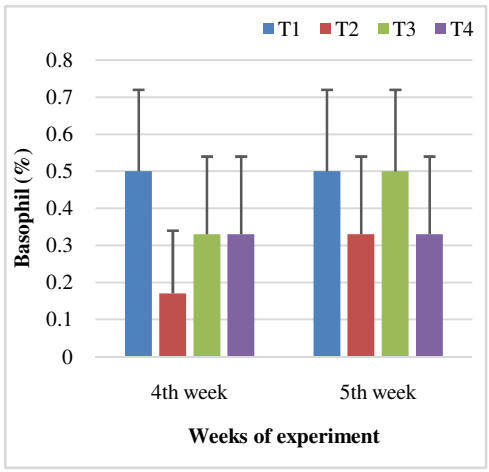
C) Heterophil (%) at the end of 4th week and 5th week



D) Monocyte (%) at the end of 4th and 5th week



E) Eosinophil (%) at the end of 4th and 5th week



F) Basophil (%) at the end of 4th and 5th week

Fig. 4.6. Hematological values related to leukocyte in different groups at the 4th and 5th week of experiment (A to F)

Similar results were observed by Chand (2011), Tadvi *et al*. (2017) in CUD treated group against the pesticide toxicity in broiler birds whereas, Naseema *et al*. (2014) and Pancha (2015) reported the immunoprotective activity of cow urine against cyclophosphamide toxicity in Swiss Albino mice.

Lymphocytic proliferation was maximum during first two weeks of life and cow urine upregulated the lymphoblastogenesis in developing stages of chicks (Kumar *et al*., 2005) and showed protective effect on lymphocytes undergoing apoptosis (Bhatele *et al*., 2016) might be the reason for the restoration of lymphocyte values towards normal values.

At the end of 5th week of experiment, non significant difference in control and other treatment group might be due to removal of toxicant from feed, which reduces the stress and restores the normal values of the lymphocytes count.

Literature scanned but did not revealed any report about the withdrawal effect of atrazine in birds or any other species.

b) Heterophil count (%)

The heterophil count of different groups at the end of 4th and 5th week were mentioned in Table 4.6 and graph represented in Fig. 4.6 (C).

At the end of 4th week of experiment, significant difference was observed in control and different treatment groups. The heterophil count were recorded as 35.33 ± 2.25, 41.17 ± 1.25, 33 ± 1.92 and 38.67 ± 1.18 in T1, T2, T3 and T4 group respectively. Significant increased in the mean values of heterophils count in T2 group was observed as compared with the T1 and T3 group. Non significant difference was noted in the mean values of heterophils count in T1 and T3 group while same finding observed in the T1 and T4 group of broiler birds at the end of 4th week. The T4 group revealed restoration towards normal levels in the values of heterophils count, suggestive of beneficial effects of the cow urine distillate group.

Similar results of significant increase in the values of heterophils were also reported by Chand (2011), Saquib *et al.* (2014) in atrazine treated broiler birds and Blahova *et al*. (2014) reported the similar results in Common Carp (*Cyprinus carpio*).

Restoration of heterophils count in cow urine distillate treated group against atrazine reveals protective effects in T4 group birds. Protective effect of cow urine distillate against various toxicities was noted by Chand (2011), Tadavi *et al.* (2017) in broiler birds, Naseema *et al*. (2014) and Pancha (2015) in mice.

At the end of 5^{th} week (7^{th} day PWP) non significant differences were observed between different treatment and control groups. The mean values of heterophils count was noted as 38.83 ± 1.17, 38.66 ± 0.71, 39.66 ± 0.66 and 39.50 ± 0.67 in T1, T2, T3 and T4 group respectively. The heterophil count was recorded towards normal levels indicates restoration. These effect might be due to removal of the atrazine toxic compound from the feed.

Literature scanned but did not revealed any relatable report about the withdrawal effect of atrazine in birds or any other species.

c) Monocyte count (%)

The monocyte count of different groups at the end of 4^{th} and 5^{th} week were mentioned in Table 4.6 and graph represented in Fig. 4.6 (D).

In the present study significant difference was observed at the end of 4^{th} week of experiment. Monocytosis was observed in T2 atrazine treated group as compared with the T1, T3 and T4 group while non significant difference noted in T1, T3 and T4 group at the end of 4^{th} week of experiment.

Similar results were observed in atrazine treated group by Chand (2011) and Saquib *et al.* (2014) in broiler birds and Blahova *et al*. (2014) reported in Common Carp (*Cyprinus carpio*).

The T4 group showed the significant alteration in the monocyte count as compared to T2 group which attributed due to administration of cow urine along with toxicant. Analogous with the present results was also reported by Chand (2011) in atrazine and cow urine distillate group in broilers.

At the end of 5th week of experiment (7th days PWP), non significant difference was noticed in all the treatment and control group. The mean values of monocyte count noted as 1.50 ± 0.22, 1.33 ± 0.33, 1.50 ± 0.22 and 1.16 ± 0.30 in T1, T2, T3 and T4 group respectively. Restoration of monocyte count towards normal range was reported in toxicity group. This effect might be due to removal of the treatment of atrazine from feed.

Literature scanned but did not revealed any result about withdrawal period of atrazine in birds or any other species.

d) Eosinophil count (%)

The eosinophil count of different groups at the end of 4th and 5th week were mentioned in Table 4.6 and graph represented in Fig. 4.6 (E).

At the end of 4th week of experiment, non significant difference in the mean values of eosinophil count were recorded in all the treatment and control group. Among these numerical decreased in the eosinophil count was recorded in T2 and T4 group as compared with the T1 and T3 group. The mean values of eosinophil were noted as 2.33 ± 0.42, 1.67 ± 0.33, 1.33 ± 0.21 and 1.00 ± 0.37 in T1, T2, T3 and T4 group respectively.

Similar finding of non significant decrease in the mean values of the eosinophil count was noted in all the treatment and control group at 4th week of experiment by Chand (2011) in broiler birds in atrazine treated groups.

At 5th week of experiment the values of mean eosinophil count were recorded as 1.17 ± 0.28, 0.83 ± 0.37, 1.00 ± 0.33 and 0.67 ± 0.19 in the T1, T2, T3 and T4 group respectively. Literature scanned but did not find any data about the residual period of atrazine in birds or any other species.

e) Basophil count (%)

The basophil count of different groups at the end of 4th and 5th week were mentioned in Table 4.6 and graph represented in Fig. 4.6 (F).

Non significant difference was observed in the mean values of basophil count in control and treatment groups at the end of 4th week. The mean values of basophil count recorded as 0.50 ± 0.22, 0.17 ± 0.17, 0.33 ± 0.21 and 0.33 ± 0.21 in T1, T2, T3 and T4 group at the end of the 4th week of experiment.

Whereas, similar finding of non significant difference was observed in the mean values of basophil count in control and treatment groups by Chand (2011) in broiler birds.

The mean values of basophil count recorded as 0.50 ± 0.22, 0.17 ± 0.17, 0.33 ± 0.21 and 0.33 ± 0.21 in T1, T2, T3 and T4 group at the end of the 5th week of experiment.

Literature scanned but did not find any relatable results about the withdrawal effect of atrazine in birds or any other species.

4.3 Biochemical observations

4.3.1 ALT (serum alanine transaminase) (IU/L)

In present experiment mean serum ALT level in different treatment are mentioned in Table 4.7 and respective graph depicted in Fig. 4.7 (A) at end of 4th week and 5th week of experiment.

At the end of 4th week of experiment, significant difference was observed in mean ALT level. The mean values of serum ALT were recorded as 11.59 ± 0.15 in T1 group, 20.28 ± 0.16 in T2 group, 10.84 ± 0.15 in T3 group and 14.75 ± 0.35 in group T4. Significant increase in the mean values of ALT in T2 group as compared to T1, T3 and T4 group whereas, T1 and T3 group showed non significant difference. The T4 group showed significant decrease value of ALT as compare with the T2 group but increase value than the T1 and T3 group. These results showed the amelioration of atrazine toxicity by the cow urine distillate.

Table 4.7. Serum ALT (IU/L), AST (IU/L), Creatinine (mg/dl), BUN (mg/dl) in different treatment groups at the end of 4th and 5th week of experiment (n=6)

Group	ALT /SGPT (IU/L)		AST/ SGOT (IU/L)		Creatinine (mg/dl)		BUN (mg/dL)	
	4th week	5th week	4th week	5th week	4th week	5th week	4th week	5th week
T1	11.59 ± 0.15^{c}	11.25 ± 0.22^{b}	175.20 ± 0.98^{c}	174.87 ± 3.92^{b}	0.50 ± 0.02^{c}	0.58 ± 0.04^{b}	11.57 ± 0.29^{c}	11.33 ± 0.23
T2	20.28 ± 0.16^{a}	12.70 ± 0.31^{a}	303.58 ± 1.22^{a}	246.77 ± 7.16^{a}	1.07 ± 0.05^{a}	0.84 ± 0.05^{a}	13.54 ± 0.20^{a}	11.56 ± 0.21
T3	10.84 ± 0.15^{d}	11.90 ± 0.19^{ab}	147.71 ± 0.92^{d}	172.55 ± 15.80^{b}	0.48 ± 0.02^{c}	0.55 ± 0.04^{b}	10.70 ± 0.18^{d}	11.03 ± 0.28
T4	14.75 ± 0.35^{b}	11.85 ± 0.39^{ab}	254.56 ± 3.72^{b}	176.74 ± 8.52^{b}	0.90 ± 0.03^{b}	0.71 ± 0.10^{ab}	12.25 ± 0.15^{b}	11.53 ± 0.32
P value	**	*	**	**	**	**	**	-
CD (0.05)	0.65	0.86	6.09	28.97	0.100	0.19	0.61	NS

Mean values with common alphabate as superscript do not differ significantly

Significance levels **P≤ 0.01, *P≤ 0.05, NS= Non significant

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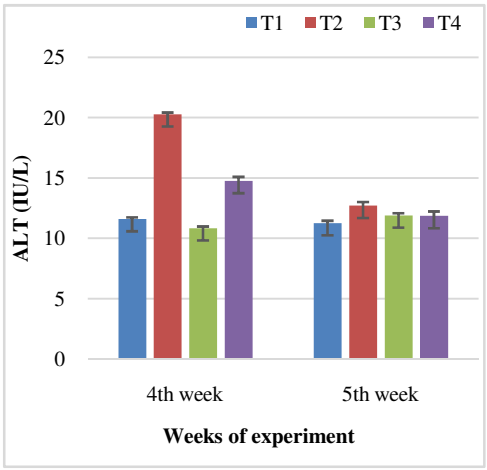
In view of the present results, similar finding of increased activity of ALT were also noted by Santa *et al*. (1987), Kanth, (2008), AL-Attabi (2012) in Wistar Albino rats, Hussain *et al*. (2012) in Japanese Quail, Chand *et al*. (2013) in broiler birds, Blahova *et al*. (2014) and Michael (2018) in Common Carp (*Cyprinus carpio*) and *Clarias gariepinus* respectively in various concentration of the atrazine treated group.

In current investigation, T3 group showed the non significant difference in ALT level when compared with control. Similar result was also recorded by Nirmala, (2010) in cow urine distillate treated rats. While, Sanganal *et al.* (2011) reported the increase in ALT level in cow urine treated rats.

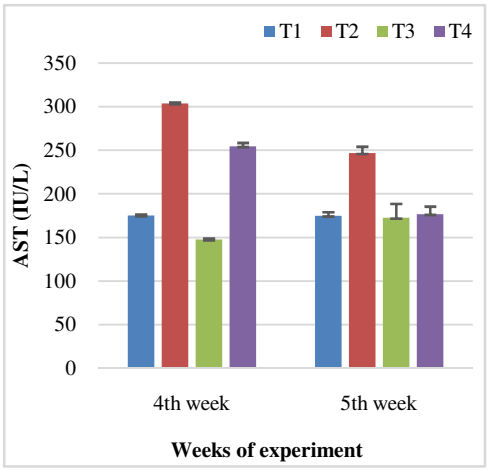
Similar results of significant reduction in the mean values of serum ALT in cow urine distillate group against atrazine toxicity were also observed by Chand (2011), Bhatele *et al*. (2016) and Tadavi *et al*. (2017) in cow urine distillate treated group against different pesticide toxicities in broilers. Gururaja *et al.* (2009) in Wistar rats also delineate that cow urine distillate decreases the level of ALT in dose dependent manner.

The mean values of ALT reveal towards normal levels in atrazine treated in combination with the CUD group in broilers attributes hepatoprotective effects of cow urine distillate due to its antioxidant property, mainly contributed by the excess production of volatile fatty acids and free radicals scavenger.

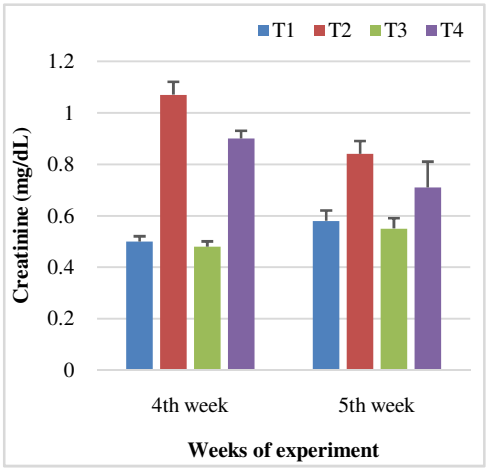
At the end of 5^{th} week of experiment, significant increase in the serum ALT values were observed in T2 group as compared with the control and other treatment groups. Non significant difference was recorded in T3 and T4 group. Numerical increase values were recorded in T3 and T4 when compared with control and numerical decrease values were recorded when compare with T2 group. The mean values of ALT were recorded as 11.25 ± 0.22 in T1 group, 12.70 ± 0.31 in T2 group, 11.90 ± 0.19 in T3 group and 11.85 ± 0.39 in T4 group. The mean values of serum ALT revealed normal levels in T4 group showed non significant difference with T1, T2 and T3



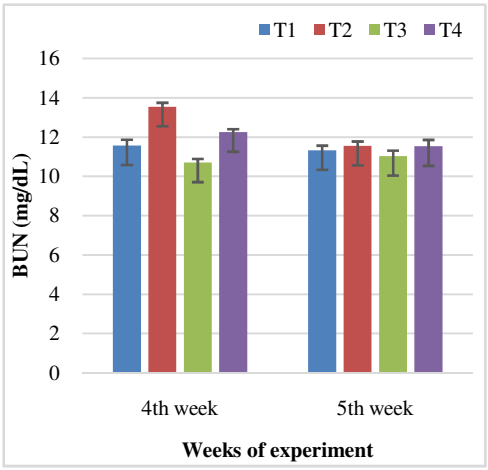
A) Serum ALT values at the end of 4th and 5th week



B) Serum AST values at the end of 4th and 5th week



C) Serum creatinine values at the end of 4th and 5th week



D) Serum BUN values at the end of 4th and 5th week

Fig. 4.7. Serum ALT (IU/L), AST (IU/L), Creatinine (mg/dl) and BUN (mg/dl) value in different treatments groups at the 4th and 5th week of experiment (A to D)

group at the end of 5th week of experiment. This could be the possible due to withdrawal of atrazine toxicity from the feed of broiler birds.

