TOXICITY STUDIES ON *JATROPHA CURCAS* (*RATANJYOT*)

Thesis

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CERTIFICATE – I

This is to certify that the thesis entitled "**Toxicity Studies on *Jatropha curcas* (Ratanjyot)**" submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** of the Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, is a record of the bonafide research work carried out by **Ku. Vijeyta Awasthy** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by her.

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This is to certify that the thesis entitled "**Toxicity Studies on *Jatropha curcas* (Ratanjyot)**" submitted by **Ku. Vijeyta Awasthy** to the Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** in the Department of Veterinary Pharmacology and Toxicology has been approved by the Student's Advisory Committee after oral examination in collaboration with the External Examiner.

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Date: November 01, 2006 Dr (Ms) Vijeyta Awasthy

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LIST OF SYMBOLS/ABBREVIATIONS

Abbreviation	Full Form
@	At the rate of
ALP/PAP	Plasma alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
β	Beta
CP	Crude protein
Cumm	Cubic millimeter
Dl	Deciliter
DLC	Differential leucocyte count
DOM	Digestible organic matter
FAO	Food and Agriculture Organization
g or gm	Gram
H and E	Hemotoxylin and eosin
Hr	Hour
Hb	Haemoglobin
JSPS	*Jatropha* seed protein supplementation
Kg	Kilogram
LD_{50}	Median lethal dose
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
ME	Metabolic energy
Mg	Milligram
MJ	Mega Jouls
m mol.	Millimol
Ml	Milliliter
µ	Micron

PCV	Packed cell volume
PGOT	Plasma glutamate oxalo-acetate transaminase
PGPT	Plasma glutamate pyruvate transaminase
RBC/rbc	Red blood cells
SE	Standard Error
TEC	Total erythrocyte count
TLC	Total leucocyte count
U	Unit
Wk	Week
%	Per cent
/	Per
±	Plus or minus

Chapter-1

INTRODUCTION

CHAPTER- I

INTRODUCTION

Man and animals mostly depend on vegetable kingdom for their food. Plants by their metabolic activities besides being the source of feed and fodder also elaborate other substances viz. alkaloids, glycosides, toxalbumins, essential oils, resins, bitter principles etc, which are important from medicinal as well as toxicological point of view. The phytotoxins found in plants adversely affect livestock production and health. The well known examples include gossypol in cotton seed cake, linamarin in linseed meal, lectin and trypsin inhibitor in soyabean meal etc. In India there are 700 poisonous plant species belonging to more than 90 different families.

Animals particularly the grazing livestock being indiscriminate eaters, when hungry they ingest food as well as nonfood plants, particularly during the scarcity periods. Absence of specific grazing areas/pasture fields in our country confounds the problem of plant toxicity due to wide distribution of non food/toxic plants in the waste lands, the so called grazing area of our livestock. In the settlement of new areas, both the livestock and ranchers were inexperienced as to the toxicity of native plants, causing serious livestock health concerns. Therefore, there is the necessity of detailed toxicological investigations of the suspected hazardous plants/feedstuffs.

Jatropha curcas (Family: Euphorbiaceae), popularly known as "Ratanjyot" is one such plants reported to possess several medicinal and toxicological properties. The root, stem, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine (Chopra *et al.*, 1956; Kirtikar and Basu, 1975; Nadkarni, 1998). Different species of Genus including *J. curcas* has been claimed to have a wide range of medicinal

properties such as tonic, analgesic, tranquilizer, abortifacient and in the treatment of rheumatism and gout (Okuyama *et al.*, 1996; Schmeda *et al.*, 1996). In addition these plants were also reported to possess anti-haemoprotozoan and vector control efficacies (Singh *et al.*,1997; Karmegam *et al.*, 1997; Ruppel and Doenhoff,1998), pisicidal action (Singh *et al* ., 2002), anti-platelet aggregation (Dutra *et al.*, 1996), anti-tumor effects (Lin *et al.*, 2003) and as molluscicide and schistosome larvicidal properties (Melanie and Andreas, 2000).

The plant *J. curcas* has recently come in limelight due to its potential as a source of much publicized "bio-diesel" in the state of Chhattisgarh. Further, it is also being contemplated to use its oil extracted seed as a source of alternative protein to meet the shortage of concentrate feed to the livestock. In addition to being a source of oil, *Jatropha* also provides a meal that serves as a highly nutritious and economic protein supplement in animal feed (defatted meal is estimated to contain 53 to 58 % crude protein), if the toxins are removed (Becker and Makkar, 1998).

A recent study (Aregheore *et al.*, 2003) revealed that *J. curcas* seed meal reduced of its phorbol ester level to a tolerable level of 0.09 mg/gm had 68 % crude protein, much higher than most of the oilseed meals. Further, some investigators have expressed existence of nontoxic and toxic varieties of *J. curcas* (Makkar *et al.*, 1998).

Many cases of poisoning with ratanjyot seed are reported in the literature. The toxicity of its ground seeds has been demonstrated in mice, rats, goats, sheep, calves and chicks (Adam and Magzoub, 1975; Ahmed and Adam, 1979 a,b; Liberalino *et al.*,1988; El-Badwi *et al.*,1995). However, the toxicological potential of *J. curcas* of Chhattisgarh region is not reported. In light of the above the present investigation is considered worthwhile to evaluate the adverse effects, if any, of feeding the local variety of *J. curcas* seeds in

experimental animals (albino rats) in order to evaluate the possibility, if any, of their utility as a source of protein supplement in animal feeds. Hence, the present research work is planned with the following objectives:

1. To study the acute oral toxicity of the aqueous extract of powdered *Jatropha curcas* seed in albino rats and to determine its oral LD_{50}.

2. To study sub acute toxicity of the powdered seed of *J. curcas* in albino rats characterized by changes in behavior Appearance of toxic signs and symptoms, feed intake and growth rate.

3. To find out the alterations, if any, in the biochemical and haematological parameters and histopathological changes in visceral organs of treated rats.

Chapter-II

REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

DESCRIPTION OF THE PLANT

Distribution

The Genus *Jatropha* belongs to the family Euphorbiaceae and thus closely related to other important commercial/cultivated plants like rubber tree, caster, *Acacia* etc. The Genus comprises of 160 -175 species, out of which nine species are recorded in India. It is believed to be native of Mexico and tropical America, but later spread to other continents of the world by Portuguese settlers (Aponte, 1978). Today it is found in almost all the tropical and subtropical regions of the world, including all over our country. The plant is popular through different vernacular names in India as listed below:

English	:	Physic nut, Purging nut, Barbados nut, Black vomit nut, Curcas bean
Local name	:	*Ratanjyot*
Hindi	:	*Jungli arandi, Pahari erand*
Sanskrit	:	*Dravanti*
Malayalam	:	*Katalavanakku, Kammati*
Tamil	:	*Kattukkottai*
Telugu	:	*Adavi-amudamu*
Bengali	:	*Bon-bheranda*
Gujarati	:	*Jamalghota, Ratanjota*

Botanical Characteristics

J. curcas is a small evergreen annual shrub or short-lived tree. The plant ranges in height from 3 to 5 meters. Stems are thick, green, glaborous, mostly herbaceous or somewhat succulent, becoming woody at the base. Leaves are alternate, long, petioled, palmately veined, cordate to truncate at the base, about 6 inches wide, with 3 to 5 shallow lobes. The flowers are small yellow, unisexual and are present in clusters in leaf axils, mostly hidden in leaf foliage. The fruits are ovoid and located in locular capsules, at first green and fleshy, becoming brownish or black and dry at maturity, containing up to 3 black seeds of about 20 mm in length.

Chemical Constituents

Seeds of *J. curcas* contain a toxic principle curcin (Chopra *et al.*, 1956). The seed kernel also contain a fatty oil, two phytoesterols, a phytosterolin (glucoside of phytoesterol), large amount of sucrose and resinous matter having nauseating, purging and griping effect.

Watt and Breyer (1962) reported that the bark, fruit, leaf, root and wood of *J. curcas* contained hydrocyanic acid.

Lampe and Fagerstrom (1968) reported the presence of dermatitis producing resin in the plant of *Jatropha curcas.*

Joubert *et al.* (1984) reported isolation of a purgative oil from the seeds of *J. curcas* (also known as hell oil, pinheon oil) containing small amount of an irritant curcanoleic acid which is related to ricinoleic acid and crotonoleic acid, the fatty acid ingredient of castor oil and croton oil respectively. Diterpenes have been isolated from seeds of *J.curcas* (Adolf *et al.*, 1984) and roots (Naengohomnong *et al.*, 1986; Chen *et al.*, 1988).

Rastogi and Mehrotra (1993) reported isolation of palmitic acid, oleic acid and linoleic acid; two flavanoid glycosides I and II along with stigmasterol and β-sitosterol from leaves; β-sitosterol-β-D-glucoside, 7-keto-sitosterol, stigmasterol, stimast-5-en-3β, 7β-diol, campesterol and 1-triacontanol from seeds of *J. curcas*.

The seeds of *J. curcas* contain toxic constituents like curcine, curcasin and a resinous toxalbumin curcin, with 6.62 % moisture, 18.02 % protein, 38 % fat, carbohydrates 17.98 % , 15.50 % fiber and 4.50 % ash and the seed oil contains 37-68 % oleic acid, 19-41 % linoleic acid and 12-17 % palmitic acid (http://www.novodboard.com/Jatropha doc, 2006).

TOXICOLOGY

Lampe and Fagerstrom (1968) reported hypersensitivity characterized by dermatitis in sensitive individuals following skin exposure to a chemical irritant present in *J. curcas* plant.

Adam (1974) reported high mortality in mice fed 40 to 50 % of *J. curcas* seed in their feed. The important symptoms of poisoning recorded by them were diarrhoea, inability to keep normal posture and depression. The most marked pathological changes observed in the mice were catarrhal enteritis, erosion of intestine, congestion or hemorrhages in small intestine, heart and lung and fatty changes in the liver and kidney.

Adam and Magzoub (1975) carried out toxicity studies of *J. curcas* in goats. Eleven Nubian goats were fed with seeds at doses ranging from 0.25 to 10 gm/kg/day. All dose levels were found to be toxic with fatal consequences within 2 to 21 days. Liver biopsy samples taken 2 days after the start of feeding and subsequent biopsies showed congestion, varying degrees of fatty change, considerable reduction in glycogen content

and necrosis of the hepatocytes. Lack of appetite, reduced water consumption, diarrhoea, dehydration, sunken eyes and a steadily deteriorating condition were reported as the important clinical signs of *Jatropha* intoxication in goats. In all animals there was a decrease in the level of glucose and a marked rise in the concentration of arginase and glutamate oxaloacetate transminase (GOT) in the serum. Post-mortem examination revealed haemorrhage in the rumen, reticulum, kidney, spleen and heart, catarrhal or haemorrhagic abomasitis and enteritis, congestion and oedema of the lung and excessive fluid in serous cavities.

Stirpe *et al.* (1976) conducted a comparative oral dosing toxicity study of curcin and crotin (*Croton tiglium*) in mice. They found that curcin, as compared to *C. tiglium* had slightly more rapid action with symptoms beginning at 12 hours and most of the deaths occurring with in 48 hours of poisoning. The toxicity signs were similar in both the toxicities except, that the neurological signs (waddling, fine-tremors, rocking occasionally convulsion etc) were more severe among the mice treated with crotin. The post-mortem examination showed degenerative lesions in liver, pancreas and spleen along with hyperemia of intestine, some times ascites. The whole picture of toxicity of curcin and crotin resembled that of rats poisoned with ricin.