Literature scanned but could not find any report about withdrawal period of atrazine in poultry or any other species.

4.3.2 AST (serum aspartate transaminase) IU/L

The mean values of AST at the end of 4th and 5th week of experiment of different groups are mentioned in Table 4.7 and graph depicted in Fig. 4.7 (B).

At the end of 4th week of experiment, the mean values of serum AST were recorded as 175.20 ± 0.98, 303.58 ± 1.22, 147.71 ± 0.92 and 254.56 ± 3.72 in T1, T2, T3 and T4 group respectively. Significant increase in AST level in T2 followed by T4 groups as compared with the T1 and T3 group. The T4 group showed significant lower value of AST due to supplementation of CUD against atrazine treated group in broiler birds.

Similar finding of increase in serum AST values in atrazine treated group were also recorded by AL-Attabi (2012) in adult male rats, Hussain *et al*. (2012) in Japanese Quail, Chand *et al*. (2013) in broiler birds, Blahova *et al*. (2014) and Michael (2018) in Common Carp (*Cyprinus carpio*) and *Clarias gariepinus* respectively.

In the present study increase in ALT and AST might be caused due to destruction of hepatocytes which was attributed by degenerative and necrotic changes in liver. Santa *et al*. (1987) stated that liver enzymes levels were frequently increased in pesticide toxicity. Higher values indicate the elevations in the activity of enzyme generally observed in massive hepatic injury. The enzyme levels increase as a result of primary liver diseases like cirrhosis, carcinoma toxic hepatitis and obstructive jaundice (Chand *et al*. 2013).

In current investigation, T3 group showed the non significant difference in AST level when compared with control. Similar result was also recorded by Nirmala, (2010) in cow urine distillate treated rats. While,

Sanganal *et al.* (2011) reported the increase in AST level in cow urine treated rats.

The T4 group showed improved values of AST as compared to T2 group in broiler birds. Significant amelioration was noticed in cow urine distillate treated group against the atrazine toxicity.

Analogous results of decreased in serum AST values were reported by Gururaja *et al.* (2009) in Wistar rats, Chand (2011), Blahova *et al*. (2016) and Tadavi *et al*. (2017) in broilers cow urine distillate used against different pesticide toxicity. The protective effect is attributed due to the presence of antioxidant Gururaja *et al.* (2009) and superoxide scavenging properties (Shukla, *et al*., 2013) present in the cow urine distillate.

At the end of 5th week (7 days PWP), significant increased in serum AST values in T2 group as compared with the T1, T3 and T4 group and non significant difference were also noted in T1, T3 and T4 group. Restoration towards normal values of serum AST was observed in T4 group. This might be due to removal of atrazine herbicide from feed restored the serum AST levels.

Literature scanned but did not find any relatable record about the withdrawal period of atrazine in birds or any other species.

4.3.3 Serum Creatinine (mg/dL)

The mean value of creatinine at the end of 4th week and 5th week noted in Table 4.7 and graph represented in Fig. 4.7 (C).

At the end of 4th week, significant increase in the mean value of creatinine in T2 group as compared to T1, T3 and T4 group were recorded. Whereas increased in creatinine value also noted T4 group as compared with T1 and T3 group and non significant difference was recorded in T1 and T3 group. The mean values of serum creatinine were noted as 0.50 ± 0.02 in T1, 1.07 ± 0.05 in T2, 0.48 ±0.02 in T3 and 0.90 ± 0.03 in T4 group.

Chand *et al*. (2013) in broilers, AL-Attabi (2012) and Liu *et al*. (2014) noted similar finding of elevated serum creatinine levels in rats in atrazine treated group.

T3 group recorded the non significant alteration in creatinine level when compared with control. Contrary finding of increase in creatinine level was reported by Sanganal, *et al*. (2011) in cow urine treated rats.

The T4 group showed significant decrease in creatinine value as compared to T2 group. This suggested the beneficial effect of cow urine distillate against toxicity.

Parallel results of significant lower levels of serum creatinine in cow urine distillate were reported by some workers against the different toxicity recorded by Shukla *et al*. (2013) in rats, Chand, (2011) and Bhatele *et al*. (2016) in cow urine distillate treated group in birds. Beneficial effect of cow urine distillate was reported against atrazine toxicity in T4 group by restoring the level towards normal values observed in birds.

At the end of 5^{th} week of experiment (7 days PWP), significant increased in the serum creatinine levels was reported in T2 group as compared with the T1, T3 and T4 group and non significant difference noted in T1, T3 and T4 group broiler birds. After withdrawal of the atrazine toxicity from the feed the values of creatinine were towards the normal levels.

Literature scanned but did not find any data regarding the withdrawal effect of the atrazine in birds or any other species.

4.3.4 Blood Urea Nitrogen (BUN) (mg/dL)

The mean value of blood urea nitrogen (BUN) at the end of 4^{th} week and 5^{th} week noted in Table 4.7 and graph represented in Fig. 4.7 (D)

Significant increase in the concentration of blood urea nitrogen (BUN) was noticed in atrazine treated group as compare to control and other treatment group. The maximum concentration of BUN was observed in T2 group followed by T4 group as compared with the T1 and T3 group.

Analogous results of significant increased in the blood urea nitrogen against atrazine treated group was observed by Chand (2013) in broilers and Liu *et al*. (2014) in rats.

Kidney is primary excretory organ which involves in elimination of exogenous substances, drug and toxins. Various studies revealed that pesticide had high risk of end stage renal disease. Atrazine directly attacked the endoplasmic reticulum (ER) and karyotheca. Administration of atrazine leads to the swelling and depletion of ribosome and partly rupture of karyotheca. These causes the renal pathological injury. Endoplasmic reticulum is target organelle of atrazine toxicity Zhang *et al*., (2019).

T3 group recorded the non significant alteration in BUN level when compared with control. Contrary finding of increase in BUN level was reported by Sanganal, *et al*. (2011) in cow urine treated rats.

The T4 group reveals decrease concentration of blood urea nitrogen as compared to the T2 group but increase than T1 and T3. These suggested the beneficial effect of cow urine distillate against the toxicity.

Similar results of decreased in values of blood urea nitrogen concentration in cow urine distillate treated groups against various toxicities were reported by Shukla *et al*. (2013) in mice and Bhatele *et al*. (2016) in birds.

In present investigation T4 group showed the significant restored the serum creatinine and BUN level which indicates the restoration of impaired renal function. Cow urine ark has the significant antioxidant and superoxide scavenging peoperties. Positive effect on enzymes suggests its nephroprotective effect of cow urine ark (Shukla, *et al*., 2013)

A non significant difference in the mean level of blood urea nitrogen were recorded in T1, T2, T3 and T4 groups of broiler birds. The mean values of serum blood urea nitrogen levels were recorded as 11.33 ± 0.23, 11.56 ± 0.21, 11.03 ± 0.28 and 11.53 ± 0.32 in T1, T2, T3 and T4 group

respectively at the end of 5th week of the experiment. Restoration of the higher values towards the normal attributed due to withdrawal effect of toxicant from the feed of broiler birds.

Literature scanned but did not revealed any information about the withdrawal period of atrazine (7th days of PWP) in birds or any other species.

4.3.5 Total protein (gm/dL)

Average mean values of total protein at the end of 4th week and 5th week are mentioned in Table 4.8 and graph represented in Fig. 4.8 (A).

Significant difference was observed in total protein levels in control and different treatment groups. Lowest level of protein was reported in T2 group (3.13 ± 0.09) as compared with control (4.32 ±0.19), T3 group (4.06 ± 0.08) and T4 group (3.70 ± 0.14). Significant decrease in the values of total protein were noted in T2 group as compared with the T4 group while, non significant difference in the mean values of total protein was recorded in T1, T3 and T4 group at the end of 4th week of the experiment.

EL-Shenawy *et al*. (2011) in mice, Chand, (2011) in broiler and Hussain *et al*. (2012) in Japanese quail, Blahova *et al*. (2014) in Common Carp (*Cyprinus carpio*) reported the similar findings of significant decreased in the levels of total protein in atrazine treated group.

In present investigation T3 group showed the non significant difference in total protein level. Similar result also reported by Nirmala, (2010) in rats when treated with cow urine distillate. While contrary findings were reported by Jojo, (2010) in broiler chicks, Mishra and Gupta (2011) in Albino rats, Panicker, *et al*. (2013) in White leghorn chicks.

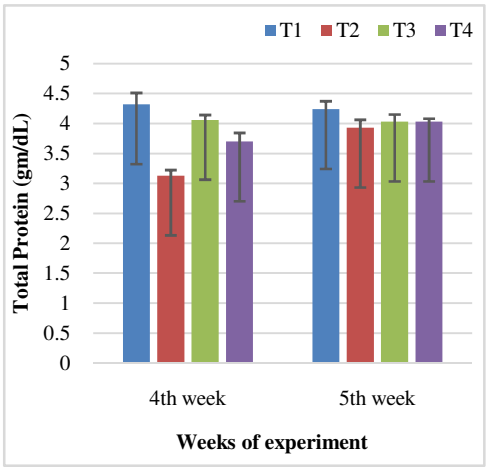
Whereas, T4 group showed the higher values of total protein than T2 group but lower values than the T1 and T3 group. This indicates the ameliorative effect of cow urine distillate on toxicity.

Table 4.8. Serum Total protein (gm/dL), Albumin (gm/dL), Globulin (gm/dL) and A:G ratio in different treatment groups at the end of 4th and 5th week of experiment (n=6)

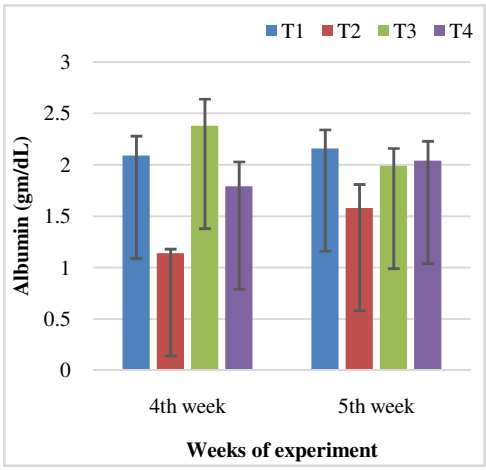
Groups	Total protein (gm/dL)		Albumin (gm/dL)		Globulin (gm/dL)		A :G ratio	
	4th week	5th week	4th week	5th week	4th week	5th week	4th week	5th week
T1	4.32 ± 0.19^{a}	4.24 ± 0.13	2.09 ± 0.19^{ab}	2.16 ± 0.18	2.23 ± 0.12	2.08 ± 0.11	0.95 ± 0.11^{b}	1.07 ± 0.12
T2	3.13 ± 0.09^{c}	3.93 ± 0.13	1.14 ± 0.04^{c}	1.58 ± 0.23	2.00 ± 0.08	2.35 ± 0.16	0.57 ± 0.03^{b}	0.72 ± 0.15
T3	4.06 ± 0.08^{ab}	4.03 ± 0.12	2.38 ± 0.26^{a}	1.99 ± 0.17	1.68 ± 0.20	2.04 ± 0.23	1.60 ± 0.31^{a}	1.08 ± 0.20
T4	3.70 ± 0.14^{b}	4.03 ± 0.05	1.79 ± 0.24^{b}	2.04 ± 0.19	1.92 ± 0.15	1.99 ± 0.18	1.02 ± 0.24^{ab}	1.13 ± 0.25
P value	**	-	**	-	-	-	*	-
CD (0.05)	0.40	NS	0.59	NS	NS	NS	0.60	NS

Mean values with common alphabet as superscript do not differ significantly

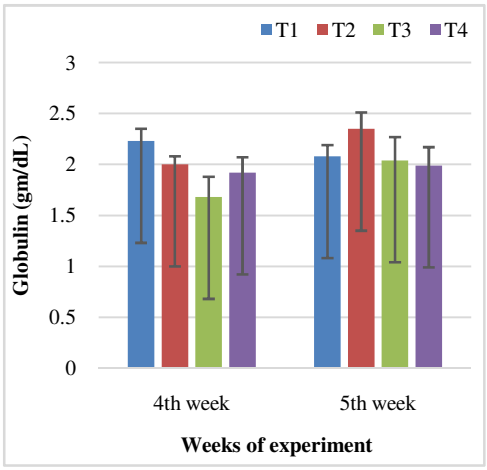
Significance levels **P≤ 0.01, *P≤ 0.05, NS= Non significant



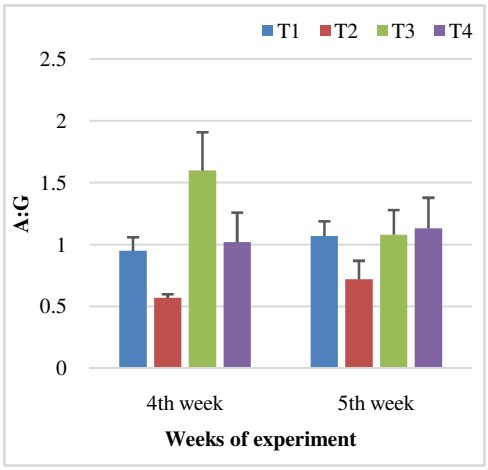
A) Serum Total Protein level at the end of 4th and 5th week



B) Serum Albumin level at the end of 4th and 5th week



C) Serum Globulin level at the end of 4th and 5th week



D) Serum A:G ratio at the end of 4th and 5th week

Fig. 4.8. Serum total protein (gm/dL), Albumin (gm/dL), Globulin (gm/dL) and A:G ratio value in different treatments groups at the 4th week age of experiment (A to D)

Similar results were observed in cow urine distillate treated group against various toxicities by Chand (2011) and Tadavi *et al*. (2017) in broilers and Pancha (2015) in mice.

At the end of 5th week of experiment a non significant difference was recorded in total protein level in control and other treatment groups. The values were 4.24 ± 0.13, 3.93 ± 0.13, 4.03 ± 0.12 and 4.03 ± 0.05 in T1, T2, T3 and T4 group respectively.

4.3.6 Serum Albumin (gm/dL)

At the end of 4th week and 5th week the albumin value of four groups was mentioned in Table 4.8 and the graph depicted in Fig. 4.8 (B).

A significant difference was reported in control and different treatment groups at the end of 4th week. Significant lower in the mean values of serum albumin were reported in T2 group as compared with the T1, T3 and T4 group whereas, non significant difference in the serum albumin levels were recorded in T1, T3 and T4 group.

Decreased in mean value of albumin in atrazine treated group was also observed by Chand (2011) in broiler, Hussain *et al*. (2012) in Japanese quail, and Blahova *et al*. (2014) in Common Carp (*Cyprinus carpio*).

In present investigation T3 group showed the non significant difference in albumin level. Similar result also reported by Nirmala, (2010) in rats when treated with cow urine distillate. While contrary findings were reported by Jojo, (2010) in broiler chicks, Mishra and Gupta (2011) in Albino rats, Panicker, *et al*. (2013) in White leghorn chicks.

However, the T4 group showed significant increased value of albumin as compared with T2 group. This indicates the beneficial effect of cow urine distillate attributed by restoring towards normal levels of serum albumin against the atrazine toxicity group.

Beneficial effects of cow urine distillate against various toxicities were also reported by Chand (2011) and Tadavi *et al*. (2017) in broilers, Pancha (2015) in mice against different toxicities.

At the end of 5th week of experiment non significant difference was detected in the mean values of serum albumin in different treatment groups at the end of 5th week of experiment. The mean values of serum albumin were recorded as 2.16 ± 0.18, 1.58 ± 0.23, 1.99 ± 0.17 and 2.04 ± 0.19 in T1, T2, T3 and T4 group respectively. Restoration of albumin values were observed in all the treatment group during the withdrawal of atrazine from the feed.

Literature scanned but did not find any data regarding the withdrawal effect of the atrazine in birds or any other species.

4.3.7 Serum Globulin (gm/dL)

The means values of globulin in control and different treatment groups at the end of 4th and 5th week of experiment are illustrated in Table 4.8 and graph depicted in Fig. 4.8 (C).

Non significant difference in the values of globulin was recorded in control and different treatment groups at the end of 4th week of experiment. The values were reported as 2.23 ± 0.12 in T1, 2 ± 0.08 in T2, 1.68 ± 0.20 in T3 and 1.92 ± 0.15 in T4 group. A non significant difference was also reported at 5th week of experiment. The values reported as 1.07 ± 0.12, 0.72 ± 0.15, 1.08 ± 0.20 and 1.13 ± 0.25 in T1, T2, T3 and T4 groups respectively.

Literature scanned but did not find any relatable data regarding the serum globulin in the atrazine toxicity in birds or any other species.

4.3.8 A:G ratio

The means values of globulin in control and different treatment groups at the end of 4th and 5th week of experiment are illustrated in Table 4.8 and graph depicted in Fig. 4.8 (D).

In the present investigation A:G ratio showed the significant difference in control and other treatment groups at the end of 4th and non significant difference at 5th week of experiment.

A significant differ in the mean values of serum albumin and globulin ratio was noted in all the treatment and control group and the values recorded were 0.95 ± 0.11, 0.57 ± 0.03, 1.60 ± 0.31 and 1.02 ± 0.24 in T1, T2, T3 and T4 group respectively at the end of 4th week of experiment.

In the present experiment reduced level of total protein and albumin was observed in atrazine treated groups. Reduction in the protein level might be due to reduce protein synthesis, nephropathy or due to liver damage. Another cause of decreased total protein and albumin is reduced feed intake with increase dose level of atrazine. Atrazine damages the gastointestinal tract which leads to anorexia, inadequate digestion or absoption which may cause the hypoproteinemia to some extent.

In T4 group treated with atrazine along with cow urine distillate curative effect of cow urine distillate was seen against the atrazine toxicity. These results might be due anabolic effect of cow urine distillate on protein metabolism

Literature scanned but did not reveal any information about the withdrawal effect of atrazine from the feed.

In the present investigation ameliorative effect of cow urine distillate on ALT, AST, creatinine, BUN, total protein, albumin and globulin was recorded against the atrazine toxicity.

4.4 Gross pathological observations

At the end of experimental period of 4th and 5th weeks (7 days withdrawal period) six birds from each group were sacrificed and detailed necropsy examination was carried out.

The birds from (T1) control group did not showed any gross pathological alterations. Liver showed the sharp borders and normal in appearence, kidney was well placed in the fossa and normal in appereance. Other visceral organs were also normal.

However, liver of birds in T2 group treated with atrazine were noted friable consistency, slight enlargement, round borders with mild

congestion, dark discolouration and hemorrhages (Plate 4.4), Distended gall bladder was noticed in atrazine treated group. Kidneys were swollen; enlarged and slight haemorrhagic while some birds showed the dilation of ureters (Plate 4.5). Heart showed epicardial hemorrhages (Plate 4.6) and hydopericardium (Plate 4.7). Generalised congestion was seen in lungs. Pinpoint haemorrhages were recorded on thymus (Plate 4.8). Spleen was swollen and enlarged in atrazine treated group.

The T3 group does not reveal any significant alteration as compared to control and other treatment groups (Plate 4.9). The borders of liver were sharp and normal in appearance. Kidney showed the normal appearance and well placed in the fossa. Other visceral organs were normal.

In T4 group, liver exhibited mild degree of congestion with pale discolouration. The magnitude and severity of the gross lesions were less pronounced in T4 group which was treated atrazine along with cow urine distillate as compared to only atrazine treated group (Plate 4.10).

In the present experiment gross lesions of liver analogous to Chand *et al*. (2013) in atrazine treated broiler birds where they found enlarged and swollen liver along with round borders. Haemorrhages and congestion was also reported. Other gross lesions observed in all the organs in the present study were comparable to Chand, (2011) and Saqib, (2009) in broiler birds. Saquib, (2009) also reported the epicardial congestion, straw coloured fluid in epicardial sac with enlarged heart in broiler birds. Enlarged and congested kidney was reported by Saquib, (2009) and Chand, (2011). Congested and reduced size of spleen was reported by Saquib, (2009) in birds

At the end of 5^{th} week (7^{th} day post withdrawal period) of experiment all the treatment groups showed the mild to moderate macroscopic pathological alterations in all organ (liver, kidney, heart, lung, thymus, bursa, spleen, brain and sciatic nerve) when compared with control group. Liver was slight congested, kidneys were slightly swollen in T2 only atrazine treated group.

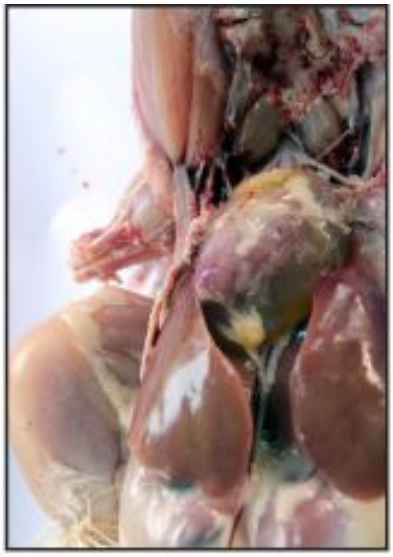


Plate 4.7. Heart (T2 group) showing hydropericardium at 4th week of age

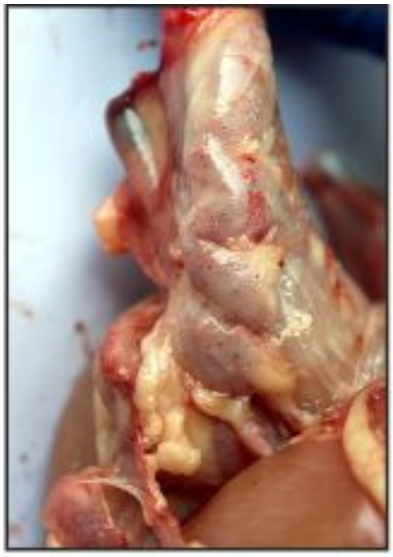


Plate 4.8. Thymus (T2 group) showing pinpoint hemorrhages at 4th week of age

Plate 4.9. Normal liver from T3 group at 4th week of age

Plate 4.10. Liver (T4 group) showing slight enlargement and slight congestion at 4th week of age

4.5 Histopathological observation

Microscopic alteration in various organs of the sacrificed birds from different groups was recorded at the end of 4^{th} and 5^{th} weeks of the experiment.

4.5.1 Liver

On microscopic examination of liver section from the control and T3 cow urine distillate treated group showed the normal histoarchitecture at both weeks of the experiment (4^{th} and 5^{th} week). Liver section from these groups revealed the polyhedral hepatocytes with large round nucleus. Normal hepatic parenchyma with normal central vein, surrounding hepatocytes and surrounded by sinusoids (Plate 4.11).

Sections from the T2 and T4 groups revealed varied degree of changes indicating adverse effect of atrazine on liver. Sections of liver from T2 group revealed the lost of histoarchitecture of the tissue, congestion of central vein, increased sinusoidal spaces with focal areas of necrosis (Plate 4.12), pyknotic nucleus (Plate 4.13) and some section showed granular and vacuolar changes with degenerative hepatocytes along with infiltration of mononuclear cells were also noticed. Bile duct hyperplasia was noted (Plate 4.14).

Section of the T4 group atrazine with cow urine distillate treated birds exhibited the congestion and infiltration of mononuclear cell with mild increased in sinusoidal dilatation (Plate 4.15), vacuolar degeneration and congestion in sinusoidal spaces (Plate 4.16) and cellular degeneration were recorded. Magnitude and severity of the lesions were less in T4 group when compared to T2 group. This suggests the reparative and regeneration process of liver tissue towards normal parenchyma due to administration of cow urine distillate.

Earlier research workers also reported the comparable lesions in section of liver in atrazine treated group recorded by Curic *et al*. (1999) in gilts, Pereira *et al*. (2012) in Wistar rats, Chand *et al*. (2013) in broiler birds,

Blahova *et al*. (2014) in Common carp, Abarikwu *et al*. (2017) in Wistar rats, Michael, (2018) in *Clarias gariepinus* . The findings suggest the liver damage when birds expose to atrazine toxicity.

At the end of 5th week of experiment (7 days of PWP), T2 group of lesions were less pronounced showing mild dilation of sinusoidal spaces, mild congestion in central vein with mild infiltration of mononuclear cells (Plate 4.17). The severity of the alterations was less after withdrawal of the toxicant from the feed. While, sections from T4 group treated with atrazine and cow urine distillate exhibited the mild congestion, sinusoidal space dilation (Plate 4.18) and normal structural integrity was maintained. These alterations indicate restorative responses, after withdrawal of the treatments from the feed. The elevated levels of liver enzymes (AST, ALT) at 5th week of experiment suggesting the mild liver damage after the removal of toxicant from feed. For complete restoration of the biochemical values and histopathological alteration more period will be required.

As atrazine administered through oral route, it absorbed into the gut and reaching directly to liver through portal circulation causing damage to liver. Adams *et al*, (1990) stated that metabolites of atrazine generate hydroxyl radicals that give dealkylated 2-chloro-s-triazine. The active oxygen radicals enter into the cytochrome p-450 mediated reactions resulting in cellular damage and leads to liver damage. These findings can be correlated with increased enzymes activities of liver in the present investigation.

In the experiment T4 group shows the lesions of less severity as compared to T2 group. Hepatoprotective role of cow urine distillate was also reported by earlier workers (Asma *et al*., 2006, Gururaja *et al*., 2009, Chand, (2011), Mishra, 2014, Tiwari *et al*., 2016, Tadavi, 2017, Sharma and Jain, 2018) against the different toxicities in different species. These might be due to presence of antioxidant in the cow urine distillate. These antioxidant scavange the free radical and act as a hepatoprotective against the toxicity.

Literature scanned but did not find any relatable results regarding the withdrawal effect of atrazine in birds or any other species.

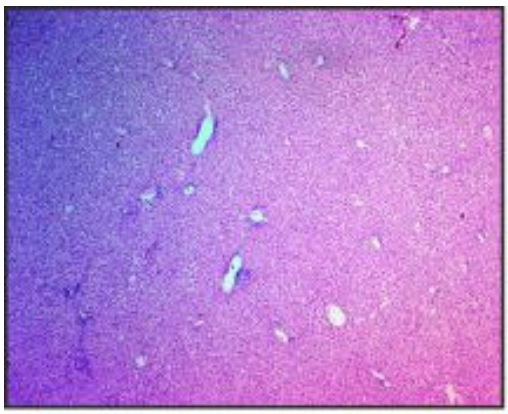


Plate 4.11. Liver (T1) showing normal histoarchitecture at 4th week (H & E × 40)

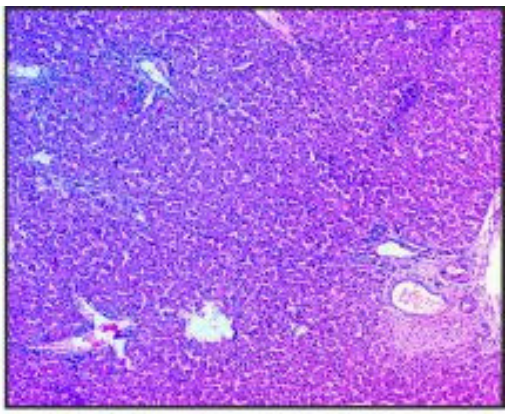


Plate 4.12. Liver (T2) showing dilation of sinusoidal with focal areas of necrosis, mononuclear cell infiltration and loss of normal parenchyma at 4th week (H & E × 100)

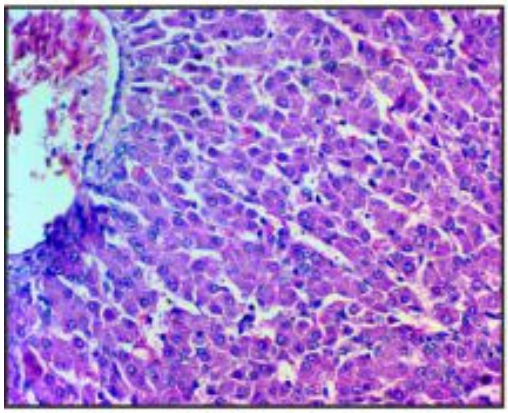


Plate 4.13. Liver (T2) showing pyknotic nuclei, degenerative changes, extensive increase in sinusoidal spaces with congestion and loss of parenchyma at 4th week (H & E × 400)

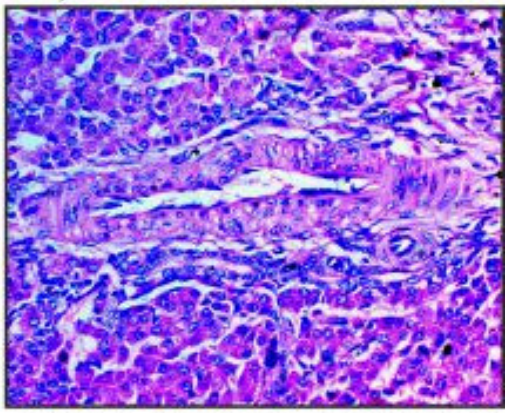


Plate 4.14. Liver (T2) showing bile duct hyperplasia with vacuolar degenerative changes in nucleus, loss of histoarchitecture at 4th week (H & E × 400)