Ahmed and Adam (1979a) evaluated toxicity of *J. curcas* in calves orally administered the water (stomach tube) in which *Jatropha* seeds were suspended at single doses of 2.5, 1 or 0.25 gm/kg in six calves and to two other calves at 0.025 gm/kg daily for 10 or 14 days. They reported that onset of toxic signs in the six calves given a single dose was rapid followed by death with in 19 hours. The two calves that received daily doses developed toxic signs and died after 10 and 14 days. The clinical signs exhibited by the animals such as diarrhoea, dyspnoea, dehydration and loss of condition were found well correlated with the pathological findings. Further, the authors recorded increases in

aspartate aminotransferase, ammonia and potassium and decreases in total protein and calcium in the serum of affected calves.

Ahmed and Adam (1979 b) reported toxicity of *J. curcas* in desert sheep and Nubian goats fed its seeds at 0.05, 0.5 and 1gm/kg/day. They recorded diarrhoea, reduced water consumption, dehydration, sunken eyes, inappetence and loss in condition as the important signs in both sheep and goats. The main pathological changes noted were haemorrhage in rumen, reticulum, lungs, kidneys and heart, catarrhal and/ or haemorrhagic enteritis, hepatic fatty changes, pulmonary congestion and oedema with straw coloured fluid in serous cavities. An increase in the concentrations of aspartate aminotransferase, ammonia, potassium and sodium and decreases in total protein and calcium were also detected in the blood serum of the animals.

Barri *et al.* (1983) conducted toxicity studies on *J. aceroides* (a plant very much similar to *J.curcas*) in sheep and goats, where the animals were fed the plant material at oral doses of 0.5 to 10 gm/kg/day. They reported death of the animals at both the dose levels between day one and 2 weeks of treatment. The clinical, haematological and pathological changes in *J. aceroides* toxicity were attributed to reduced ability of liver to synthesize protein. Renal dysfunction and haemoconcentration were also observed. The important post-mortem and histological findings observed by the authors included lesions in the liver, pancreas and spleen; hyperemia of the intestine and some ascites.

Joubert *et al.* (1984) based on an *in vitro* study showed that a phytotoxin of *J. curcas* seeds caused agglutination of erythrocytes. They did also report a case of acute poisoning of purging nut in children.

Abdu-Aguye *et al.* (1986) also reported *J. curcas* seed poisoning in children, where the toxicity signs such as restlessness, drowsiness, vomition, diarrhoea and moderate

dehydration were recorded. The laboratory examinations revealed normal haemoglobin, normal liver function tests and mild alkalosis among the affected children. The authors also recorded toxicity of *J. curcas* seeds in mice, which were fed diets mixed with the seeds. Post-mortem examination revealed infarction of various parts of the gastrointestinal tract with congested vessels. Sodium chloride solution (150 mmol saline) extract of the dried seed administered intraperitoneally in mice caused death at doses as low as 1 mg/kg. Post-mortem studies in this case also showed widespread haemorrhages involving the colon, lungs as well as infarction of the liver. Larger intraperitoneal doses (greater than 30 mg/kg) were lethal rapidly, but not associated with gross gastrointestinal haemorrhage.

Fojas *et al.* (1986) reported that the extract of the leaves of *J. curcas* showed potent cardiovascular action in guinea pigs and might be possible source of β blocker agents.

El-Badwai *et al.* (1992) studied toxicity of low level dietary intake of *J. curcas* seeds in Brown Hisex chicks. The seeds at 0.1 and 0.5% to level in feed resulted in reduced growth rate, hepatonephropathies and widespread hemorrhages and congestion in internal organs in the chicks.

El-Badwai *et al.* (1995) carried out comparative toxicity study of *Ricinus communis* and *J. curcas* in Brown Hisex chicks fed diets containing 0.5 % of seeds of the plants. Symptoms, lesions and changes in growth, haematology and clinical chemistry were also investigated in Brown Hisex chicks. High mortality and more severe changes occurred in chicks on *Ricinus* diet than *Jatropha* feed.

Goonasekera *et al.* (1995) investigated fertility-regulating effects of *J. curcas* fruit extracts in pregnant rats orally administered @ 0.12 to 3.13 gm/kg for different periods. Foetal resorption was observed with methanol, petroleum ether and dichloromethane

extracts indicating abortifacient properties of its fruit. The results also suggested that the interruption of pregnancy occurred at an early stage after implantation. This effect was observed even when the extracts were given from the 6th to the 8th days of pregnancy. Loss of body weight during the dosing period, ranging from slight to severe was seen in the treated animals. Marked toxicity was observed with some extracts when given over a relatively longer period of 10 days or more.

Gandhi *et al.* (1995) studied the toxicity of Ratanjyot oil. The proximate composition of the kernels and physicochemical characteristics of its oil were also determined. The kernels constituted 62% of the seed and contained 52% oil (phorbol esters). A toxic fraction (2.4%) containing the phorbol esters was isolated from the oil. The acute oral LD_{50} of the oil was found to be 6 ml/kg body weight in rats. The oil caused severe diarrhoea and gastro-intestinal inflammation. The isolated toxic fraction, when applied to the skin of rabbits and rats, produced a severely irritant reaction followed by necrosis. The fraction was also found to cause death of mice following dermal application. The oil and the toxic fraction at 25 and 1 mg respectively in 10 ml saline showed haemolytic activity, disrupting the integrity of red blood cells *in vitro*.

Makonnen *et al.* (1997) reported antifertility effect of *J. curas* seeds in guinea pigs. They observed that a crude extract of seeds orally administered @ 0.3 gm/kg daily to mature female albino guinea pigs for one week before mating significantly reduced the number of births. Further they reported that when the extract was administered at the same dose for 10 days before mating and for 27 days after mating resulted in anti-implantation and abortifacient effects. The extract when administered over a period of 27 days was also noted to prolong oestrus cycle in guinea pigs. The weight of the uterus was found reduced in animals treated with the extract, while that of the ovaries did not show any significant change from that of the control.

Oluwole *et al.* (1997) investigated the effects of methanolic extract of *J. curcas* on haematological parameters in male albino rats. The extract was found to cause a progressive reduction in packed cell volume, haemoglobin concentration and red cell count. The reduction in the values of red blood cell counts and haemoglobin concentration were significantly pronounced following 8 to 10 days of treatment. It was suggested that *J. curcas* caused macrocytic hypochromic anaemia in rats. The LD_{50} value of the extract in rats was found as 25.19 mg/kg.

Makkar *et al.* (1998) evaluated non-toxic and toxic varities of *J. curcas* for chemical composition, digestibility, protein degradability and toxic factors. They reported that the meal (defatted kernels) had a CP content of 57.13, 61.9, 56.1 and 64.4% for Cape Verde, Nicargua, Ife-Nigeria and non-toxic Mexico verities, respectively and about 90% of this CP as true protein. The amino acid composition of meals from Cape Verde, Nicargua and non-toxic Mexico varieties was similar. The levels of essential amino acid except lysine were higher than that for the FAO reference protein. The meal from the toxic variety (Cape-Verde) did not have any anti-fermentative activity on rumen microbes. The estimated digestible organic matter (DOM) and ME for the shells were low (26.2-27.1% and 2.4-2.8 MJ/kg), where as these values for *Jatropha* meals were 77.3-78.4% and 10.7-10.9 MJ/kg. Tannins, Cyanogens, glucosinolates and amylase inhibitors were not detected in meals of the 4 varities. A small amount of tannins were present in shells (2.0-2.9%as tannic acid equivalent). High levels of trypsin inhibitor activity, lectin and phytate were observed in the meals. The concentrations of phorbol esters in kernels of Cape Verde, Nicargua and Ife-Nigeria varieties were 2.70, 2.17 and 2.30 mg/g, where as kernels of non-toxic Mexican had a very low level (o.11mg/g) of phorbol esters. Further, they reported *Jatropha curcas* toxicity in rodents, man and livestock. Phorbol esters were identified main toxic agents. The

important clinical signs include lower average metabolic growth rate, faecal mucus production and rejection of feed, after feeding these phorbol esters to the carps.

Adeyemi *et al.* (2001) studied toxic effect of feeding boiled *J. curcas* seeds in chicks. Feeding boiled *J. curcas* seeds to the chicks produced growth depression, hepatonephropathy and hemorrhages in visceral organs. Increasing the concentration of boiled seeds in the diet was noted to decrease feed intake, weight gain and protein efficiency. It also decreased the gross blood features, serum total protein, albumin and globulin accompanied by increased serum creatinine levels.

Singh *et al.* (2002) showed fatal effect of some common Indian plants of Euphorbiaceae family including *Jatropha curcas* on fresh water fish.

The comparative toxicity of *Croton macrostachys, J. curcas* and *Piper abyssinica* seeds was investigated in Nubian goat kids by Abdel- Gadir *et al.* (2003). The kids were treated with *C. macrostachys* seeds at 1 or 0.25 gm/kg/day, *J. curcas* seeds at 1 or 0.25 gm/kg/day and *P. abyssinica* seeds at 1 or 0.25 gm/kg/day. Both oral dose levels of *C. macrostachys* and *J. curcas* seeds were lethal for kids between 7 and 21 days, following bloody diarrhoea, dyspnoea, dehydration, loss in condition, paresis of the hind limbs and recumbency. Lesions noted in the affected animals included widespread haemorrhages and congestion, enterohepatonephrotoxicity, pulmonary haemorrhage, emphysema and cyanosis, tracheal froths, ascites and hydropericardium. These lesions were accompanied by increases in the activity of serum AST and in the concentration of urea, decreases in total protein and albumin, and anaemia and leukopenia.

Poisoning from ingestion of seeds of *Jatropha* plant is very well known in veterinary practice area. Autopsy includes severe gastroenteritis, nephritis, myocardial degeneration, haemagglutination and sub-epicardial sub-endocardial hemorrhage as well as renal sub-

cortical and sub-plural bleeding (http://www.fiuoridealert.org/pesticides/fluorine. eu.report. 2003).

Abd-Elhamid (2004) carried out toxicological and histopathological investigations on the acetonitrile extract from *J. curcas* seeds in comparison to praziquantel, the known antischistosomal drug. On a constant weight dose bases (single dose of 50 mg/kg body weight administered orally to albino rats), the acetonitrile extract showed significant increase in serum AST, ALT and creatinine and non-significant changes in lipid and protein profiles of in rats.

Kulkarni *et al.* (2005) reported accidental poisoning among children manifested predominantly by gastro intestinal signs, headache and fever.

Chapter-III

MATERIALS & METHODS

CHAPTER - III
MATERIALS AND METHODS

The present investigations consisted of screening local variety of *Jatropha curcas* seeds for their toxicological actions in albino rats. The various materials used and methods employed are described in this Chapter.

PLANT MATERIAL

The seeds of *J.curcas* were locally collected in bulk in the month of March-April from the premises of College of Veterinary Science & A.H., Anjora, Durg campus, The plant species was authenticated after botanical identification (Fig.1). The seeds (Fig. 2) were properly cleaned to free from any extraneous dust or other material. The cleaned seeds were shade dried and reduced to fine powder with the help of an electrical grinder. The seed powder so obtained was stored in air tight containers and used as such for preparation of the extract or experimental diets, whenever required.

De-oiling Process

A part of the powder was de-oiled, for the removal of fat. Twenty- five gm of the powder was taken in a 500 ml capacity conical flask. Two hundred ml of organic solvent diethyl ether was added into the flask and kept in refrigerator for 72 hr for maceration. The contents of the flask was shaken intermittently daily during the maceration period. Meanwhile in this period, the supernatant solvent was separated and again 50 ml of fresh solvent was added to remove any left portion of the fat, if any. At the end of 72 hr, the remaining portion of the solvent was completely expelled from the seed powder.

Preparation of Extracts

The whole and the de-oiled portion of powdered seeds of *J. curcas* were processed to obtain aqueous extracts as described below:

Fig.1: Plant of *Jatropha curcas (Ratanjyot)* with mature fruits

Fig.2: Whole seeds of *Jatropha curcas (Ratanjyot)*

Cold Aqueous Extracts: Twenty-five gm of powder of whole seeds and defatted seeds was taken in 500 ml capacity conical flasks. One hundred ml of water was added into the flasks and kept in refrigerator for 72 hours for maceration. The contents of the flasks was shaken intermittently daily during the maceration period. After the end of 72 hr, the contents of the flasks was filtered through Whatman No. 1 filter paper and its volume was made to 50 ml. The extracts were stored in air tight bottles and preserved in refrigerator for use whenever required.