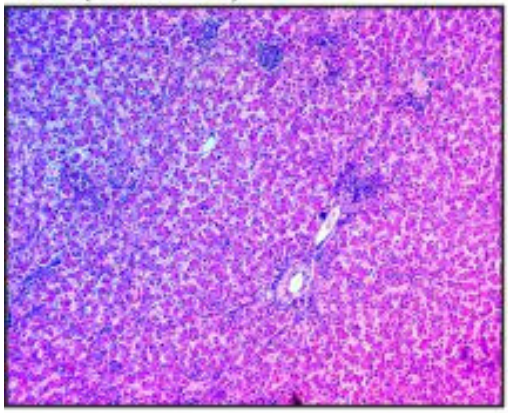


Plate 4.15. Liver (T4) showing increase in sinusoidal spaces with infiltration of mononuclear cells and degenerative changes at 4th week (H & E × 100)

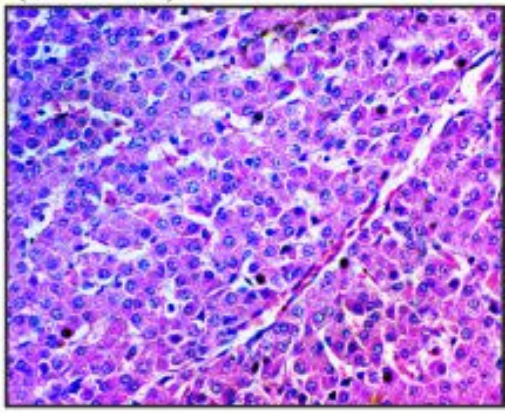


Plate 4.16. Liver (T4) showing vacuolar degeneration, increase in sinusoidal spaces with congestion in sinusoidal spaces at 4th week (H & E × 400)

4.5.2 Kidney

Sections of kidney from control group showed normal histoarchitecture at the end of 4th week and 5th week of experiment (Plate 4.19). The renal parenchyma was divided into lobules the outer cortical region and the inner medullary region. The glomeruli were of normal size and shape. Normal tubule with normal tubular epithelium and normal renal parenchyma was observed.

At the end of 4th week of experiment, sections of the kidney from T2 group exhibited shrunken and degeneration of glomeruli, desquamation of lining epithelium of tubules along with focal areas of necrosis, interstitial haemorrhages, infiltration of mononuclear cells (Plate 4.20), appearance of protein casts in lumen (Plate 4.21), increased in glomerular cellularity with increase bowman's capsule space (Plate 4.22) with loss of parenchyma (Plate 4.22).

The T3 cow urine treated group showed the mild intertubular congestion. The parenchyma was normal with normal tubular epithelium. The T4 group treated with atrazine and cow urine distillate consists of mild to moderate degenerative changes in glomerular and tubular epithelium along with congestion in interstitial tissue (Plate 4.24), varying size of glomeruli and degeneration (Plate 4.25) with normal renal parenchyma. From the current investigation findings it can be concluded that cow urine distillate showed the nephroprotective role against the atrazine induced toxicity.

Similar findings in kidney of atrazine treated group were also noted by Curic *et al*. (1999) in gilts, Saquib, (2008) in broiler, Chand *et al*. (2013) in broiler birds, Abarikwu *et al*. (2017) in Wistar rats when treated with atrazine at different doses. The histopathological alteration observed in kidney due to feeding of atrazine were accompanied with increased in the serum creatinine and blood urea nitrogen (BUN).

However, Mishra, (2014) and Tadavi, (2017) reported restoration of the histopathological alterations in kidney of rats and broiler

birds respectively when intoxicated with different toxicity and amelioration done with cow urine distillate.

At the end of 5th week of experiment section from the group T2 (atrazine @ 250 mg/kg of feed) revealed the mild congestion, mild granular and vacuolar degeneration in tubules (Plate 4.26) with interstitial hemorrhages. Sections of kidney from T3 group exhibited the normal histoarchitecture. Kidney from T4 group showed the mild degenerative changes in glomeruli with interstitial haemorrhages indicative of restoration towards normal renal parenchyma. The microscopic changes can be correlated with biochemical profile indicating the restorative effect compared with 4th week. The serum BUN and creatinine values suggest the restoration towards normal. The microscopic and biochemical investigation at 5th week indicate the presence of mild kidney damage. For complete restoration more period will be require.

The literature was scanned, but no relevant findings about the withdrawal effect of atrazine in birds or other species were found.

4.5.3 Heart

On microscopic examination, heart section from the control and T3 cow urine distillate treatment groups birds revealed the normal histoarchitecture at the end of 4th week and 5th week of experiment (Plate 4.27). The heart was showed the normal cardiac muscle fibers, normal cross striations with prominent nuclei.

At the end of 4th week of experiment, section of heart from T2 group revealed congestion of the vessels, degeneration, separation of myocardial fibers (Plate 4.28), infiltration of mononuclear cells accompanied with muscular hemorrhages, degenerative changes in nuclei (Plate 4.29) and focal necrosis was noted. Heart from T4 group exhibited mild to moderate separation of muscle fibers with mild mononuclear cells infiltration (Plate 4.30). The lesions were less severe as compared to T2 group indicating reparative quality of cow urine distillate on cardiac muscle fiber.

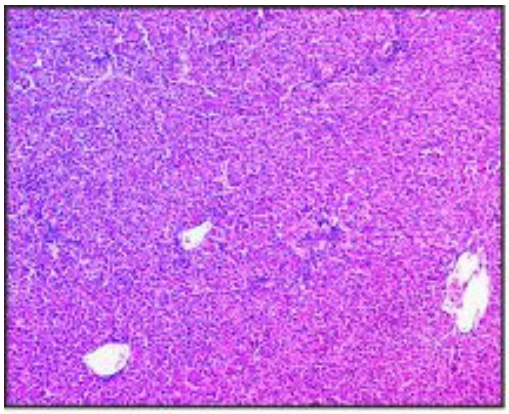


Plate 4.17. Liver (T2) showing mild dilation of sinusoid spaces and monocyte infiltration at 5th week (H & E × 100)

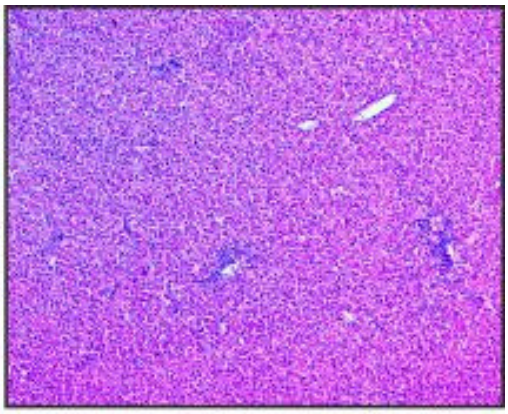


Plate 4.18. Liver (T4) showing mild increase in sinusoidal spaces with mild infiltration of mononuclear cells restoration towards normal at 5th week (H & E × 100)

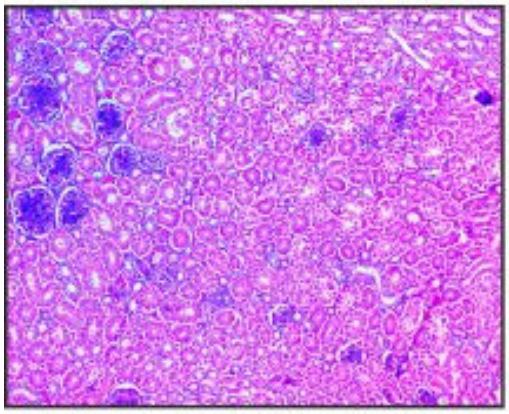


Plate 4.19. Kidney from control (T1) group showing normal histoarchitecture except mild swelling at 4th week (H & E × 100)

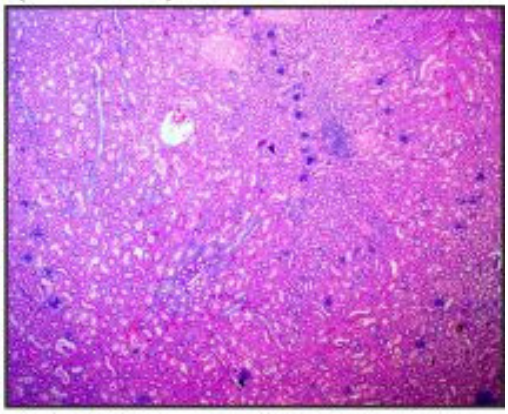


Plate 4.20. Kidney (T2) showing disruption of epithelium, focal areas of necrosis, degenerating glomeruli and interstitial haemorrhage at 4th week (H & E × 40)

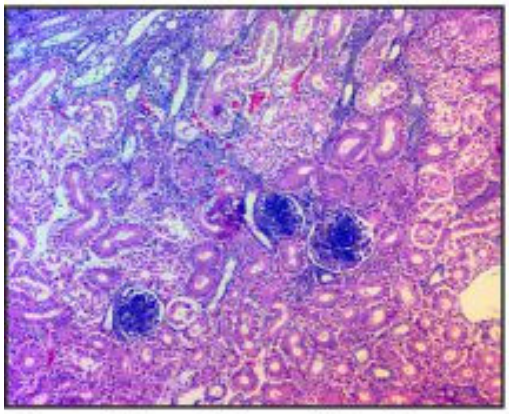


Plate 4.21. Kidney (T2) showing desquamation of epithelial lining, protein casts in lumen, interstitial hemorrhage with lost of histoarchitecture at 4th week (H & E × 100)

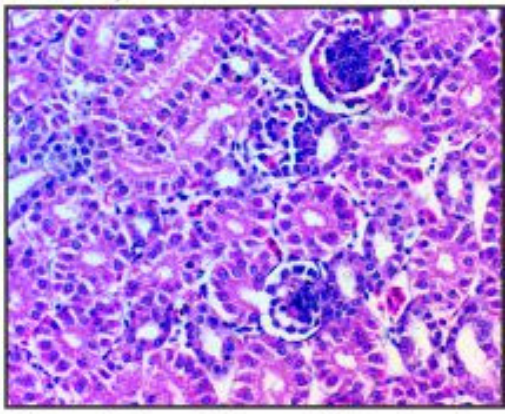


Plate 4.22. Kidney (T2) showing increase cellularity of glomeruli, increase bowman's capsule space and disrupted epithelium with congestion at 4th week (H & E × 400)

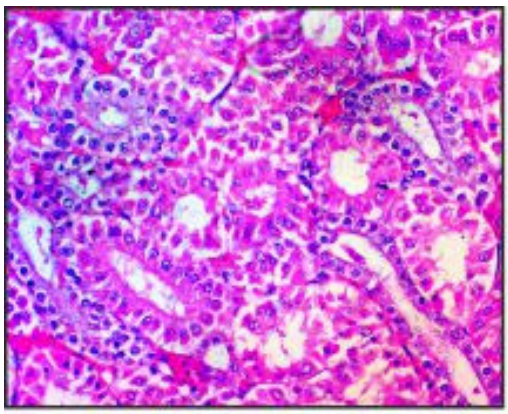


Plate 4.23. Kidney (T2) showing degenerative changes in nuclei and disruption of tubular epithelium at 4th week (H & E × 400)

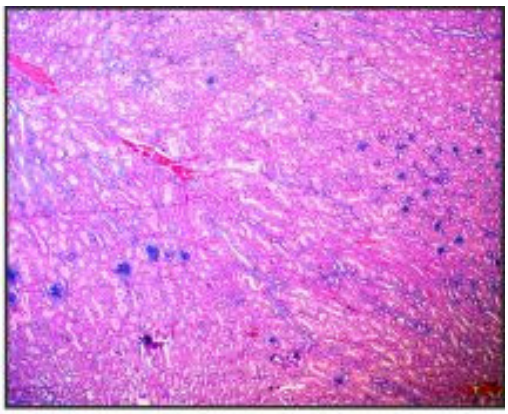


Plate 4.24. Kidney (T4) showing degenerative glomeruli with interstitial congestion at 4th week (H & E × 40)

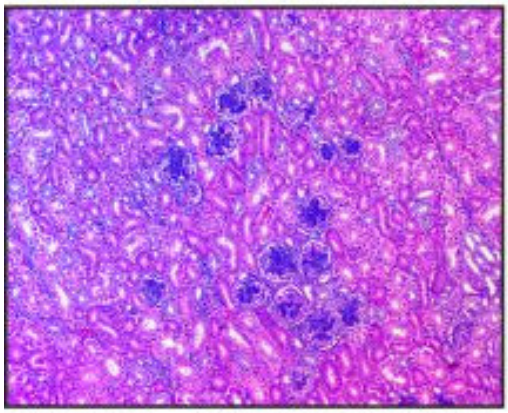


Plate 4.25. Kidney (T4) showing pleomorphic glomeruli with comparatively normal parenchyma compared to T2 group at 4th week (H & E × 100)

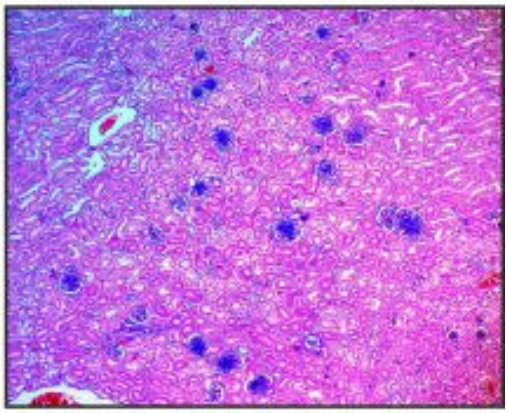


Plate 4.26. Kidney (T2) showing mild congestion and mild degenerative changes in tubules restoration towards normal at 5th week (H & E × 40)

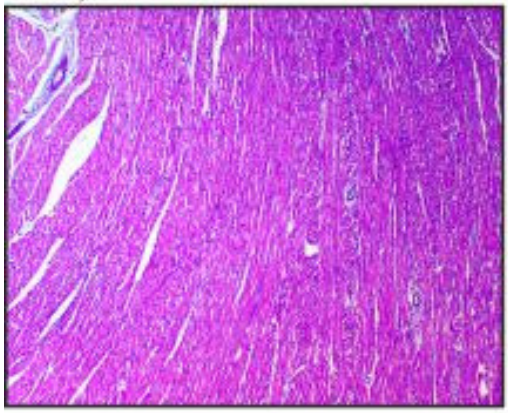


Plate 4.27. Heart from control (T1) showing normal histoarchitecture of cardiac muscle fibres at 4th week (H & E × 40)

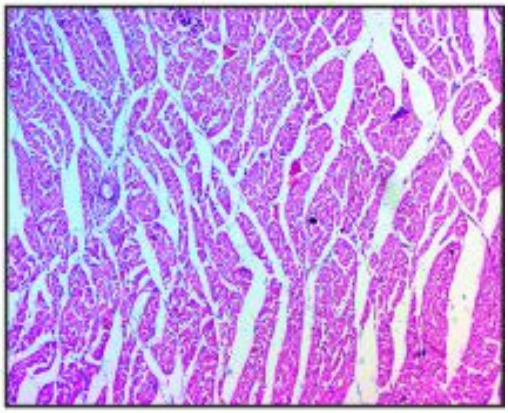


Plate 4.28. Heart (T2) showing disordered and separation of muscle fibres infiltration of mononuclear cells at 4th week (H & E × 100)

Curic *et al*. (1999) in gilts, Kanth, (2008) in Wistar rats, Saquib (2009) and Chand (2011) in boiler birds and Li *et al*. (2017) in mice observed the similar results in various levels of atrazine treated group.

At the end of 5th week of experiment sections of heart from T2 group were less pronounced when compared with 4th week. Mild hemorrhages with infiltrating cells and mild separation of muscles fibers reported in T2 group (Plate 4.31). The T4 group showed the mild myocardial hemorrhages with infiltration of mononuclear cells. Alterations in heart at 5th week indicate the restoration towards normal after withdrawal of the toxicant. The literature was scanned, but no relevant findings about the withdrawal effect of atrazine in birds or any other species were found.

4.5.4 Lung

Sections from the lung of control and T3 groups revealed normal parenchyma at the end of 4th week and 5th week of experiment group (Plate 4.32). The bronchi and bronchiole were normal, alveoli filled with air and interlobular septa was thin.

At the end of 4th week of experiment, section of lung from T2 group revealed congestion in interstitial tissue and in vessel, desquamation of bronchial epithelium (Plate 4.33) with infiltration of mononuclear cells, the rupture of alveolar wall, thin alveolar wall, thickened interlobular septa, emphysema and severe congestion all over the lung (Plate 4.34) and disruption of bronchial epithelium was evident.

The T4 group showed mild focal oedema, ruptured alveloli, thick interlobular septa with infiltration of mononuclear cells and vessel congestion (Plate 4.35). The lesions were noted less severe as compared to T2 group.

In the present experiment, findings from sections of lung were correlated with the finding recorded by Saquib, (2008) in broiler birds treated with atrazine at different dose rates.

Similar ameliorative effect of cow urine distillate (T4) on the lung parenchyma was also reported by Mishra, 2014 and Tadavi, 2017 against the different pesticide intoxication.

At the end of 5th week of experiment, sections of lung from T2 (Plate 4.36) and T4 group (Plate 4.37) showed mild congestion with restoration towards normal lung parenchyma indicating withdrawal effect of the atrazine toxicity from the feed. The literature was scanned, but no relevant findings about the withdrawal effect of atrazine in birds or any other species were found.

4.5.5 Brain

At the end of 4th week and 5th week of experiment section of brain from group T1 (Plate 4.38) and T3 revealed the normal parenchyma. Sections showed the well distinguished grey matter and white matter with normal neuronal architecture.

At the end of 4th week of experiment, histopathological findings in brain of T2 group reveals swelling of neurons, vacuolar changes in brain matrix (Plate 4.39), multifocal necrosis, satellitosis, mild increase in Virchow-Robin spaces with congestion and degenerative changes in neurons (Plate 4.40). Some sections of T4 group revealed the necrotic changes, swelling of neurons and mild degenerative changes (Plate 4.41) was noted.

The present investigation findings were in accordance with Kanth (2008) in Wistar rats treated with atrazine at different doses. The metabolites of atrazine are toxic and have been able to cross the blood brain barrier which leads to brain damage and produce lesions in the present study.

The T4 groups recorded lesions was in accordance with the finding of Mishra, (2014) and Tadavi, (2017) in cow urine distillate treated group showed the ameliorative effect against the different pesticide toxicity in Wistar rats and broiler birds.

At the end of 5th week of experiment, brain of different treatment and control groups revealed mild degenerative changes in neurons

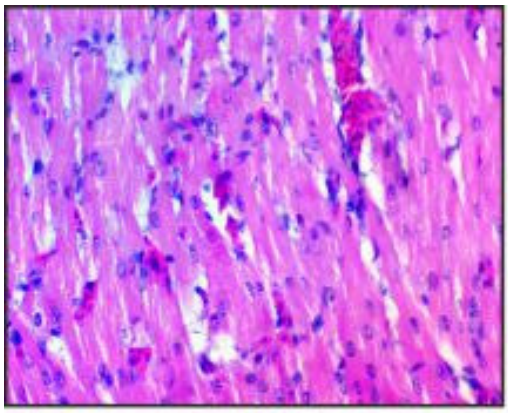


Plate 4.29. Heart (T2) showing degeneration of nuclei, hemorrhages with loss of striation at 4th week (H & E × 400)

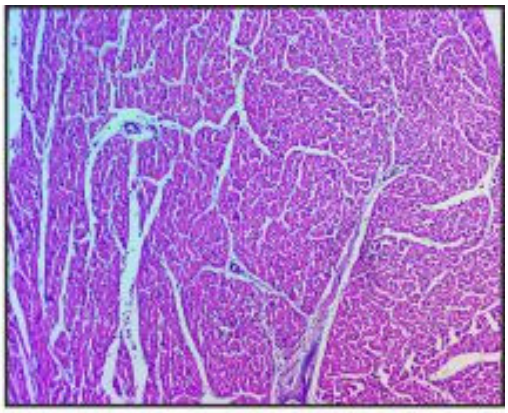


Plate 4.30. Heart (T4) showing moderate separation of muscle fibres with mild mononuclear cell infiltration at 4th week (H & E × 100)

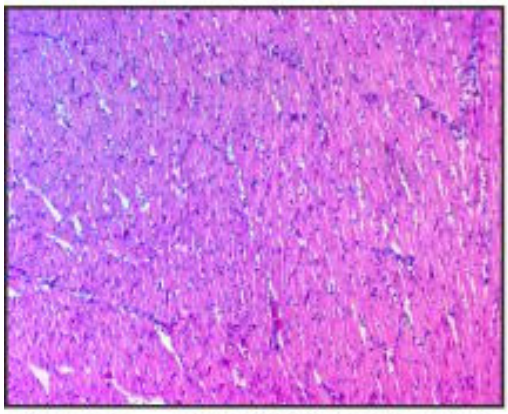


Plate 4.31. Heart (T2) showing mild separation of fibres with infiltration of mononuclear cells at 5th week (H & E × 100)

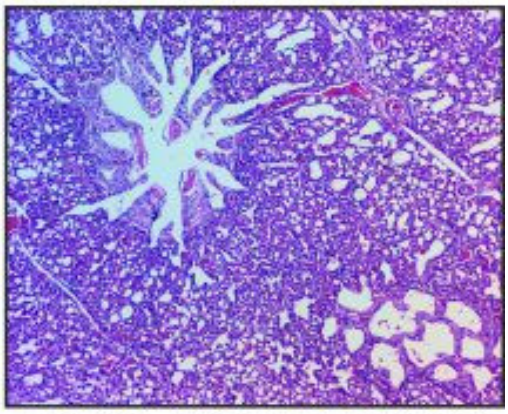


Plate 4.32. Lung (T1 group) showing normal parenchyma at 4th week (H & E × 40)

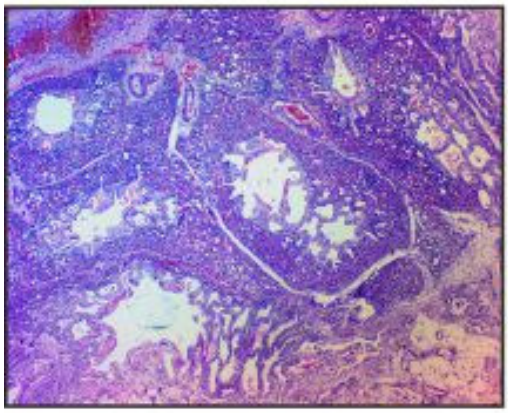


Plate 4.33. Lung (T2) showing congestion in vessel and interstitial tissue, rupture of alveoli with desquamation of bronchial epithelium and loss of parenchyma at 4th week (H & E × 40)

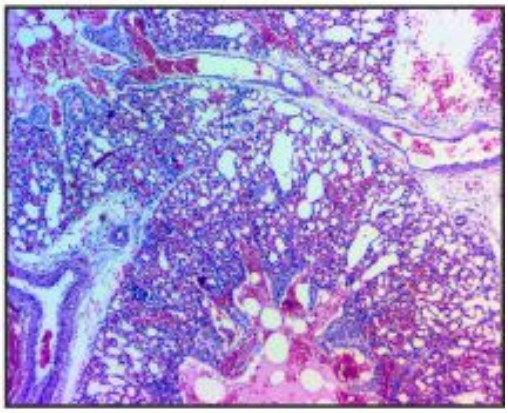


Plate 4.34. Lung (T2) showing severe congestion thickened interlobular septa, emphysema, rupture of alveoli with monocyte infiltration at 4th week (H & E × 100)

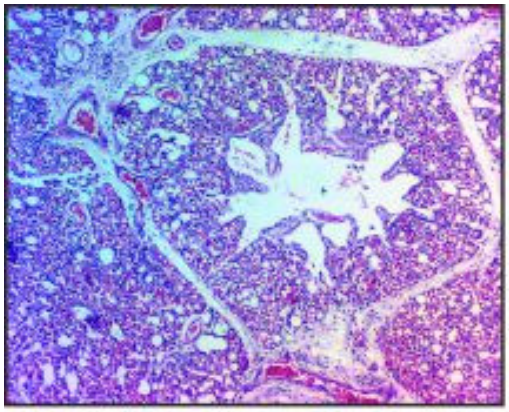


Plate 4.35. Lung (T4) showing ruptured alveloli, thick interlobular septa with infiltration of mononuclear cells and vessel congestion at 4th week (H & E × 40)

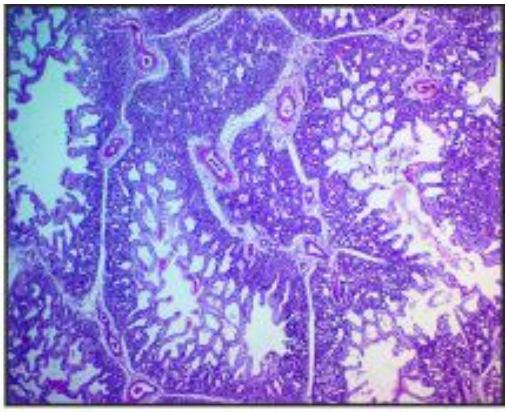


Plate 4.36. Lung (T2) showing mild congestion and hemorrhages with normal parenchyma suggesting towards restoration at 5th week (H & E × 40)

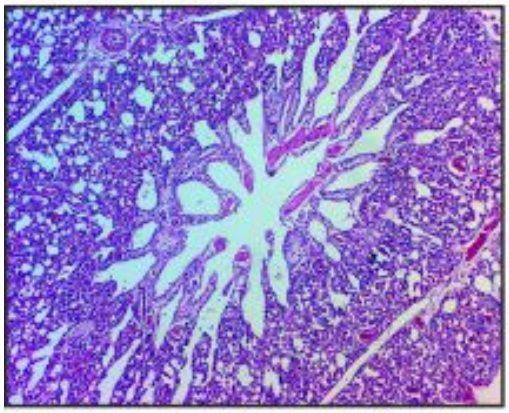


Plate 4.37. Lung (T4) showing normal parenchyma indicating the restoration of lung parenchyma towards normal at 5th week (H & E × 100)

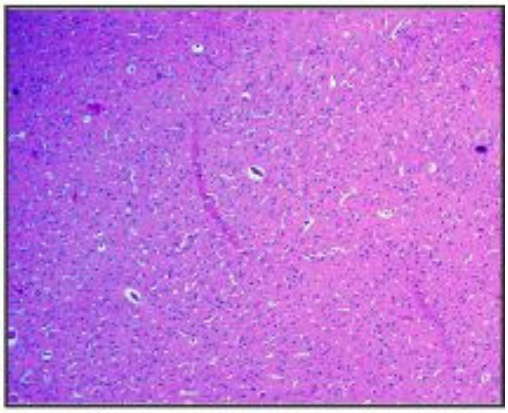


Plate 4.38. Brain from control (T1) group showing normal histoarchitecture at 4th week (H & E × 40)

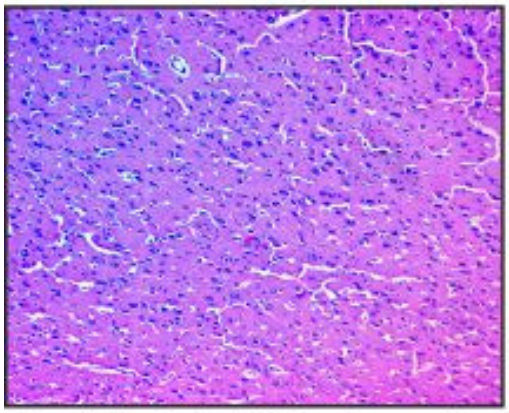


Plate 4.39. Brain (T2) showing swelling of neurons, vacuolar degenerative changes in neurons at 4th week (H & E × 100)

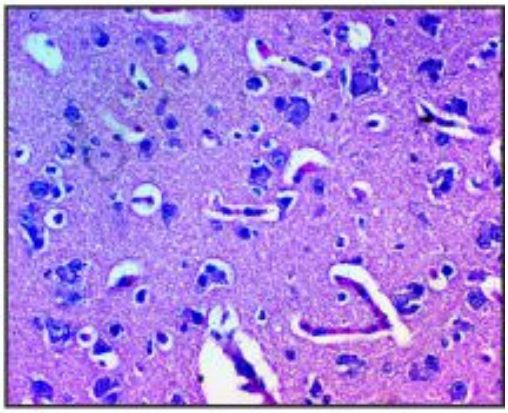


Plate 4.40. Brain (T2) showing degeneration of neurons and congestion at 4th week (H & E × 400)

and comparatively normal parenchyma (Plate 4.42) in the T2 group. The T4 group revealed the mild degenerative changes at some sections and normal neuronal parenchyma. This indicates the restoration of the tissue towards normal after withdrawal of the toxicant.

The literature was scanned, but no relevant findings about the withdrawal effect of atrazine in birds or any other species were found.