Hot Aqueous Extract: Twenty-five gm of whole seed powder was taken in a 500 ml capacity conical flask. Two hundred ml of water was added to the flask and kept under boiling till the water volume was reduced up to 100 ml. After cooling, the contents of the flask were filtered through Whatman No.1 filter paper. The volume of the filtrate was made up to 50 ml and stored in air tight bottle in a refrigerator for use whenever required.

PREPARATION OF EXPERIMENTAL ANIMAL DIETS

The experimental diets for feeding to the animals were prepared after consulting with the Department of Animal Nutrition, College of Veterinary Science & AH, Anjora, Durg. The control diet (standard diet) provided 22% CP containing normal feed ingredients. The modified diets were prepared by replacing the normal feed ingredients to an extent of 50 and 25% of the CP with *J. curcas* whole seed powder. The composition of the experimental diets is shown in Table 1.

Table 1: Composition of experimental diets

Ingredient	Proportion (100 gm)		
	Control Diet	Diet with *Jatropha* protein supplementation (%)	
		25	50
Maize	52.0	29.0	8.0
Soyabean	34.0	20.0	16.0
Rice Polish	6.0	4.0	5.0
Groundnut Cake	7.0	14.0	7.0
***Jatropha* Seed**	**0.0**	**32.0**	**63.0**
Vitamin-Mineral Pre-mix	1.0	1.0	1.0
Total	100	100	100

TOXICITY STUDIES

The toxicity investigations included acute and sub-acute toxicity studies. The hot and cold aqueous extracts of seeds of *J. curcas* were selected for screening their acute toxicity study as described below.

Experimental Animals

Young weaned Wistar rats (60-75 gm) of either sex were obtained from a laboratory animal breeder. The animals were housed in the Lab Animal House attached to the Department of Veterinary Pharmacology and Toxicology under normal housing conditions and fed with standard feed with free access to clean drinking water. After sufficient period of acclimatization to the experimental conditions, the animals were randomly selected for the acute and sub-acute toxicity testing.

Acute Oral Toxicity Study

Acute oral toxicity test was undertaken to find out the nature of toxicity, if any, the intensity of acute toxic potential and to determine the median lethal dose of aqueous extract of whole and de-oiled (defatted) *Jatropha* seed powder.

The acute toxicity was studied in rats using the whole seed powder as well as the defatted seed powder of *J. curcas*. The seed powders were processed to obtain cold and

hot aqueous extracts as explained above. Initially, pilot trials were conducted by orally administering the cold aqueous extracts (whole seeds and defatted seeds) at a random dose level corresponding to 1000 or 3000 mg/kg in one male and female rat. As there was no toxicity in any of the rats, the maximum possible volume for single dosing of the extracts i.e. one ml/rat, corresponding to 7000 mg/kg was administered in two more rats. No mortality was also noted up to 48 hr of post-administration of 7000 mg/kg dose of the extract. Therefore, it was decided to repeat the maximum dose of 7000 mg/kg using 10 rats (five male and five female rats) for the two cold extracts (Groups II and III). The same schedule was repeated using the hot aqueous extract of whole seed powder (Group IV). A control group of similar number of animals, was like-wise administered normal saline @ one ml/rat was simultaneously run (Group I). All the rats were kept under observation for noting change in behaviour and development of toxicity signs and symptoms, if any, up to a period of seven days thereafter. The body weight of the rats in the four groups was recorded prior to dosing of the extracts and on 7th day of post-treatment.

Subacute Toxicity Study

Subacute toxicity test was undertaken to find out the effect of phytotoxins present in *J. curcas* seeds on various systems of the body following repeated exposure at sub-toxic doses. For this purpose, weanling albino rats of either sex were taken. Fifty-two rats were randomly assigned to three groups as summarized in Table 2.

Table 2: Experimental design for subacute toxicity of *J. curcas*. seeds

Group No.	No. of Rats	Treatment *
I	20 (10 male + 10 female)	Normal diet (Control diet)
II	16 (8 male + 8 female)	*Jatropha* protein supplementation (25%)
III	16 (8 male + 8 female)	*Jatropha* protein supplementation (50%)

The first group rats were fed normal standard ration, which served as control group. The second and third group rats were fed modified ration. The toxicity trial was initially designed for a period of 90 consecutive days. The following observations were recorded in the three groups:

a. General Studies:

The general observations which were recorded included, behaviour of the animals, toxic signs and symptoms and feed intake (daily observation) and body weight at weekly intervals. The animals were provided with liberal but measured quantity of the feed daily as per the experimental protocol and water was provided *ad libitum.* Quantity of the feed left over on subsequent day was also measured before providing fresh feed. The mean intake of feed (in gm) per rat per day for each group was calculated week wise. Individual body weight (in gm) were recorded at the beginning of the study and then at weekly intervals thereafter.

b. Biochemical Studies:

The following biochemical parameters were estimated using blood plasma on day 0 (pre-treatment), 15 (mid-treatment) and 21 days (post-treatment).

1. Glucose (mg/dl)
2. Total protein (gm/dl)
3. Creatinine (mg/dl)
4. Glutamate pyruvate transaminase (U/L)
5. Glutamate oxalo-acetate transaminase (U/L)
6. Alkaline phosphatase (U/L)

The blood samples collected either through cardiac puncture or after decapitation were heparinized. The above estimations were carried out by standard procedures using the diagnostic kits (Bayer's Diagnostics, Baroda) with the help of a semi-automated analyzer (RA-50 Chemistry).

c. Haematological Studies:

The haematological parameters were studied using whole blood on day 0 (pre-treatment), 15 (mid-treatment) and 21 days (post-treatment).The blood was collected from retro orbital bleeding method (Talwar, 1983). Heparin was used to collect the blood as an anticoagulant (0.2 mg/ml). The rats were held against a wire grid under the left hand and restricted by holding its tail with right hand. It is immobilized by pressing down on to the back with left hand holding the scalp of neck and exerted traction by forefinger and thumb on the skin close to the eye. Using the right hand, tip of the clean and fine capillary tube was inserted gently in the inner angle of eye. It was slided under the eye ball at 45^0 angel and over the bony socket to rupture the fragile venous capillaries of the ophthalmic venous plexus. The tip of the capillary tube slightly retracted and the blood accumulated in the orbital cavity was collected in a fresh and sterilized glass vial. To have smooth flow of the blood and to avoid clotting, the capillary tube was rotated slightly during bleeding. After collecting the desired volume of blood, capillary tube was removed with simultaneous release of pressure by forefinger and thumb. Any residual blood droplet around the eye ball was wiped off by dry cotton wool. The following haematological parameters were carried out by the standard methods of Jain (1986) and expressed as follows:

1. Total erythrocyte count (TEC; millions/cumm)
2. Total leucocyte count (TLC; thousands/cumm)
3. Haemoglobin (Hb; gm %)
4. Mean corpuscular volume (MCV; cubic microns)
5. Mean corpuscular haemoglobin (MCH; picograms)
6. Mean corpuscular haemoglobin concentration (MCHC; %)
7. Packed cell volume (PCV; %)

In addition blood smears were made from all the animals and DLC was carried out by conventional microscopy. The smears were stained with Leishman stain (Jain, 1986).

d. Pathological Findings:

All the animals which succumbed to the toxicity were critically and closely examined for appearance of gross pathological lesions, if any, in comparison to the sacrificed control group rats. The lesions in the different organs were noted down for each animal separately. The tissue samples, which appear abnormal, were collected in 10 % formal saline solution for histopathology. The routine procedure adopted at Department of Pathology, College of Veterinary Science and AH, Anjora, Durg was employed for histopathological examination. Sections were cut at 3-5 μ and stained with haemotoxylin and eosin (H and E) as per the standard method (Luna, 1968; Culling, 1974).

STATISTICAL ANALYSIS

The data of body weight, feed intake, biochemical and haematological analyses were subjected to ANOVA to find out statistical variation between the mean values of different groups at different intervals of the observation period using the software SPSS 10 for Windows.

Chapter-IV

RESULTS & DISCUSSION

CHAPTER - IV

RESULTS AND DISCUSSION

The present investigations included study of acute and short-term toxicity of *J. curcas* seeds in Wistar rats. The various observations and results are presented and discussed in this Chapter.

ACUTE TOXICITY

Toxicity Signs and Symptoms

The rats administered the cold aqueous extracts of whole seeds or defatted seeds @ 7000 mg/kg showed circling movement after about 15 to 20 min of oral administration followed by depression and remained off feed for about 2 to 3 hr thereafter. The rats in both the groups (II and III) showed blood-tinged loose feces on the next day. All the rats became normal after 2 to 3 days of treatment and there was no mortality up to seven days of post-treatment.

The rats administered the hot aqueous extract of whole seeds (Group IV) were dull and depressed after about 15 to 20 min of administration. All these rats became normal about 4 to 6 hr thereafter and did not show blood-tinged feces. There was also no mortality in this group up to seven days of post-treatment. All the rats in control group remained healthy all through the observation period.

Since there was no mortality at the highest possible dose of the prepared extracts, the acute oral LD_{50} was presumed to be more than 7000 mg/kg. The toxic principle of the seeds may not be water soluble, and therefore, the *J. curcas* seed crude aqueous extracts were considered as relatively non-toxic. There are no reports of acute toxicity of aqueous extract of *Jatropha* seeds in laboratory animals.

Effect on Body Weight

The body weight data of rats treated with acute doses of aqueous extracts of *J. curcas* seeds are represented in Table 3. The pre-treatment body weights of the rats in the four groups 73.6 ± 1.06, 70.2 ± 0.74, 70.4 ± 0.69 and 70.6 ± 0.52 gm respectively. At the end of the post-treatment observation period (seven days) their respective body weights were 78.3 ± 1.53, 75.1 ± 0.72, 76.0 ± 1.04 and 76.3 ± 0.73 gm. However, the variation in the mean body weights between the groups was statistically similar. The gain in body weight on 7th post-treatment day among the four groups ranged between 4.7 and 5.7 gm.

Table 3: Body weight of rats following acute oral administration of *J. curcas* seed aqueous extracts

Group [a] No.	*J. curcas* Seed Aqueous Extract (@7000 mg/kg) [b]	Body Weight (gm) ± SE	
		Pre-treatment	Post-treatment 7th day
I	Normal saline (Control)	73.6 ± 1.06	78.3 ± 1.53
II	Cold: Whole seeds	70.2 ± 0.74	75.1 ± 0.72
III	Cold: Defatted seeds	70.4 ± 0.69	76.0 ± 1.04
IV	Hot: Whole seeds	70.6 ± 0.52	76.3 ± 0.73

a: Each group contained five male and five female rats
b: Administered orally in volume of 1 ml/rat
Variation in mean body weights between different groups is non-significant

SUBACUTE TOXICITY

General Observations

Toxicity Signs and Symptoms

The rats in both the groups which received the *Jatropha* seed protein supplementation (Groups II and III) were dull and depressed from 3rd day of treatment and showed passage of blood-tinged loose feces from 6th post-treatment day onwards. The rats in both the groups became weak and gradual loss of condition. Two rats of Group II (25 % JSPS) and

four rats of Group III (50 % JSPS) died during 12th to 13th day of the trial. Further, two rats of Group III and one rat of Group II succumbed to the toxicity on 16th and 20th days of treatment, respectively. Almost similar toxicity signs and symptoms were reported by other workers. Adam (1974) observed diarrhea, inability to keep normal posture and depression in mice fed on diets containing 40 to 50 % of *J. curcas* seeds. Anorexia, diarrhea, dehydration, sunken eyes and deteriorating condition of goats fed with *J. curcas* seeds @ 0.25 to 10 gm/kg/day up to 2 to 21 days were reported by Adam and Magzoub (1975) and among calves administered orally the seed suspended water @ 0.025 to 2.5 gm/kg/day for 10 or 14 days (Ahmed and Adam, 1979a).