4.5.6 Spleen

At the end of 4^{th} and 5^{th} week of experiment section of spleen from control group revealed normal parenchyma showing even distribution of lymphocyte population and well distinguished white and red pulp (Plate 4.43). The capsule was thick around the spleen.

Sections from the T2 group revealed moderate to severe depletion of lymphoid population, severe congestion along with focal areas of necrosis and loss of normal histoarchitecture (Plate 4.44) was recorded.

The T3 group receiving cow urine distillate, some sections of spleen showed the normal structure of white pulp and red pulp and evenly distributed lymphocyte population as it was observed in control.

Sections from atrazine and cow urine distillate treated T4 group revealed mild to moderate lymphoid depletion in lymphoid follicle along with mild congestion (Plate 4.45). Improvement in the spleenic tissue alteration was noticed as compared to T2 only atrazine treated group.

Comparable lesion was also reported by Curic *et al*. (1999) and Saquib, (2009) during atrazine toxicity in gilts and broiler birds respectively. The depletion in the lymphoid population in present study supported by the lymphocytopenia in atrazine treated group. These indicate the immunotoxic effect of the atrazine on bird physiology.

In current experiment atrazine along with cow urine distillate treated (T4) group noted the improvement in the cellular structure and lesions. These finding were also reported by various workers (Pancha, 2015 and Tadavi, 2017) in cow urine distillate treated group against the different

toxicity. Chauhan *et al*. (2001) in mice and Kumar *et al*. (2005) in chicks reported the significant increase in B and T cell blastogenesis in CUD treated group.

At the end of 5th week of experiment, spleen from T2 group revealed mild depletion in lymphocyte follicle and degenerative changes (Plate 4.46) were also noticed with comparatively normal histoarchitecture was evident. The T3 group were revealed the normal parenchyma with well differentiated white pulp and red pulp and abundant lymphoid population as that of control.

The T4 group showed the regeneration of lymphoid follicle and restoration towards normal (Plate 4.47). The less severity of the lesions suggests restoration towards normal after withdrawal period of seven days.

4.5.7 Thymus

At the end of 4th and 5th week of experiment section of thymus from control and T3 cow urine treated group revealed the normal histoarchitecture (Plate 4.48). The cross section of thymus revealed the dark colour in the cortex and lighter colour at the periphery. The thymic lobules were even in size with abundant lymphoid population.

Section from the T2 group showed the severe depletion in the lymphoid population, proliferation of connective tissue, loss in the uniformity of the thymic lobules, disruption in thymus organisation and hemorrhages (Plate 4.49) and vacuolar degenerative changes (Plate 4.50) was noted.

T4 group showed the mild depletion in the lymphoid population with mild hemorrhages (Plate 4.51), uniformity in thymic lobules, marked recovery was observed in thymus suggestive of beneficial effects of cow urine distillate.

The present findings in thymus of T2 group were in accordance with the finding of Saquib (2009) in atrazine treated group in broiler birds.

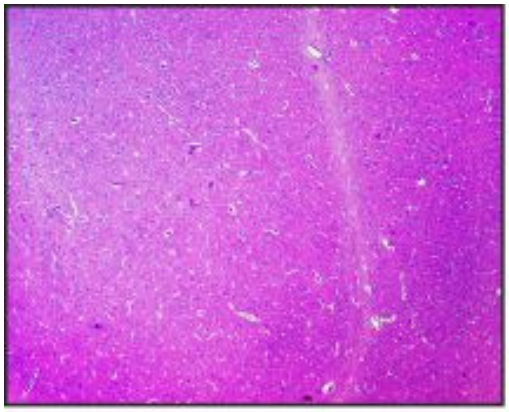


Plate 4.41. Brain (T4) showing swelling of neurons and mild degenerative changes at 4th week (H & E × 40)

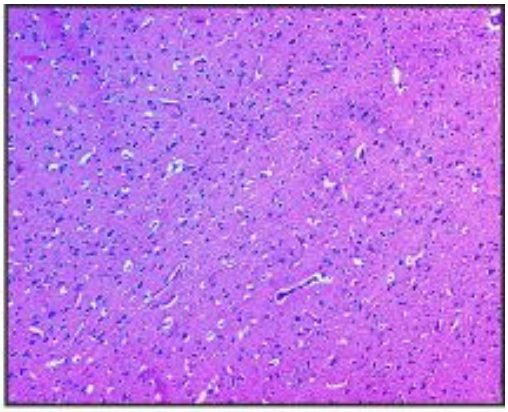


Plate 4.42. Brain (T2) showing mild degenerative changes in neurons and comparatively normal parenchyma at 5th week (H & E × 100)

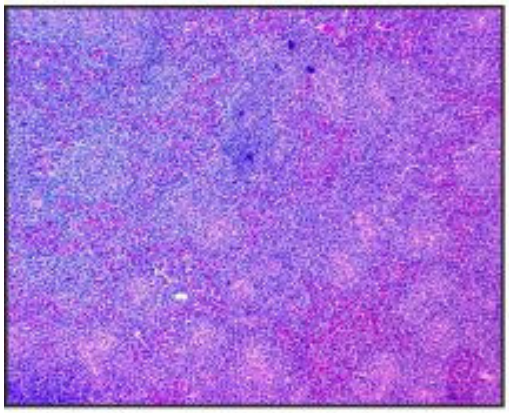


Plate 4.43. Spleen from control (T1) group showing normal parenchyma showing even distribution of white and red pulp at 4th week (H & E × 100)

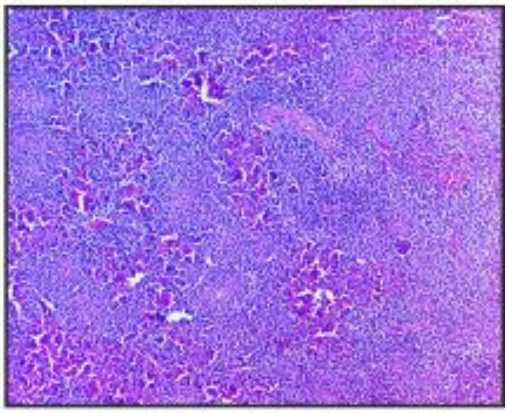


Plate 4.44. Spleen (T2) showing severe congestion with depletion in lymphoid population and loss of normal parenchyma at 4th week (H & E × 100)

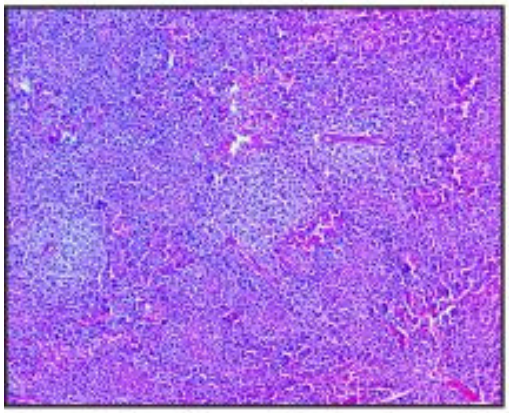


Plate 4.45. Spleen (T4) showing mild congestion and mild depletion in lymphoid population at 4th week (H & E × 100)

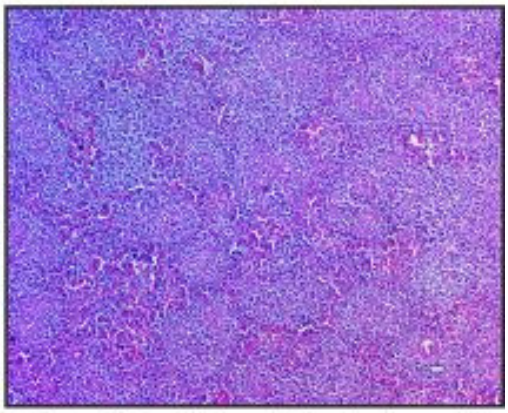


Plate 4.46. Spleen (T2) showing mild congestion with depletion in lymphocyte with comparative normal histoarchitecture at 5th week (H & E × 100)

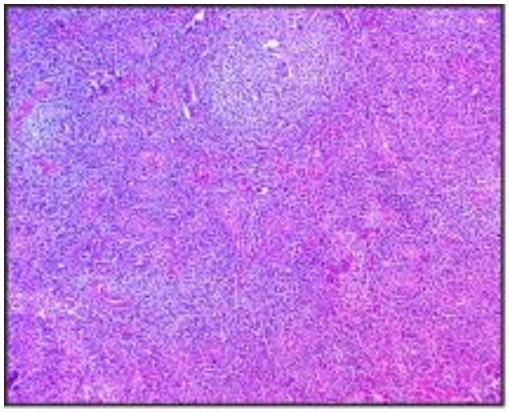


Plate 4.47. Spleen (T4) showing normal parenchyma restoration towards normal at 5th week (H & E × 100)

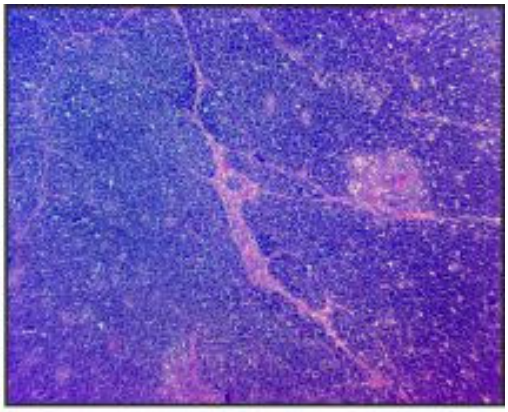


Plate 4.48. Thymus from control (T1) group showing normal histoarchitecture with normal lymphoid population at 4th week (H & E × 100)

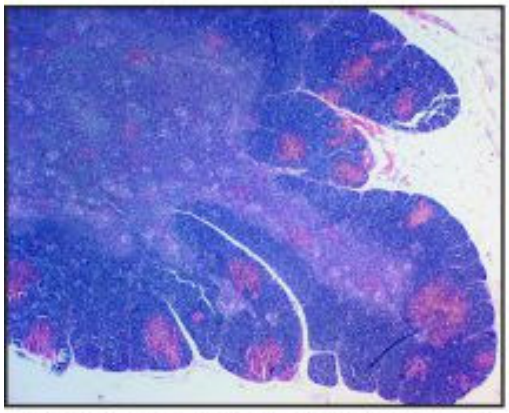


Plate 4.49. Thymus (T2) showing severe haemorrhages disorganization of thymic lobule at 4th week (H & E × 40)

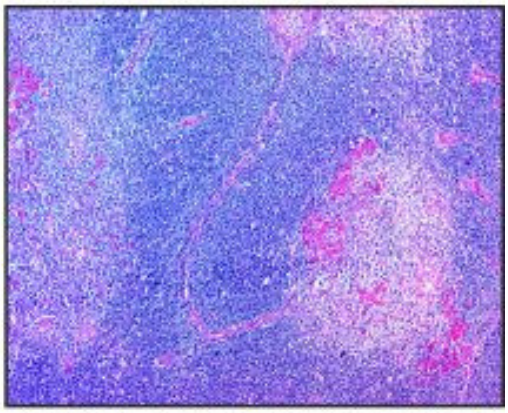


Plate 4.50. Thymus (T2) showing severe hemorrhages with depletion of lymphoid population and vacuolar degenerative changes at 4th week (H & E × 100)

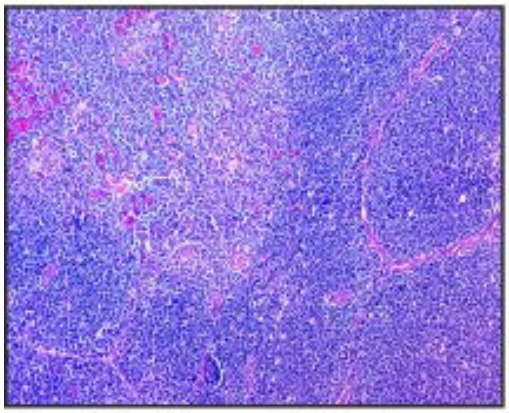


Plate 4.51. Thymus (T4) showing mild hemorrhages with depletion in lymphoid population at 4th week (H & E × 100)

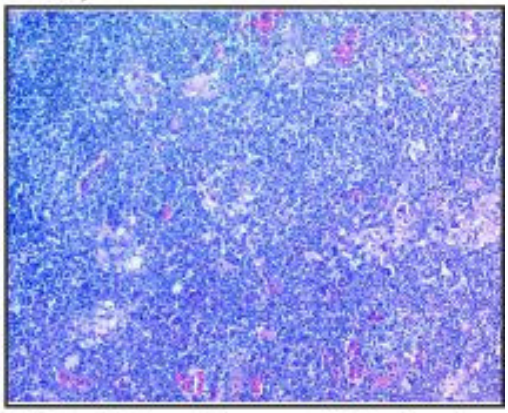


Plate 4.52. Thymus (T2) showing restoration of lymphoid population towards normal with mild degeneration and hemorrhages at 5th week (H & E × 100)

Whereas, findings in thymus of T4 group was in accordance with the finding recorded by Pancha (2015) and Tadavi (2017) in different intoxication and in different species.

However at the end of 5th week of experiment, these changes were mild in the treatment groups. Restoration of lymphoid population towards normal with mild degeneration and haemorrhages was noted in T2 group (Plate 4.52). This indicates the restoration of the changes towards normal histoarchitecture.

4.5.8 Bursa of Fabricius

At the end of 4th and 5th week of experiment, sections of bursa of fabricius from control and T3 group showed the uniformity in follicle with normal lymphoid population and histoarchitecture (Plate 4.53).

At the end of 4th week of experiment, bursa of fabricius from T2 group exhibited lesion of moderate congestion in follicle, thickened interfollicular septa, varying sized follicle (Plate 4.54) and depletion of lymphocyte population (Plate 4.55) was also observed.

Whereas, T4 group (atrazine with cow urine distillate treated group) reveals mild depletion in lymphocyte population (Plate 4.56). Changes were mild when compared with T2 atrazine intoxicated group.

Saquib (2009) reported the degenerative changes in lymphocyte cells and follicles with moderate congestion in bursa of fabricius of the atrazine fed broilers.

The changes observed in bursa of fabricius in T4 group were reveals similar finding reported by Tadavi (2017) in CUD treated group in broiler birds indicated ameliorative effect against the chlorpyrifos toxicity.

At the end of 5th week of experiment, control and cow urine distillate treated groups showed the normal histoarchitecture. The lymphocyte population was evenly distributed and abundant lymphocyte was observed with uniformity in bursal follicle. The atrazine induced toxicity group does not reveal any recognizable lesions except mild depletion in lymphocyte

population indicating restoration towards normal parenchyma (Plate 4.57). The T4 group did not reveal any noticeable change in bursa of fabricius. These changes at the end of 5th week indicate the restoration of lymphoid population towards normal with abundant lymphoid population and normal histoarchitecture.