Effect on Growth Rate

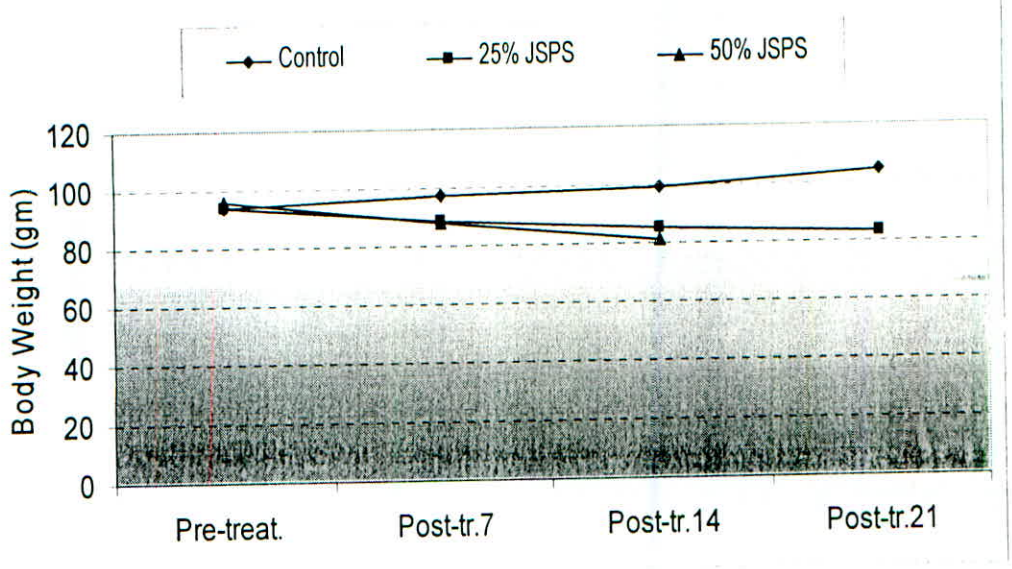
The mean body weight of rats at pre-treatment and at different post-treatment intervals in the control group and those fed *Jatropha* seed protein supplemented groups (II and III) is explained in Table 4 and Fig. 3. At pre-treatment the body weight among the three groups varied between 94.0 ± 4.52 and 96.3 ± 6.52 gm. During post-treatment the body weight in Groups I, II and III varied from 97.1 ± 5.06 to 105.0 + 6.51 gm, 88.7 ± 5.24 to 83.3 ± 9.61 gm and 88.0 ± 5.09 to 81.6 ± 4.01 gm, respectively. The rats in Group I which received the normal feed showed a gradual increase in body weight. Whereas, the rats which received *Jatropha* seed protein supplementation at 25 % (Group II) and 50% (Group III) level in feed exhibited a progressive reduction in their body weight; where the reduction was 11 gm on 21st post-treatment day and 14.7 gm on 14th post-treatment day in these two groups, respectively. Therefore, it is apparent that *Jatropha* protein supplementation exerted a negative balance on the growth rate of the rats. The loss in body weight was related to the level of protein supplementation. However, it was statistically significant in Group III after two weeks of feeding.

Table 4: Effect of feeding *Jatropha curcas* seed protein supplemented diet on body weight of Wistar rats

Group No.	Treatment	Mean Body Weight (gm) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	94.0 ± 4.52 (20)	97.1 ± 5.05 (15)	99.4 ± 5.99 (10)	105.0 ± 6.51 (5)
II	*Jatropha* Protein Supplementation (25% of CP)	94.3 ± 5.66 (15)	88.7 ± 5.24 (15)	85.1 ± 4.82 (8)	83.3 ± 9.61 (3)
III	*Jatropha* Protein Supplementation (50% of CP)	96.3 ± 6.52 (15)	88.0 ± 5.09 (15)	81.6 [a] ± 4.01 (6)	--

Figures in parentheses refer to No. of observations
a: Lower than Group I ($P < 0.05$)

Figure 3: Effect of *Jatropha* seed protein supplementation (JSPS) on growth rate of rats



The retarded growth rate of rats due to *Jatropha* protein replacement may due to reduced feed intake (see below). The *Jatropha* seeds have been reported to possess anti-nutrients such as trypsin inhibitor (s), amylase inhibitor, lectins, phytates, phorbol esters and tannins (Makkar *et al.*, 1998)). The anti-nutritional factors are non-nutrient chemicals present in foods which have undesirable properties with respect to food safety. The anti-nutrients might have contributed to non-utilization (digestion and/or absorption) edible

protein and carbohydrates in the diet. The tannins (also present in *Jatropha* seeds) either form complex with nutrients or interfere with enzymatic action of digestive juices resulting in improper utilization of nutrients (Salunkhe *et al.*, 1982). A steadily deteriorating condition was also observed in Nubian goats fed *J. curcas* seeds @ 0.25 to 10 gm/kg/day up to 21 days (Adam and Magzoub, 1975). Goonasekera *et al.* (1995) also noticed loss in body weight of rats fed *J. curcas* fruit extracts @ 0.12 to 3.13 gm/kg. Several studies also indicated growth reduction in rats (Mole *et al.*, 1993; Pusztai *et al.*, 1992, 1993; Grant *et al.*, 1995; Mori *et al.*, 1998), pigs (Li-Shaoyan *et al.*, 1998) and poultry (Balmar *et al.*, 1999) fed on feeds containing anti-nutrients.

Effect on Feed Intake

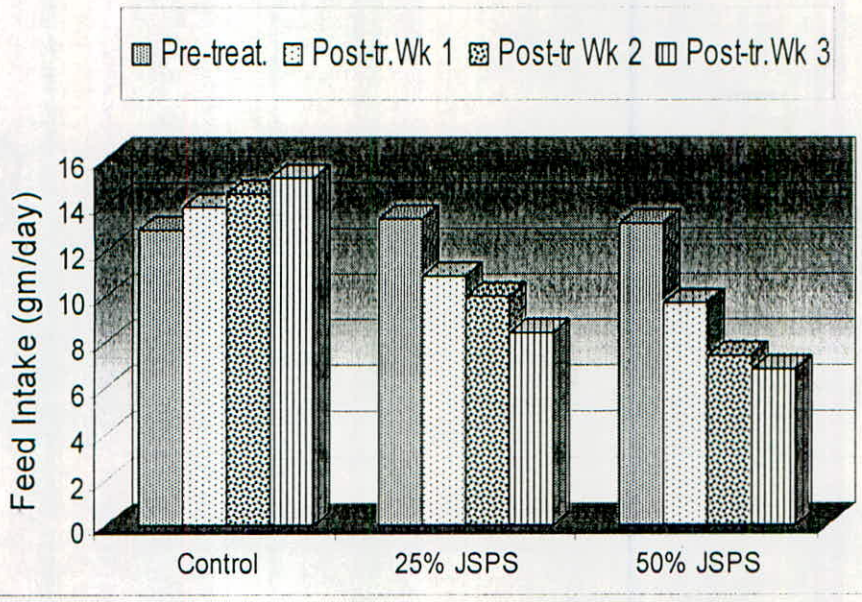
Table 5 and Fig. 4 describe the mean feed intake/day in the three groups during different intervals of the experiment. During pre-treatment the feed intake among the three groups was statistically similar, where it varied between 12.9 ± 0.18 and 13.4 ± 0.14 gm/rat/day. The feed intake by the control group rats on first, second and third post-treatment weeks was 13.9 ± 0.04, 14.5 ± 0.07 and 15.2 ± 0.11 gm, respectively, where it was significantly higher than the pre-treatment level (12.9 ± 0.18 gm). The feed intake by the Group II rats, which received 25 % protein supplementation through *Jatropha* seeds during the three post-treatment weeks, was 10.9 ± 0.05, 10.0 ± 0.09 and 8.4 ± 0.16 gm, respectively, where it was significantly lower than the pre-treatment level (13.4 ± 0.14 gm). The feed intake by the Group III rats, which received 50 % protein supplementation through *Jatropha* seeds during the three post-treatment weeks, was 9.7 ± 0.19, 7.4 ± 0.09 and 6.8 ± 0.25 gm, respectively, where it was significantly lower than the pre-treatment level (13.2 ± 0.08gm). Further, at all intervals, the feed intake in Group II was significantly lower than the respective intervals of the control group and the feed intake in Group III was significantly lower than the respective intervals of Group II and control group rats.

Table 5: Effect of feeding *Jatropha curcas* seed protein supplemented diet on feed intake of Wistar rats

Group No.	Treatment	Mean Feed Intake (gm/day) ± S.E.			
		Pre-treatment	Post-treatment (Weeks)		
			First	Second	Third
I	Normal Feed	12.9 ± 0.18 [a]	13.9 [a, d] ± 0.04	14.5 [a, e] ± 0.07	15.2 [a, f] ± 0.11
II	*Jatropha* Protein Supplementation (25% of CP)	13.4 ± 0.14 [b]	10.9 [b, d] ± 0.05	10.0 [b, e] ± 0.09	8.4 [b, f] ± 0.16
III	*Jatropha* Protein Supplementation (50% of CP)	13.2 ± 0.08 [c]	9.7 [c, d] ± 0.19	7.4 [c, e] ± 0.09	6.8 [c, f] ± 0.25

Means with similar superscripts within rows or columns are significantly different ($P < 0.05$)

Figure 4: Effect of dietary supplementation of *Jatropha* seed protein (JSPS) on feed intake of rats



The reduced feed intake might be due to non-palatability of the diet as a result of supplementation of *Jatropha* seed protein. The fact that reduced feed intake was more pronounced in Group III with 50 % protein supplementation than that in Group II with 25 % of protein supplementation may also concur with the above observation.

Biochemical Profile

The effect of dietary supplementation of *Jatropha* seed protein on various blood plasma biochemical parameters such as glucose, total protein, glutamate pryruvate transaminase, glutamate oxalo-acetate transaminase and alkaline phosphatase of rats were determined. The pre-treatment values (normal/control) were determined by blood collection following decapitation of randomly selected five rats (three males and two females) of the control group. Since no variation is anticipated in the normal biochemical profiles in a homogenous population, the same were considered as the pre-treatment values (in order to avoid further sacrificing 10 animals) and the changes during the post-treatment, if any, were compared with the pre-treatment levels in all the three groups.