The literature was scanned, but did not found any relevant findings about the withdrawal effect of atrazine in birds or any other species.

4.5.9 Sciatic Nerve

At the end of 4th week and 5th week of experiment birds from T1 and T3 group exhibited the normal histoarchitecture of the sciatic nerve (Plate 4.58). The myelinated nerve fibers were normal with axons and normal schwann cells was observed.

At the end of 4th week of experiment, longitudinal section of sciatic nerve from T2 atrazine induced toxicity group were showed moderate separation of nerve fibres (Plate 4.59), vacuolar changes with areas of infiltration of mononuclear cells (Plate 4.60), focal areas of demyelination and degeneration of nerve fibres was observed.

Sections from T4 (atrazine with cow urine distillate treated) group were recorded mild degenerative changes with separation of nerve fibers (Plate 4.61) as compared to T2 (only atrazine) group. The lesions indicate the protective effect of cow urine distillate on atrazine induced toxicity.

At the end of 5th week of experiment, microscopic examination of sciatic nerve revealed mild lesions like separation of the nerve fiber in T2 and T4 group as compared to normal architecture in T1 and T3 groups. These suggest the restoration of the tissue architecture towards the normal after withdrawal of toxicant through feed.

Literature scanned but did not report any reference related to withdrawal of atrazine toxicity in sciatic nerve in broilers.

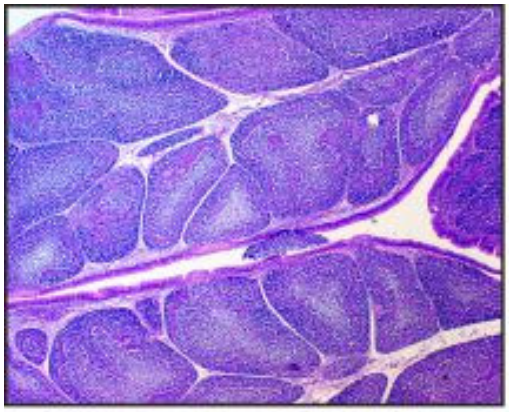


Plate 4.53. Bursa of fabricius from control (T1) group showing uniformity in follicle with normal lymphoid population and histoarchitecture at 4th week (H & E × 40)

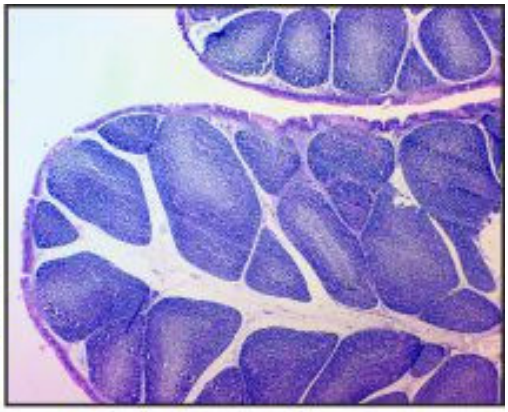


Plate 4.54. Bursa of fabricius (T2) showing varying size of follicle with depletion of lymphoid population and thickened inter follicular space at 4th week (H & E × 40)

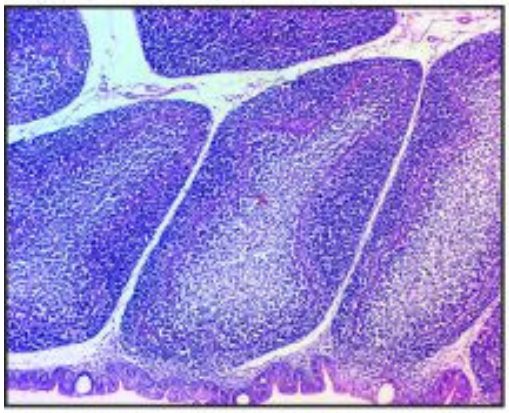


Plate 4.55. Bursa of fabricius (T2) showing depletion of lymphocyte population at 4th week (H & E × 400)

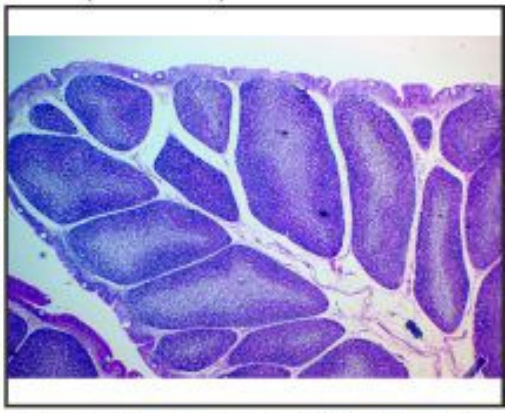


Plate 4.56. Bursa of fabricius (T4) showing depletion of lymphocyte population and mild increase in inter lobular space at 4th week (H & E × 40)

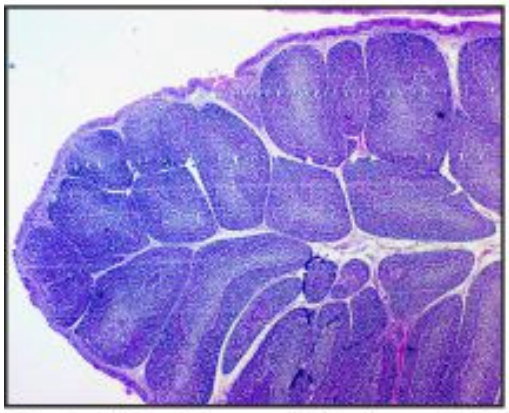


Plate 4.57. Bursa of fabric us (T2) showing restoration of lymphoid population towards normal with mild haemorrhage at 5th week (H & E × 40)

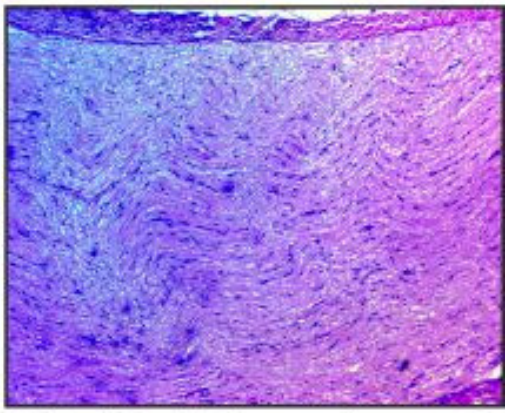


Plate 4.58. Sciatic nerve from control (T1) group showing normal normal nerve fibre and histoarchitecture at 4th week (H & E × 100)

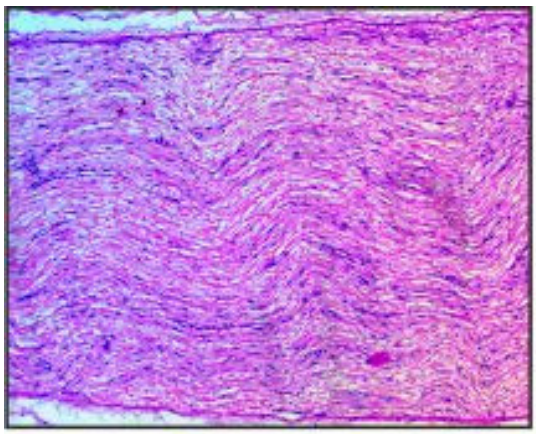


Plate 4.59. Sciatic nerve (T2) showing separation of nerve fibres, vacuolar degeneration and infiltration of mononuclear cells at 4th week (H & E × 100)

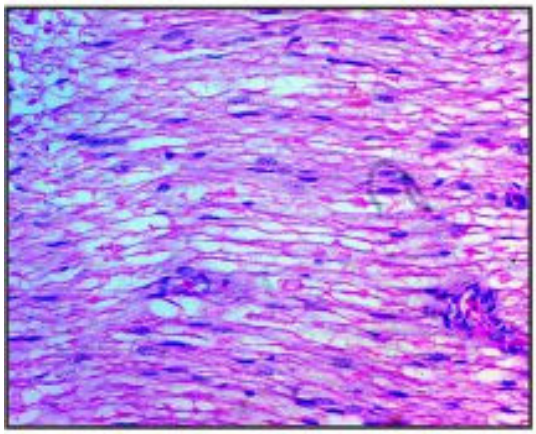


Plate 4.60. Sciatic nerve (T2) showing vacuolar degenerative changes and mononuclear cells infiltration at 4th week (H & E × 400)

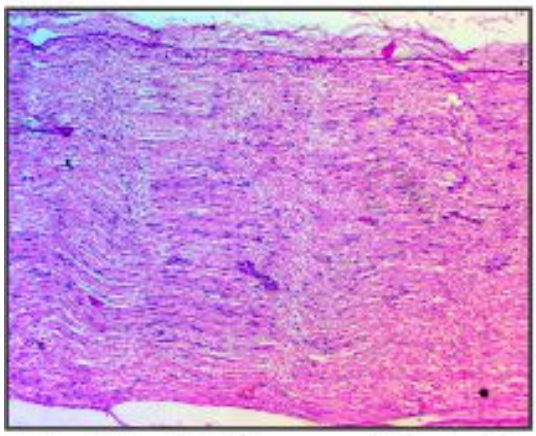


Plate 4.61. Sciatic nerve (T4) showing mild degenerative and mild separation of nerve fibres at 4th week (H & E × 100)

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation entitled "Ameliorative effect of cow urine distillate on atrazine induced toxicity in broilers" was conducted at Poultry Research Centre and Department of Veterinary Pathology, Post Graduate Institute of Veterinary and Animal Sciences, Akola (Maharashtra) India.

The current experimental trial was designed to study the beneficial effect of cow urine distillate on atrazine induce toxicity and also to elucidate the alterations occurred in general performance, haematobiochemical and pathological parameters at the end of 4^{th} and 5^{th} week of experiment. For the present study eighty day old broiler chicks were procured from M/s. Amruta Hatcheries Pvt. Ltd. Amravati. The investigation was carried with prior approval of Institutional Animal Ethical Committee (IAEC), PGIVAS, Akola. The chicks were acclimatized for a period of one week and randomly divided into four groups. Each group comprising of 20 birds (n=20). The T1 group served as control group fed with normal feed, T2 group treated with atrazine @ 250mg/kg of feed, T3 group administered with cow urine distillate @ 10 mL/lit of water and T4 group treated with atrazine @ 250mg/kg of feed along with cow urine distillate @ 10mL/lit of water. The treatment was given for period of three week (21 days) and during 5^{th} week the respective dietary treatment was withdrawn and all the groups were fed with normal feed for the period of seven days (7^{th} days of PWP).

During the experimental period, all the birds were closely observed for clinical sign, symptoms and general performance if any. The control and T3 group birds did not exhibited any clinical signs and symptoms throughout the experiment whereas, T2 group fed with atrazine @ 250 mg/kg of feed exhibited the untoward signs like dullness, depressed, closed eyes at 2^{nd} week, lower feed intake, pasty faeces with one mortality and day by day severity of the clinical sign were found to be increase at 3^{rd} week in atrazine

intoxicated group. The T4 group bird showed more or less, clinical signs as compare to T2 group. During the withdrawal period (7^{th} days of PWP) did not revealed any untoward clinical signs in any of the treatment and control groups.

Significant decreased in body weight, body weight gain and numerical decreased in feed consumption and FCR values were recorded in T2 group when compared with the T1 and T3 group. The T3 group reveals non significant difference and significant difference as compared with T1 and T2 group respectively. The T4 group revealed non significant difference in body weight, body weight gain, feed consumption and FCR values at 4^{th} week indicated beneficial effect of CUD on general performance in broiler

Significant decrease in Hb, PCV, TEC, and lymphocyte count while significant increase in MCV, MCH, heretophil and monocyte count were observed in T2 group. The T4 group showed significant improvement in above all haematological parameters values as compared to T2 group indicated hematinic role of CUD against the atrazine toxicity. Whereas, T3 group revealed non significant difference with control group in all the haematological parameters at the end of 4^{th} week of experiment. At the end of 5^{th} week of experiment, significant difference in the values of Hb, PCV, TEC, MCH and MCHC were recorded in T2 and T4 group as compared with the T1 and T3 group whereas, non significant difference noted MCV, TLC, lymphocyte, heterophils and monocytes count in T2 group as compared with T4 group. The T3 group showed significant difference in Hb, PCV and TEC and non significant difference in rest of the haematological parameters with T1 group. T4 group revealed significant beneficial improvement in Hb, PCV, TEC, MCH and MCHC when compared with T2 group indicated beneficial effect of withdrawal of atrazine from the feed.

Significant increase in the AST, ALT, creatinine and BUN values and significant deceased in the total protein, albumin and creatinine values however, non significant difference was noted in serum globulin, serum albumin and globulin ratio in T2 group as compared with T4 group.

Significant improvement recorded in above all the biochemical parameters in T4 group. The T3 group revealed significant difference in AST, ALT, serum creatinine, BUN and serum albumin levels with the T2 group at the end of 4th week indicated beneficial effect of the CUD in broilers. Significant difference in values of AST, ALT, serum creatinine, and non significant difference in BUN, total protein, serum albumin, globulin and albumin and globulin ratio was recorded in T2 group as compared with T1, T3 and T4 group. The T3 group reveals non significant differ in ALT, AST, serum creatinine, BUN, total protein, albumin, globulin and albumin to globulin ratio when compared with the T1 and T4 group at the end of 5th week (7th days of PWP) .

Liver of T2 group were revealed friable consistency, slight enlargement with mild congestion and haemorrhages, distended gall bladder, Kidneys were swollen, enlarged and slight haemorrhagic. Heart showed epicardial hemorrhages and hydopericardium. Generalised congestion was seen in lungs. Pinpoint haemorrhages were recorded on thymus in atrazine treated group. The T3 group does not reveal any significant alteration as compared to control. Liver of T4 group exhibited lesions of mild degree of congestion with pale discolouration. At the end of 5th weeks of experiment all the treatment groups did not reveal any macroscopic pathological alterations in any organ (liver, kidney, heart, lung, thymus, bursa, spleen, brain and sciatic nerve) as compared with control group.

Liver of T2 group revealed the lost of histoarchitecture, congestion of central vein, increased sinusoidal spaces with focal areas of necrosis, pyknosis of nucleus, granular and vacuolar degenerative changes in hepatocytes along with infiltration of mononuclear cells and bile duct hyperplasia were noticed. The T3 group revealed normal histoachitecture similar to control group birds whereas T4 group noted the mild congestion, infiltration of mononuclear cell and mild sinusoidal dilatation when compared with T2 group at the end of 4th week. The control and T3 group reveals normal liver parenchyma whereas, T2 group showed lesions were more or less pronounced such as mild dilation of sinusoidal spaces, congestion in central vein and infiltration of mononuclear cells. Sections from T4 group exhibited

the mild congestion, sinusoidal space dilation and normal structural integrity was maintained at 5th week. (7th days of PWP).