Effect on Plasma Glucose

Table 6 and Fig. 5 show the effect of *Jatropha* seed protein supplementation on blood plasma glucose levels in rats. The glucose levels during pre-treatment and at all the three post-treatment intervals were statistically similar, which ranged from 56.2 $\pm$ 1.78 to 57.0 $\pm$ 1.67 mg/dl. Following protein supplementation at 25 % level in the feed with *Jatropha* seed protein, the glucose levels at 7^{th}, 14^{th} and 21^{st} post-treatment intervals were 55.4 $\pm$ 0.50, 52.9 $\pm$ 0.72 and 47.4 $\pm$ 0.30 mg/dl, respectively, where the 21^{st} day glucose level was significantly lower than the pre-treatment level. Following protein supplementation at 50 % level in the feed with *Jatropha* seed protein, the glucose levels at 7^{th} and 14^{th} post-treatment intervals were 54.7 $\pm$ 1.11 and 45.5 $\pm$ 1.21 mg/dl, respectively, where the 14^{th} day glucose level was significantly lower than the pre-treatment level. At pre-treatment and on 7^{th} day post-treatment the glucose levels in the three groups were statistically at par. The glucose level at 21^{st} day post-treatment in Group II was significantly lower than the level of Control Group (I). Similarly, the 14^{th} day post-treatment

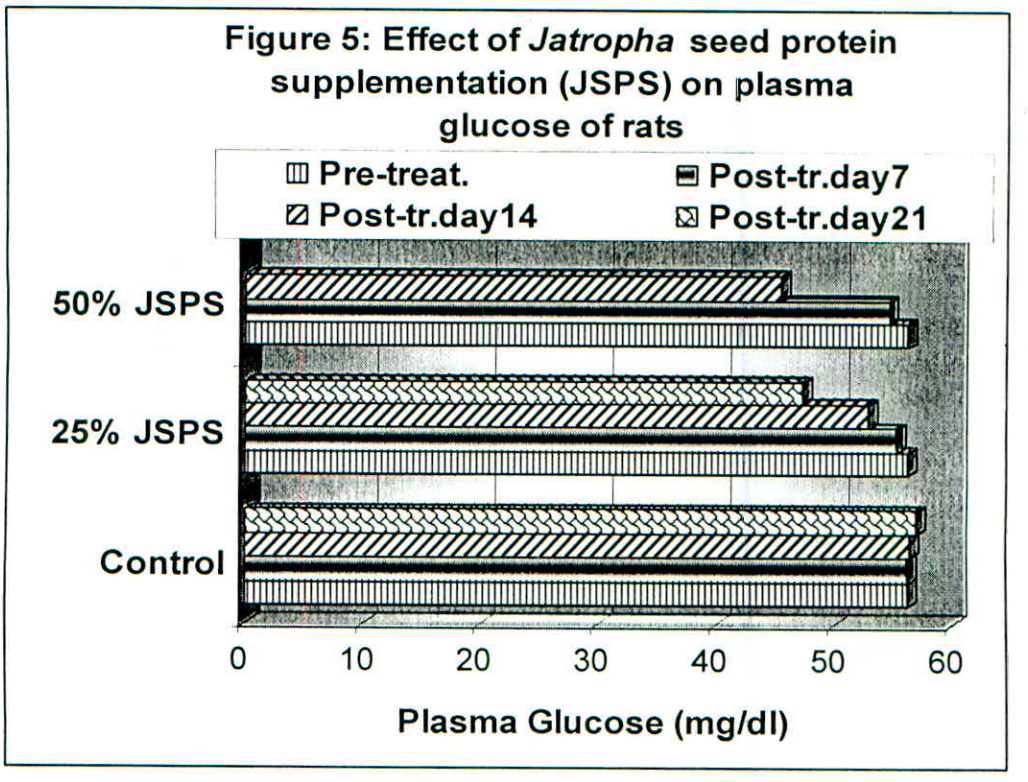
glucose level in Group III was also significantly lower than the 14th post-treatment glucose level of control group and Group II.

The reduced blood sugar levels of rats following *Jatropha* seed protein supplementation might be due to impairment of carbohydrate digestion due to presence of anti-nutrient amylase inhibitor in the seeds and absorption of glucose. The amylase inhibitors (tannins/polyphenols) in legume feeds were also reported to hinder carbohydrate (starch) digestion and glucose metabolism in animals (Glik and Joslyn, 1970; El-Sayed *et al.*, 1997). Decrease in hepatic glycogen (liver biopsy) and reduced blood sugar levels were also observed in Nubian goats fed *J. curcas* seeds @ 0.25 to 10 gm/kg/day (Adam and Magzoub, 1975).

Table 6: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma glucose of Wistar rats

Group No.	Treatment	Mean Plasma Glucose (mg/dl) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	56.2 ± 1.78	56.3 ± 1.95 (5)	56.5 ± 1.98 (5)	57.0 ± 1.67 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		55.4 ± 0.50 (5)	52.9 ± 0.72 (4)	47.4 [a, c] ± 0.30 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		54.7 ± 1.11 (5)	45.5 [b, d] ± 1.21 (4)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment and post-treatment 7th and 14th days ($P < 0.05$)
b: Lower than pre-treatment and post-treatment 7th day ($P < 0.05$)
c: Lower than Group I ($P < 0.05$)
d: Lower than Groups I and II ($P < 0.05$)



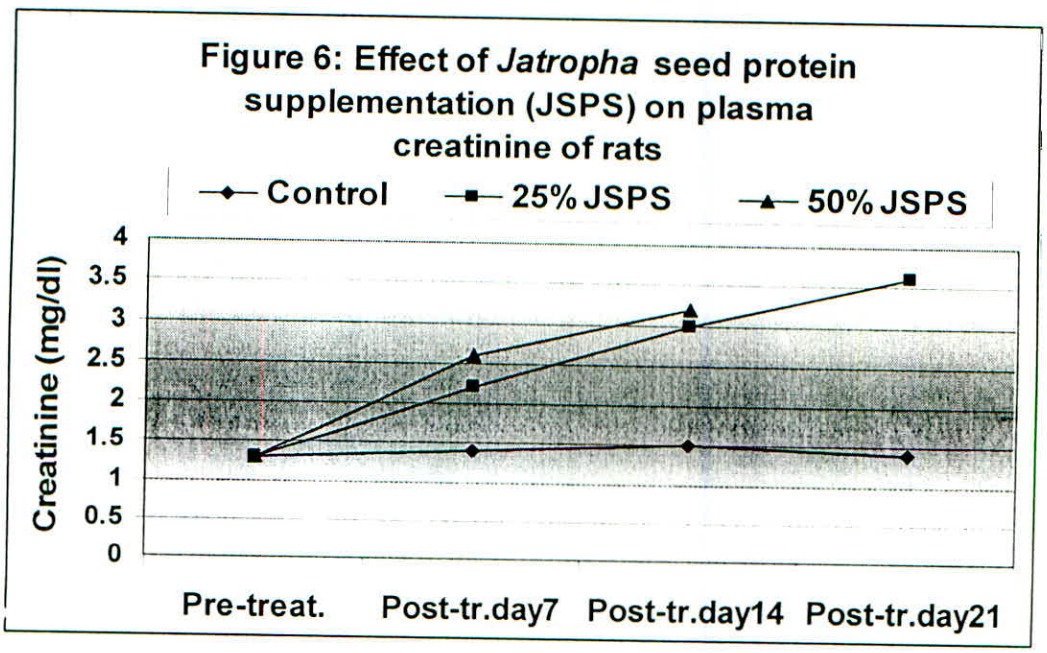
Effect on Plasma Creatinine

The effect of *Jatropha* protein supplementation in feed on blood plasma creatinine levels in the three groups of the experimental animals is summarized in Table 7 and Fig. 6. In control group the creatinine levels during the 7th to 21st post-treatments (1.4 ± 0.21 to 1.5 ± 0.23 mg/dl) were identical to the pretreatment level of 1.3 ± 0.24 mg/dl. The rats in Group II (25% *Jatropha* protein supplementation) showed creatinine levels in the range of 2.2 ± 0.17 to 3.6 ± 0.20 mg/dl which were significantly higher than the pre-treatment level and their 14th or 21st post-treatment day creatinine levels were also higher than the 7th post-treatment level. In Group III (50% *Jatropha* protein supplementation) the creatinine levels at both the post-treatment intervals were also significantly higher than pre-treatment level (1.3 ± 0.24 mg/dl). The 14th and 21st post-treatment levels in Group II and 7th and 14th post-treatment levels in Group III were significantly higher than control group (I).

Table 7: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma creatinine of Wistar rats

Group No.	Treatment	Mean Plasma Creatinine (mg/dl) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	1.3 ± 0.24	1.4 ± 0.26 (5)	1.5 ± 0.23 (5)	1.4 ± 0.21 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		2.2 [a] ± 0.17 (5)	3.0 [b,c] ± 0.19 (4)	3.6 [b,c] ± 0.20 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		2.6[a,c] ± 0.28 (5)	3.2 [a,c] ± 0.20 (4)	--

Figures in parentheses refer to No. of observations
a: Higher than pre-treatment ($P < 0.05$)
b: Higher than pre-treatment and post-treatment 7th day ($P < 0.05$)
c: Higher than Group I ($P < 0.05$)



The blood creatinine or urea nitrogen levels are indicative of renal function (Owen, 1991; Finco, 1997)). The elevated plasma creatinine levels are indicative of renal impairment following *Jatropha* seed protein supplementation. The histopathological observation of degenerative changes in kidney (see below) also corroborates elevated plasma creatinine levels. Renal dysfunction or nephropathy was also reported in short-

term oral toxicity with *J. curcas* seeds in chicks (El-Badwai *et al.*, 1992) or rodents or livestock (Makkar *et al.*, 1998).

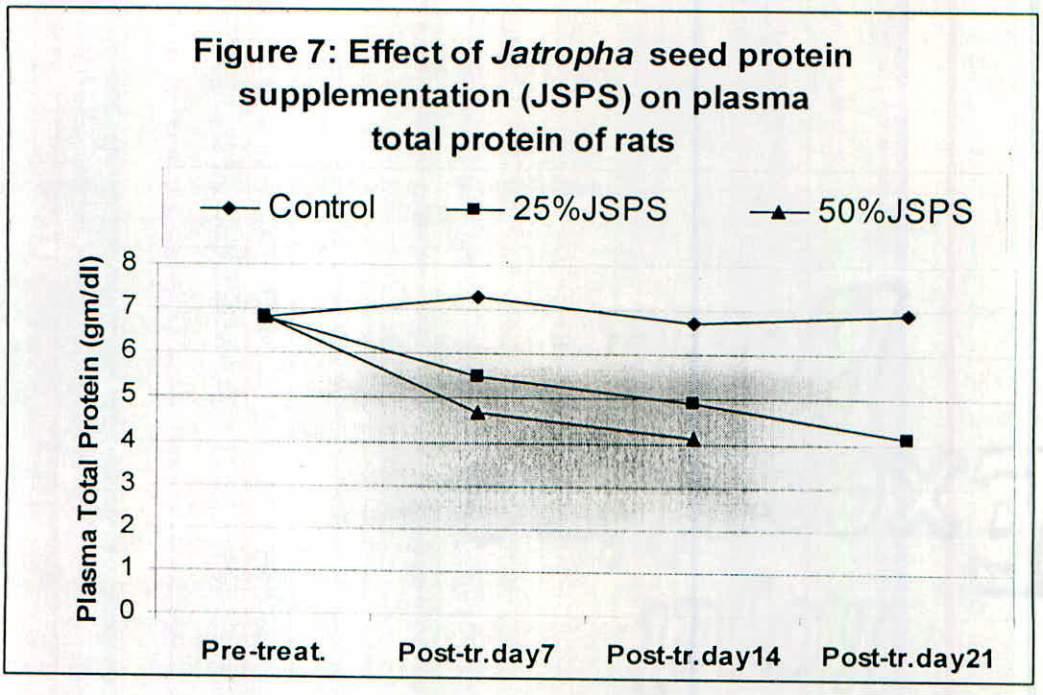
Effect on Plasma Protein

Table 8 and Fig. 7 explain the effect of *Jatropha* seed protein supplementation on the plasma total protein level of rats. The protein levels during pre-treatment (6.8 ± 0.35 gm/dl) and at the three post-treatment intervals were statistically at par (6.7 ± 0.30 to 7.3 ± 0.19 gm/dl). Following 25 % *Jatropha* protein supplementation (Group II) the variation between pre-treatment and different post-treatment intervals was significant, where the levels on day 7th (5.5 ± 0.36 gm/dl) and day 14th (4.9 ± 0.41 gm/dl) of post-treatment were significantly lower than at pre-treatment and the 21st post-treatment level (4.1 ± 0.05 gm/dl) was also lower than that of pre-treatment and post-treatment 7th day. The protein levels of Groups II and III were lower during post-treatment as compared to respective levels of control group (I). Further, the 7th and 14th days' protein levels of Group III were also significantly lower than the respective levels of Group II.

Table 8: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma total protein of Wistar rats

Group No.	Treatment	Mean Plasma Protein (gm/dl) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	6.8 ± 0.35	7.3 ± 0.19 (5)	6.7 ± 0.30 (5)	6.9 ± 0.26 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		5.5 a, c ± 0.36 (5)	4.9 a, c ± 0.41 (4)	4.1 a, b, c ± 0.05 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		4.7 a, d ± 0.16 (5)	4.1 a, d ± 0.07 (4)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment ($P < 0.05$) b: Lower than post-treatment 7th day ($P < 0.05$)
c: Lower than Group I ($P < 0.05$) d: Lower than Groups I and II ($P < 0.05$)



The hypoproteinaemic effect of *Jatropha* seed protein supplementation may also correlated to presence of anti-nutrients such as trypsin inhibitor and tannins in the *Jatropha* seeds. The trypsin inhibitor interferes with digestion of dietary protein (Liner, 1982; Salunkhe, 1982) and the tannins complex with and inactivate proteins in general, including the digestive enzymes that help in protein digestion and utilization (Arora and Luthra, 1974; Satwadhar *et al.*, 1981; Barroga *et al.*, 1985). Reduced plasma protein levels were also recorded in calves following repeated exposure through oral ingestion to *J. curcas* seed soaked water @ 0.025 gm/kg/ day for 10 to 14 days (Ahmed and Adam, 1979a) or in Nubian goats and desert sheep fed the seeds @ 0.05 to 1 gm/kg/day (Ahmed and Adam, 1979 b). Barri *et al.* (1983) also attributed reduced ability of liver to synthesize protein as the cause of clinical, haematological and pathological changes in *J. aceroides* (another species of *Jatropha*) toxicity in sheep and goats fed the plant material @ 0.5 to 10 g/kg/day for one or two weeks.

Effect on Transaminases

The investigations included estimation of plasma glutamate pyruvate transaminase (PGPT) and glutamate oxalo-acetate transaminase (PGOT).

PGPT: Table 9 and Fig. 8 illustrate the effect of dietary supplementation of *Jatropha* seed protein at 25 and 50 % levels on PGPT of rats.

Table 9: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma glutamate pyruvate transaminase activity of Wistar rats

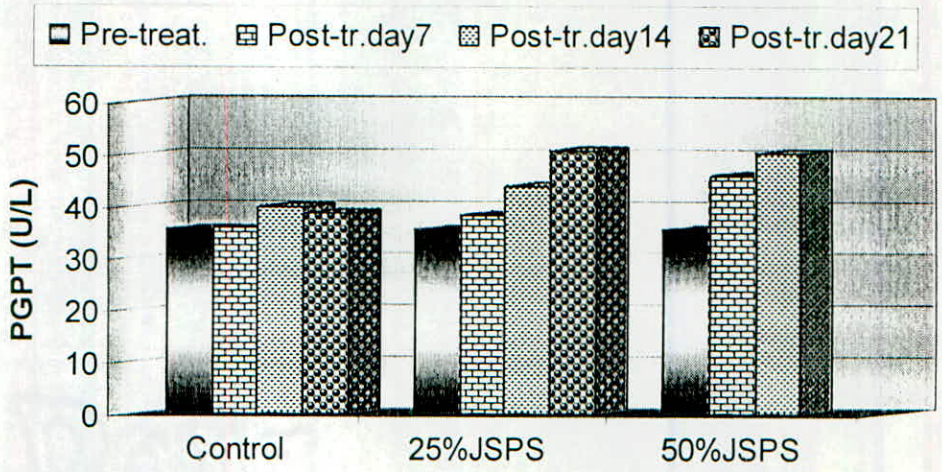
Group No.	Treatment	Mean Plasma Glutamate Pyruvate Transaminase (U/L) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	35.6 ± 0.67 (5)	35.8 ± 0.73 (5)	40.0 ± 1.70 (5)	38.8 ± 0.86 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		38.4 ± 1.16 (5)	44.1 [a, b] ± 1.95 (4)	50.6 [c, d] ± 1.76 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		45.8 [a, e] ± 0.96 (5)	50.5 [a, b, e] ± 0.64 (4)	--

Figures in parentheses refer to No. of observations
a: Higher than pre-treatment ($P < 0.05$)
b: Higher than post-treatment 7th day ($P < 0.05$)
c: Higher than pre-treatment and post-treatment 7th and 14th days ($P < 0.05$)
d: Higher than Group I ($P < 0.05$)
e: Higher than Groups I and II ($P < 0.05$)

In control group (I) the enzyme activity at different post-treatment intervals (35.8 ± 0.73 to 40.0 ± 1.70 U/L) were statistically similar to the pre-treatment activity (35.6 ± 0.67 U/L). In Group II, where 25 % protein supplementation was done the PGPT level at 14th and 21st post-treatment days were significantly higher (44.1 ± 1.95 and 50.6 ± 1.76 U/L, respectively) than at pre-treatment or on 7th post-treatment day (38.4 ± 1.16 U/L). In Group III, where 50 % protein supplementation was done the PGPT levels at 7th and 14th post-treatment days were significantly higher (45.8 ± 0.96 and 50.5 ± 0.64 U/L,

respectively) than at pre-treatment (35.6 ± 0.67 U/L) and the 14th post-treatment activity was also higher than that of 7th post-treatment day. The day 21st post-treatment enzyme activity in Group II was significantly higher than the respective level of control group. Further, the 7th and 14th days post-treatment enzyme activity of Group III was also significantly higher than the respective values of control group and Group II.

Figure 8: Effect of *Jatropha* seed protein supplementation (JSPS) on plasma glutamic pyruvate transaminase of rats



PGOT: Table 10 and Fig. 9 illustrate the effect of dietary supplementation of *Jatropha* seed protein at 25 and 50 % levels on PGOT of rats. In control group (I) the enzyme activity at different post-treatment intervals (41.2 ± 1.39 to 45.2 ± 1.59 U/L) were statistically similar to the pre-treatment activity (36.8 ± 2.70 U/L). In Group II, where 25 % of protein supplementation was done the PGOT level at 14th (51.0 ± 0.91 U/L) was significantly higher than pre-treatment (36.8 ± 2.70 U/L) and post-treatment 7th day (37.4 ± 1.77 U/L) and the PGOT activity on 21st post-treatment day (71.6 ± 2.02 U/L) was significantly higher than pre-treatment and post-treatment 7th and 14th days. In Group III, where 50 % protein supplementation was done the PGOT levels at 7th post-treatment day (46.0 ± 0.94 U/L) were significantly higher than that of pre-treatment and the 14th post-treatment activity was also significantly higher (72.7 + 2.17 U/L) than at pre-treatment and post-treatment 7th day. The enzyme activity in Group II on 14th and 21st post-treatment

days was significantly higher than that of control group. Similarly the 7th and 14th post-treatment PGOT activities of Group III were also significantly higher the activities in Group II and control group (I).

Table 10: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma glutamate oxalo-acetate transaminase activity of Wistar rats

Group No.	Treatment	Mean Plasma Oxalo-acetate Transaminase (U/L) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	36.8±2.70 (5)	41.2 ±1.39 (5)	42.6 ±0.97 (5)	45.2 ±1.59 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		37.4 ±1.77 (5)	51.0 [a, d] ±0.91 (4)	71.6 [b, d] ± 2.02 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		46.0 [c, e] ±0.94 (5)	72.7 [a, e] ±2.17 (4)	--

Figures in parentheses refer to No. of observations
a: Higher than pre-treatment and post-treatment 7th day ($P < 0.05$)
b: Higher than pre-treatment and post-treatment 7th and 14th days ($P < 0.05$)
c: Higher than pre-treatment ($P < 0.05$)
d: Higher than Group I ($P < 0.05$)
e: Higher than Groups I and II ($P < 0.05$)

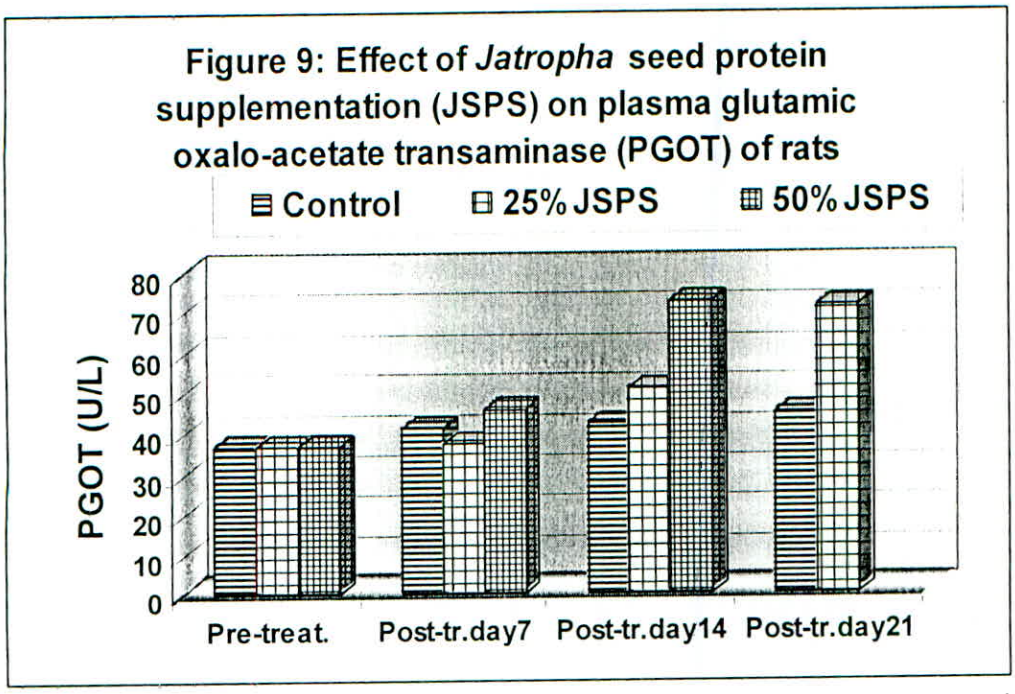


Figure 9: Effect of *Jatropha* seed protein supplementation (JSPS) on plasma glutamic oxalo-acetate transaminase (PGOT) of rats

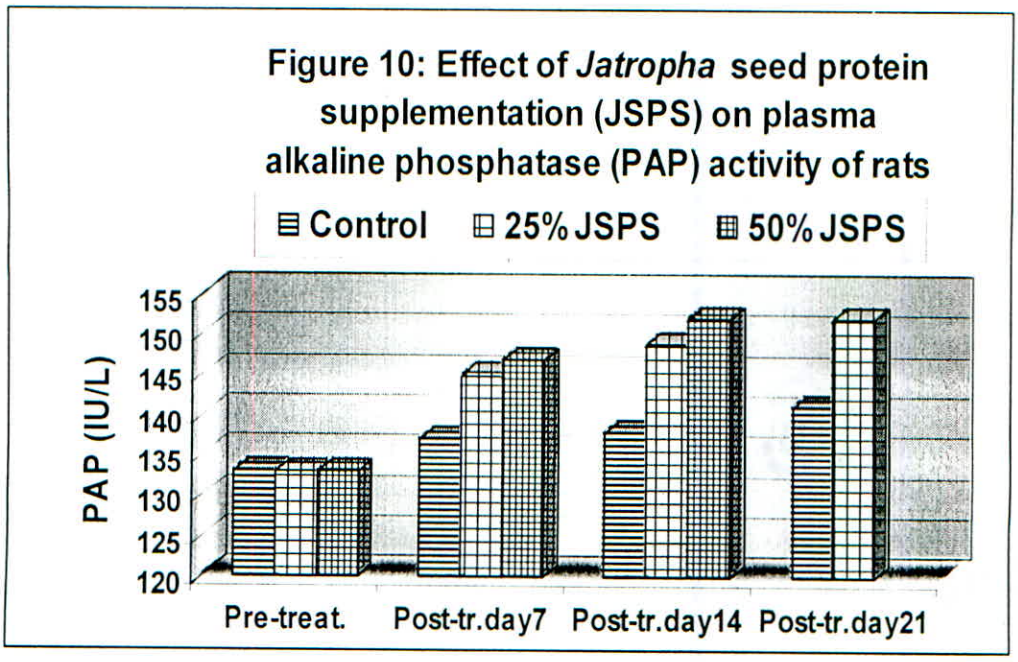
Alkaline Phosphatase

The plasma alkaline phosphatase (PAP) activity of rats in control as well as in *Jatropha* seed protein supplemented groups is explained in Table 11 and Fig. 10. The PAP activity in control group during pre-treatment and post-treatment intervals was statistically identical which ranged between 133.0 ± 1.48 and 141. 0 ± 2.02 U/L. The enzyme activity in Group II (25 % protein supplementation by *Jatropha* seed) the PAP at the three post-treatment intervals (144.6 ± 1.02 U/L to 152.0 ± 2.08 U/L) was significantly higher than that of pre-treatment. Further, the PAP levels at 14th and 21st post-treatment days in Group II were also significantly higher than the PAP level at 7th post-treatment day. The enzyme activity in both the *Jatropha* seed protein supplemented groups at the post-treatment intervals was significantly higher than the respective PAP activities of control group.