Kidney of control and T3 group showed normal histoarchitecture and T2 group exhibited lesions of shrunken, degeneration of glomeruli, desquamation of lining epithelium of tubules along with focal areas of necrosis, interstitial hemorrhages and infiltration of mononuclear cells, protein casts in lumen, increase glomerular cellularity with increase bowman's capsule space with loss of parenchyma however, T4 group reveals mild to moderate degenerative changes in glomerular and tubular epithelium along with congestion in interstitial tissue, degeneration of glomeruli and varying sizes of glomeruli with normal renal parenchyma were observed. The T2 group revealed mild congestion, granular and vacuolar degeneration in tubules with interstitial haemorrhages, whereas, T4 group showed mild degenerative changes in glomeruli with interstitial haemorrhages was evident at 5th week.

Heart of T2 group revealed congestion of blood vessels, degeneration and separation of myocardial fibers, infiltration of mononuclear cells accompanied with muscular hemorrhages, pyknotic nuclei and focal areas of necrosis whereas, T4 group exhibited lesion of mild to moderate separation of muscle fibers with infiltration of MNC at 4th week indicated beneficial effect of CUD in broilers. Heart from T2 group noted mild lesion such as mild haemorrhages, infiltration of MNC and separation of muscles fibers whereas, T4 group showed the mild myocardial hemorrhages with infiltration of mononuclear cells and T3 group revealed normal histoarchitecture of heart indicated withdrawal effect of atrazine from the feed at 7th days of PWP.

The control and T3 group showed the normal parenchyma of lung. Lung of T2 group revealed congestion in interstitial tissue and in blood vessel, desquamation of bronchial epithelium with infiltration of mononuclear cells, thin alveolar wall, rupture of alveolar wall, thickened interlobular septa, emphysema, severe congestion throughout lung and disruption of bronchial epithelium. Whereas, T4 group showed the mild focal oedema, ruptured

alveoli, thick interlobular septa with infiltration of mononuclear cells and congestion of blood vessel was noted at the end of 4th week. Lung from T2 and T4 group showed normal parenchyma except mild congestion whereas; T1 and T3 group reveals normal lung parenchyma at 5th week.

Brain of T1 and T3 group revealed the normal histoarchitecture. Histopathological examination of brain of T2 group consists of swelling of neurons, vacuolar changes, multifocal necrosis, satellitosis and mild increase in Virchow-Robin spaces with congestion and degenerative changes in neurons whereas, T4 group revealed the swelling of neurons, necrotic changes and degenerative changes at the end of 4th week of experiment. At the end of 5th week of experiment, brain tissue from different treatment groups revealed mild degenerative changes in neurons as comparative to control group.

Section of spleen from control and T3 group revealed normal parenchyma showing even distribution of white and red pulp. The T2 group revealed moderate to severe depletion of lymphoid population, congestion along with focal areas of necrosis and loss of normal histoarchitecture. The T4 group noted mild to moderate lymphoid depletion in lymphoid follicle along with mild congestion at the end of 4th week. Spleen of T2 group revealed mild depletion in lymphocyte follicle and degenerative changes and parenchyma close to normal. The T4 group showed the regeneration of lymphoid follicle and restoration towards normal while T3 group was observed the normal parenchyma as that of control at the end of 5th week of experiment.

Thymus of control group were noted the normal histoarchitecture. Section from the T2 group showed severe depletion in the lymphoid population, proliferation of connective tissue, lost in the uniformity of the thymic lobules, hemorrhages and vacuolar degeneration whereas, T4 group showed the mild depletion in the lymphoid population with mild haemorrhages and uniformity in thymic lobules. The T3 group no microscopic alteration was observed and lymphoid population were normal at the end of 4th week. However mild alteration in all the treatment groups as compared with

control group. Restoration of lymphoid population towards normal except mild degeneration and hemorrhages present in T2 group was noted at the end of 5th week.

Bursa of fabricius of control group showed the uniformity in follicle with normal lymphoid population and normal histoarchitecture. The T2 group exhibited histological lesion of moderate congestion in follicle, thickened interfollicular septa, varying sized follicle and depletion of lymphocyte population. Whereas, in T4 group mild depletion in lymphocyte population was observed. The T3 group does not reveal any recognizable lesion at the end of 4th week. The T1 and T3 groups showed the normal histoarchitecture whereas; T2 group reveal mild depletion in lymphocyte population and T4 group did not reveals any noticeable change at the end of 5th week of experiment.

The T1 and T3 group exhibited normal histoarchitecture of the sciatic nerve. The T2 group showed moderate separation of nerve fibres, vacuolar changes with areas of infiltration of mononuclear cells, focal areas of demyelination and degeneration of nerve fibres whereas, T4 group exhibited lesions of mild degenerative changes and separation of nerve fibers at 4th week indicated beneficial effect of CUD. At 5th week of experiment microscopic examination of sciatic nerve revealed mild lesions in T2 and T4 group as compared to normal architecture in T1 and T3 groups suggested withdrawal effect of atrazine from feed of broilers.

On the basis of observation were recorded in present investigation it is thus concluded:

1) Atrazine induced toxicity @ 250 mg/kg of feed results in significant decreased in body weight, body weight gain and numerical decreased in feed consumption and FCR values affect general performance of birds at 4th week whereas restoration effect recorded in body weight gain at 7th days of PWP.

2) Cow urine distillate @ 10 mL/lit of water showed the better improvement in general performance of birds against atrazine fed @ 250 mg/kg of feed at 4^{th} and 5^{th} week.

3) Atrazine causes detrimental effect on haematological parameters (Hb, PCV, TEC, TLC and DLC). Restorative effect on haematological parameters was recorded in CUD given against atrazine treated group at 4^{th} week indicates hematinic property of cow urine distillate.

4) Significant increased in AST, ALT, creatinine, BUN and decreased in total protein, albumin levels were noted on atrazine treated group, whereas significant improvement recorded in CUD treated group against atrazine toxicity group suggesting the ameliorative hepatoprotective and nephroprotective property of cow urine distillate.

5) Pathological examination of visceral organs reveals hepatoprotective, nephroprotective and immunomodulatory effect of CUD against atrazine toxicity at 4^{th} week and restores towards normal levels at 5^{th} week.

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THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	**:**	**AMELIORATIVE EFFECT OF COW URINE DISTILLATE ON ATRAZINE INDUCED TOXICITY IN BROILERS**
b)	**Full name of student**	**:**	**Patil Anjali Arun**
c)	**Name and address of Major advisor**	**:**	**Dr. P. R. Rathod** Assistant Professor, Department of Veterinary Pathology, PGIVAS, Akola
d)	**Degree to be awarded**	**:**	M. V. Sc. (Veterinary Pathology)
e)	**Year of award of degree**	**:**	2021
f)	**Major subject**	**:**	Veterinary Pathology
g)	**Total number of pages In the thesis**	**:**	113
h)	**Number of words in the abstract**	**:**	300
i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

The present investigation was conducted on "Ameliorative Effect of Cow Urine Distillate on Atrazine Induced Toxicity in Broilers." Eighty day old broilers were selected and divided into four groups; T1 (control), group T2 (Atrazine@250 mg/kg of feed), group T3 (CUD @ 10 mL/lit of water) and group T4 (Atrazine combine with CUD) received treatment for 3 weeks and withdrawal of treatment after seven days.

Clinical sign of dullness, closed eyes; pasty faeces and mortality recorded whereas, significant difference in general performance (body weight and body weight gain), haematological (Hb, PCV, TEC, MCV, MCH, MCHC, TLC, heterophils, lymphocyte and monocyte count) and biochemical parameters (AST, ALT, BUN, Total protein, Albumin and Creatinine) observed in T2 group.

Grossly liver, kidney, brain, bursa, spleen and thymus revealed enlargement, congestion and hemorrhages. Microscopic examination of liver showed congestion, sinusoidal dilatation, granular and vacuolar degeneration, kidney revealed degeneration, desquamation of tubular epithelium, haemorrhages and increase glomerular cellularity, heart showed degeneration, muscular haemorrhages, lung showed congestion, bronchial epithelium desquamation and thickened interlobular septa, brain showed satellitosis and increase Virchow-Robin spaces, spleen, bursa and thymus revealed severe depletion of lymphoid population and sciatic nerve showed demyelination and degeneration of nerve fibres in T2 group. However, T1 and T3 groups did not reveal any changes in general performance, haematobiochemical and pathological lesion. T4 group showed significant improvement almost in all parameters indicated ameliorative effect of CUD against toxicity at 4^{th} week. Significant difference recorded in hematobiochemical parameters (except MCV, TLC, lymphocyte, heterophil, monocyte, serum BUN, total protein, albumin, globulin) in T4 group at 5^{th} week indicating withdrawal effect of atrazine.

It is concluded that atrazine @ 250 mg/kg of feed causes detrimental effect of hepatotoxicity, nephrotoxicity and neurotoxicity whereas, CUD treated group recorded protective effect against atrazine toxicity in broilers. After withdrawal of toxicity mild restoration effect observed.

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	: **मांसल कोंबड्यांमध्ये अट्रेझिन प्रेरित विषाच्या तीव्रतेवर गोमूत्र अर्काचा सुधारित परिणाम**
२.	**विद्यार्थ्याचे पूर्ण नांव**	: **पाटील अंजली अरुण**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	: **डॉ. प्र. रा. राठोड** सहाय्यक प्राध्यापक पशुविकृतीशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला
४.	**प्रदान केली जाणारी पदवी**	: एम.व्ही.एस्सी.
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०२१
६.	**मुख्य विषय**	: पशुविकृतीशास्त्र विभाग
७.	**प्रबंधामधील एकुण पाने**	: ११३
८.	**प्रबंध सारांशामधील एकुण शब्द**	: ३३५
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

(डॉ. र. सु. इंगोले)
सहाय्यक प्राध्यापक व विभाग प्रमुख
पशुविकृतीशास्त्र विभाग
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अकोला.

सारांश

सदरील प्रयोगात "मांसल कोंबड्यांमध्ये अट्रेझिन प्रेरित विषाच्या तीव्रतेवर गोमूत्र अर्काचा सुधारित परिणाम" याचा अभ्यास करण्यात आला. या मध्ये एक दिवस वयाचे ऐंशी मांसल पक्ष्यांची निवड करून त्यांना चार समान गटात विभागण्यात आले; टी 1 (नियंत्रण), गट टी 2 (ॲट्राझिन@250 मिग्रॅ/किग्रॅ खाद्याद्वारे), गट टी 3 (गोमूत्र अर्क@10 मिली /लिटर पाण्यात) आणि गट टी 4 (ॲट्राझिन@250 मिलीग्राम/किलो खाद्याद्वारे + गोमूत्र अर्क@10 मिली/लिटर) 3 आठवडे उपचार केला गेला आणि सात दिवसांनी ॲट्राझिनचा विषक्तपणा खाद्यामधून काढून टाकला.

चिकित्सालयीन लक्षणे जसे मंदपणा, बंद डोळे, चिकट विष्ठा आणि मृत्यूची नोंद केली गेली आणि सामान्य कामगिरी (शरीराचे वजन आणि वजन वाढ), रक्तवाहिन्यासंबंधी (एचबी, पीसीव्ही, टीईसी, एमसीव्ही, एमसीएच, एमसीएचसी, टीएलसी, हेटरोफिल्स, लिम्फोसाइट आणि मोनोसाइट गणना) आणि जीवरासायनिक मापदंडांत (एएसटी, एएलटी, बीयूएन, एकूण प्रथिने, अल्बुमिन आणि क्रिएटिनिन) लक्षणीय फरक गट टी 2 मध्ये नोंदविण्यात आले.

स्थूल परीक्षणामध्ये यकृत, मूत्रपिंड, मेंदू, बर्सा, प्लीहा आणि हृदोधिष्ठग्रंथी ग्रंथी मध्ये रक्तसंचय, रक्तस्राव आणि अवयवाचा आकार वाढलेला दिसून आला. यकृताच्या सूक्ष्म तपासणीमध्ये रक्तसंचय, साइनसॉइडचा फुगीरपणा, रवाळ तथा पोकळपनाचा ऱ्हास, मूत्रपिंडाचा ऱ्हास, नलीकेच्या अधिस्तराचा कोंडा पडणे, रक्तस्राव, सूक्ष्म मूत्रनलिकेच्या केशवाहिन्यांचा जुडग्याच्या पेशीमध्ये वाढ झालेली दिसून आली. हृदयच्या स्नायू मध्ये रक्तस्त्राव व स्नायूचा ऱ्हास झाल्याचे दिसून आले, फुफ्फुसामध्ये रक्तसंचय, श्वासनलिकेचा अधिस्तराचा कोंडा पडणे आणि दोन लोब्युल मधील पडदा जाड होणे, मेंदूमध्ये सेटेलायटोसीस आणि व्हर्चोरोबिन जागा वाढणे, प्लीहा, बर्सा आणि हृदोधिष्ठग्रंथी यांच्या परीक्षणामध्ये लिम्फोसाईटची संख्या आणि सायटिक मज्जातंतूच्या मायेलिन अवरणांचा नाश होणे अश्या प्रकारची व्रण टी 2 गटामध्ये दिसून आली. तथापि, टी 1 आणि टी 3 गटाच्या सामान्य कामगिरी, रक्तवाहिन्यासंबंधी, जीवरासायनिक मापदंडांत आणि अवयवांच्या विकृतीत कोणताही बदल झालेला आढळून आलेला नाही, तथापी टी 4 गटाने जवळजवळ सर्व मापदंडांमध्ये चौथ्या आठवड्यात विषक्तपणा विरूद्ध गोमूत्र अर्काचा सुधारित परिणाम नोंदविण्यात आला. 5 व्या आठवड्यात टी 4 ग्रुपमध्ये महत्त्वपूर्ण फरक जवळपास सर्व हेमॅटोबायोकेमिकल मापदंडांमध्ये (वगळता एमसीव्ही, टीएलसी, लिम्फोसाइट, हेटरोफिल, मोनोसाइट, सीरम बीयूएन, एकूण प्रथिने, अल्बमिन, ग्लोब्यलीन) अट्रेझिन चा प्रभाव कमी होताना नोंदवला गेला.

सदर प्रयोगांमधून असा निष्कर्ष काढण्यात येतो कि, अट्रेझिन @ 250 मिलीग्राम/किग्रा खाद्यामध्ये दिले असता यकृत, मूत्रपिंड आणि मज्जातंतू यांवर विषबाधा होते आणि गोमूत्र अर्काच्या वापरामुळे अट्रेझिनणे निर्माण केलेल्या विषबाधेच्या विरोधात संरक्षणात्मक परिणाम दिसून आला. सात दिवसानंतर विषक्तपणाचा प्रभाव कमी होताना आढळून आला.