Table 11: Effect of feeding *Jatropha curcas* seed protein supplemented diet on plasma alkaline phosphatase activity of Wistar rats

Group No.	Treatment	Mean Plasma Alkaline Phosphatase Activity (U/L) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	133.0±1.48 (5)	136.8 ±0.73 (5)	137.6 ±1.43 (5)	141.0 ±2.02 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		144.6 a,c ±1.02 (5)	149.2 b,c ±1.10 (4)	152.0 b,c ±2.08 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		146.6a,c ±2.61 (5)	152.0a,c ±1.77 (4)	--

Figures in parentheses refer to No. of observations
a: Higher than pre-treatment ($P < 0.05$)
b: Higher than pre-treatment and post-treatment 7th day ($P < 0.05$)
c: Higher than Groups I ($P < 0.05$)



The elevated serum or plasma transaminases (GPT and GOT) and alkaline phosphatase activities is suggestive of hepatic impairment (Denman *et al.*, 1983; Tennant, 1997), as these enzymes are organ specific in rats. The histopathological examination indicating degenerative changes in liver sections of rats that succumbed to toxicity of *Jatropha* seed protein supplementation support the above results. Marked rise in serum arginase and GOT was also reported in Nubian goats fed *J. curcas* seeds @ 0.25 to 10 g/kg/day up to 21 days (Adam and Magzoub, 1975) or in calves orally administered water in which *J. curcas* seeds were suspended @ 0.25, 1 or 2.5 gm/kg within 10 or 14 days (Ahmed and Adam, 1979 a) or in desert sheep fed the seeds @ 0.05, 0.5 and 1 gm/kg/day (Ahmed and Adam, 1979 b). While investigating the short-term oral toxicity (14 days) of crude extract of some plants of *Euphorbia* Adedapo *et al.* (2004) also reported significant elevation in serum transaminase activities (GOT and GPT).

Haematological Profile

The effect of *Jatropha* seed protein supplementation on haematology profile of rats was investigated. The various haematology parameters included were cell counts (red blood cells, leucocytes and differential leucocytes), haemoglobin, PCV, MCV, MCH and MCHC.

Effect on Red Blood Cell Count:

Table12 and Fig. 11 illustrate the effect of feeding *Jatropha* seed protein supplemented diet on the red blood cell (rbc) count of rats. The mean rbc count in the control group (I) during pre-treatment and at different post-treatment intervals varied from 7.5 $\pm$ 0.38 to 8.0 $\pm$ 0.26 millions/cumm and the variation between different intervals was insignificant. In Group II (25 % *Jatropha* protein supplementation) the rbc counts at 14th and 21st post-treatment intervals were 6.1 $\pm$ 0.34 and 5.2 $\pm$ 0.24 millions/cumm, respectively, which were significantly lower than the pre-treatment count. Further, in this group the rbc count at 21st day post-treatment was also significantly lower than the count at 7th post-treatment day. In Group III (50 % *Jatropha* protein supplementation) the rbc count at 7th and 14th post-treatment days (5.6 $\pm$ 0.21 and 5.1 $\pm$ 0.17 millions/cumm, respectively) were significantly lower than pre-treatment. The rbc counts at the three post-treatment intervals of Groups II and III were significantly lower than the respective counts of control group. Similarly, the 14th post-treatment rbc count of Group III was also significantly lower than the 14th post-treatment count of Group II.

Table 12: Effect of feeding *Jatropha curcas* seed protein supplemented diet on total red blood cell count of Wistar rats

Group No.	Treatment	Mean Red Blood Cell Count (million/cumm) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	7.5 ± 0.38 (5)	7.6 ± 0.31 (5)	7.9 ± 0.15 (5)	8.0 ± 0.26 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		6.2 [c] ± 0.50 (5)	6.1 [a, c] ± 0.34 (4)	5.2 [b, c] ± 0.24 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		5.6 [a, c] ± 0.21 (5)	5.1 [a,d] ± 0.17 (4)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment ($P < 0.05$)
b: Lower than pre-treatment and post-treatment 7th day ($P < 0.05$)
c: Lower than Group I ($P < 0.05$)
d: Lower than Groups I and II ($P < 0.05$)

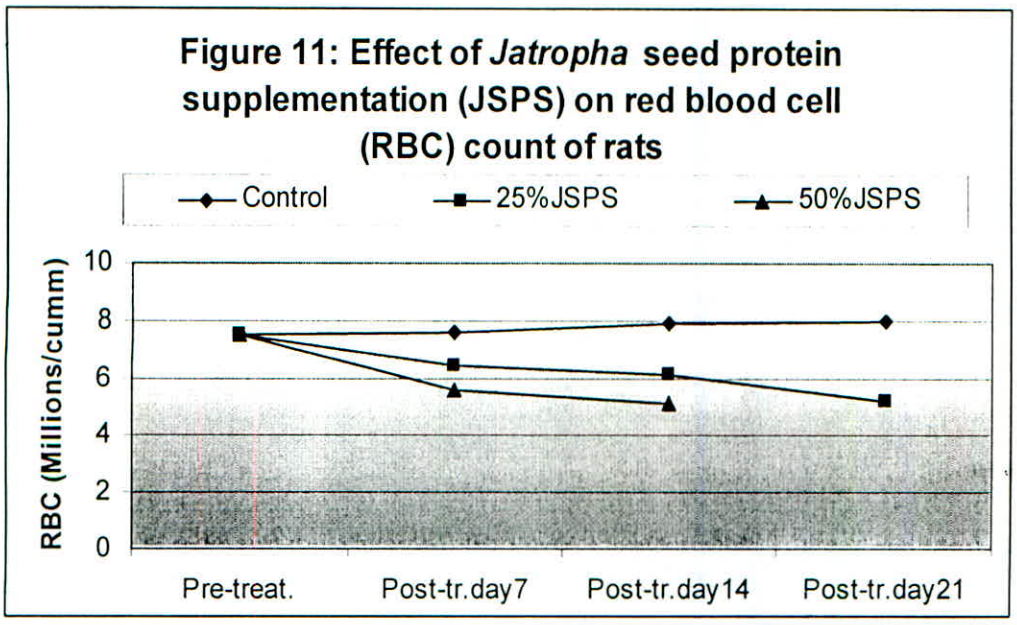


Figure 11: Effect of *Jatropha* seed protein supplementation (JSPS) on red blood cell (RBC) count of rats

The reduction in red blood cell count of rats on *Jatropha* seed protein supplementation suggests inhibitory effect on erythropoiesis by the phytotoxins and anti-nutrients present in the seeds.

Effect on Haemoglobin

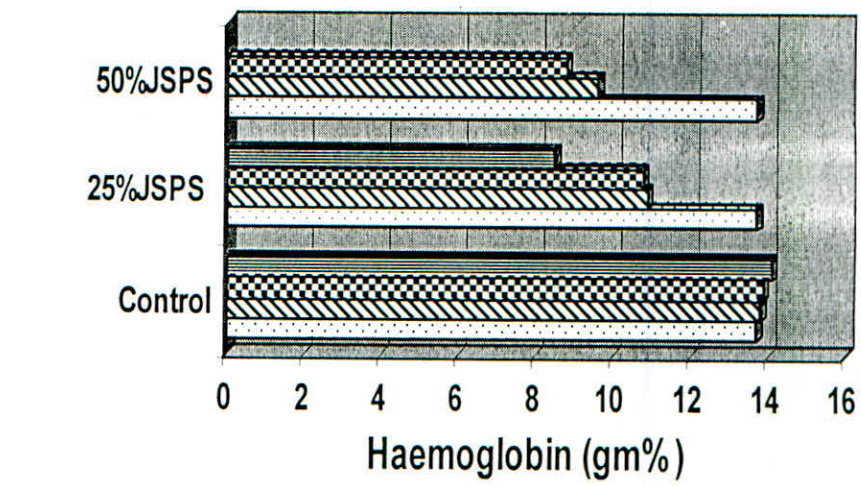
The effect of *Jatropha* feed supplementation on haemoglobin levels of rats are explained through Table 13 and Fig. 12. The mean haemoglobin level in the control group (I) during pre-treatment and at different post-treatment intervals varied from 13.7 ± 0.57 to 14.1 ± 0.55 gm % and the variation in haemoglobin levels between different intervals was insignificant. The haemoglobin levels in Group II (25 % protein supplementation) during the three post-treatment intervals were 11.1 ± 0.71, 10.6 ± 0.69 and 8.5 ± 0.29 gm %, respectively, which were significantly lower than the pretreatment level of 13.7 ± 0.57 gm %. The mean haemoglobin levels in Group III at 7th (9.6 ± 0.23 gm %) and 14th (8.8 ± 0.43 gm %) post-treatment intervals were also significantly lesser than the pre-treatment haemoglobin level. The post-treatment haemoglobin levels of Group II were significantly lower than the respective haemoglobin levels of control group. Similarly, the post-treatment levels of Group III were also significantly lesser than those of control (I) as well as 25 % protein supplemented group (II).

Table 13: Effect of feeding *Jatropha curcas* seed protein supplemented diet on haemoglobin of Wistar rats

Group No.	Treatment	Mean Haemoglobin (gm %) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	13.7 ± 0.57 (5)	13.8 ± 0.50 (5)	13.9 ± 0.55 (5)	14.1 ± 0.55 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		11.1 $^{a, b}$ ± 0.71 (5)	10.6 $^{a, b}$ ± 0.69 (4)	8.5 $^{a, b}$ ± 0.29 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		9.6 ^{a,c} ± 0.23 (5)	8.8 ^{a,c} ± 0.43 (4)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment ($P < 0.05$)
b: Lower than Group I ($P < 0.05$)
c: Lower than Groups I and II ($P < 0.05$)

Figure 12: Effect of *Jatropha* seed protein supplementation (JSPS) on haemoglobin of rats



The reduction in haemoglobin of rats fed on *Jatropha* seed protein supplemented feed may be due to reduction in absorption of dietary iron, since tannic acid and phytates interfere with absorption of minerals and metals in intestines (Salunkhe *et al.*, 1982).

Effect on Packed Cell Volume

Table 14 and Fig 13 summarize the effect of *Jatropha* seed protein supplementation on packed cell volume of (PCV) of rats. The mean PCV values at pre-treatment and during the entire post-treatment were statistically at par (41.4 ± 1.93 to 45.4 ± 0.40 %). The mean PCV values of Group II at 7th, 14th and 21st post-treatment intervals were 37.0 ± 1.78, 36.5 ± 1.55 and 31.6 ± 0.88 % respectively. The PCV values in Group III at 7th and 14th post-treatment intervals were 32.0 ± 0.89 and 31.7 ± 1.18 % respectively. *Jatropha* seed protein supplementation at 25 and 50 % levels resulted in significant reduction in the PCV, where the reduction in Group II was significantly different compared to control group and in Group III as compared to

respective PCV levels in control group or Group II. Moreover, the 21st day post-treatment level in Group II and 7th and 14th post-treatment days' level in Group III were also significantly lower than the pretreatment levels. The reduction in PCV was due to reduction in red blood cells of the *Jatropha* seed protein rats. Oluwole *et al.* (1997) also reported progressive reduction in PCV, haemoglobin level and red blood cell count in rats fed methanolic extract of *J. curcas* seeds.

Table 14: Effect of feeding *Jatropha curcas* seed protein supplemented diet on packed cell volume of Wistar rats

Group No.	Treatment	Mean Packed Cell Volume (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	41.4 ± 1.93 (5)	42.2 ± 1.39 (5)	44.4 ± 0.42 (5)	45.4 ± 0.40 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		37.0 [b] ± 1.78 (5)	36.5 [b] ± 1.55 (4)	31.6 [a, b] ± 0.88 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		32.0 [a, c] ± 0.89 (5)	31.7 [a, c] ± 1.18 (4)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment ($P < 0.05$) b: Lower than Group I ($P < 0.05$)
c: Lower than Groups I and II ($P < 0.05$)

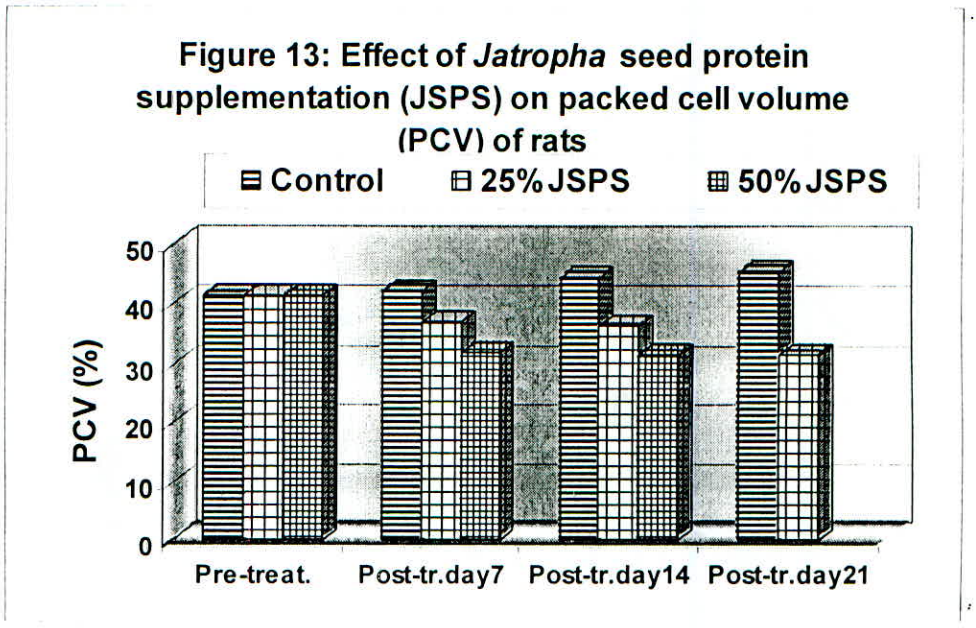


Figure 13: Effect of *Jatropha* seed protein supplementation (JSPS) on packed cell volume (PCV) of rats

Effect on Mean Corpuscular Volume

The effect of *Jatropha* feed supplementation on mean corpuscular volume (MCV) of rats depicted in Table 15 and Fig. 14. The MCV in control group (I) during pre-treatment and at different post-treatment intervals varied from 55.1 ± 0.97 to 57.0 ± 2.55 cubic microns and its variation between different intervals was insignificant. The MCV in Group II (25 % protein supplementation) during the three post-treatment intervals was 57.6 ± 1.31, 59.7 ± 1.22 and 60.2 ± 1.61 cubic microns, respectively, where the values 14 and 21 days of post-treatment were significantly higher than the pretreatment MCV of 55.1 ± 0.97 cubic microns. The MCV values in Group III (50 % *Jatropha* protein supplementation) at 7th and 14th post-treatment days were 56.9 ± 0.65 and 61.4 ± 0.63 cubic microns respectively, where the MCV at 14th post-treatment day was significantly higher than the pre-treatment MCV. The post-treatment MCV levels of Groups II and III at 14th post-treatment were significantly higher than the MCV of control group.

Table 15: Effect of feeding *Jatropha curcas* seed protein supplemented diet on mean corpuscular volume of Wistar rats

Group No.	Treatment	Mean Corpuscular Volume (μ^3) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	55.1 ± 0.97 (5)	55.2 ± 0.75 (5)	55.6 ± 1.32 (5)	57.0 ± 2.55 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		57.6 ± 1.31 (5)	59.7 a, b ± 1.22 (4)	60.2 a ± 1.61 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		56.9 ± 0.65 (5)	61.4 a, b ± 0.63 (4)	--

Figures in parentheses refer to No. of observations
a: Higher than pre-treatment ($P < 0.05$)
b: Higher than Group I ($P < 0.05$)

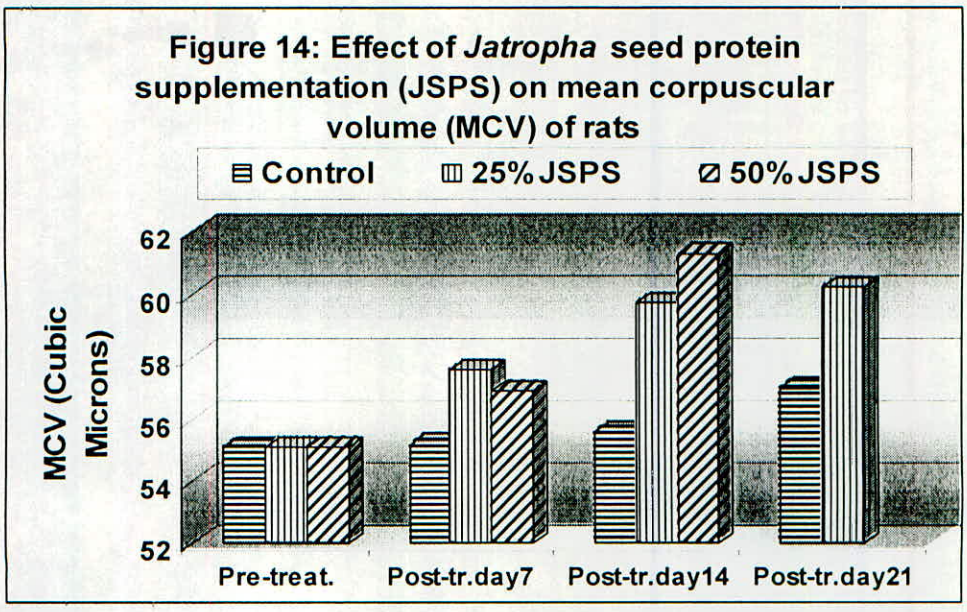


Figure 14: Effect of *Jatropha* seed protein supplementation (JSPS) on mean corpuscular volume (MCV) of rats

Effect on Mean Corpuscular Haemoglobin

Table 16 shows the effect of *Jatropha* seed protein supplementation on mean haemoglobin concentration (MCH) of rats. The mean MCH values at post-treatment in Group I (Control), Group II (25 % JSPS) and Group III (50 % JSPS) were in the range of 17.5 ± 0.60 to 18.2 ± 0.20 picograms, 16.1 ± 0.21 to 17.8 ± 0.62 picograms and 17.1 ± 0.26 to 17.5 ± 0.41 picograms, respectively. The variation in MCH values between intervals or between groups was not statistically significant as compared to the pre-treatment MCH of 18.1 ± 0.23 picograms.

Table 16: Effect of feeding *Jatropha curcas* seed protein supplemented diet on mean haemoglobin concentration of Wistar rats

Group No.	Treatment	Mean Haemglobin Concentration (pg) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	18.1 ± 0.23 (5)	18.2 ± 0.20 (5)	17.5 ± 0.60 (5)	17.6 ± 0.45 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		17.8 ± 0.62 (5)	17.7 ± 1.10 (4)	16.1 ± 0.21 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		17.1 ± 0.26 (5)	17.5 ± 0.41 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

Effect on Mean Corpuscular Haemoglobin Concentration

Table 17 shows the effect of *Jatropha* seed protein supplementation on mean corpuscular haemoglobin concentration (MCHC) of rats. The mean MCHC values at post-treatment in Group I (Control), Group II (25 % *Jatropha* protein supplementation) and Group III (50 % *Jatropha* protein supplementation) were in the range of 31.1 ± 1.43 to 32.8 ± 0.14 %, 26.9 ± 0.62 to 29.3 ± 1.45 % and 30.1 ± 0.22 to 30.5 ± 0.55 %, respectively. In both the *Jatropha* protein-treated groups, the MCHC values during post-treatment were lower than the pre-treatment value (33.2 ± 0.18 %). The MCHC level at 7th post-treatment day in Groups II and III was lower than the MCHC value of control group (32.8 ± 0.14 %).

Table 17: Effect of feeding *Jatropha curcas* seed protein supplemented diet on mean corpuscular haemoglobin concentration of Wistar rats

Group No.	Treatment	Mean Corpuscular Haemoglobin Concentration (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	33.2 ± 0.18 (5)	32.8 ± 0.14 (5)	31.3 ± 1.10 (5)	31.1 ± 1.43 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		29.3 [b] ± 1.45 (5)	29.0 [a] ± 1.43 (4)	26.9 [a] ± 0.62 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		30.1 [a, b] ± 0.22 (5)	30.5 [a] ± 0.55 (3)	--

Figures in parentheses refer to No. of observations
a: Lower than pre-treatment ($P < 0.05$)
b: Lower than Group I ($P < 0.05$)

The data of erythrocytic indices clearly demonstrated increase in MCV and decrease in MCHC suggesting macrocytic-hypochromic anaemia in the rats following feeding diets containing 25 and 50 % protein supplementation through *Jatropha* seeds. The present results are supported by an earlier observation of macrocytic-hypochormic

anaemia in rats fed methanolic extract of *J. curcas* seeds for 8 to 10 days (Oluwole *et al.*, 1997).

Leucocyte Count

Table 18 shows the effect of *Jatropha* seed protein supplementation on mean total leucocyte count (TLC) of rats. The TLC counts at post-treatment in Group I (Control), Group II (25 % *Jatropha* protein supplementation) and Group III (50 % *Jatropha* protein supplementation) were in the range of 5.8 ± 0.21 to 5.9 ± 0.21 thousands/cumm,5.6 ± 0.24 to 6.0 ± 0.22 thousands/cumm and 5.3 ± 0.29 to 6.0 ± 0.28 thousands/cumm, respectively. The variation in TLC counts between intervals or between groups was not statistically significant as compared to the pre-treatment TLC count of 5.7 ± 0.25 thousands/cumm.

Table 18: Effect of feeding *Jatropha curcas* seed protein supplemented diet on total leucocyte count of Wistar rats

Group No.	Treatment	Mean Total Leucocytes (Thousands/cumm) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	5.7 ± 0.25 (5)	5.8 ± 0.21 (5)	5.8 ± 0.24 (5)	5.9 ± 0.21 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		5.6 ± 0.24 (5)	6.0 ± 0.22 (4)	5.9 ± 0.31 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		6.0 ± 0.28 (5)	5.3 ± 0.29 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

Effect on Differential Leucocyte Counts

The differential leucocyte counts (DLC) of control group rats (I) and those fed on *Jatropha* seed protein supplemented group rats (II and III) is explained in Tables 19 to 22.

The mean lymphocyte counts during post-treatment in the three groups varied between 73.7 ± 1.49 and 75.4 ± 0.54 % as compared to the pre-treatment lymphocyte count of 74.6 ± 1.24 %. The variation in lymphocyte counts between intervals or between groups was not statistically significant as compared to the pre-treatment lymphocyte count (Table 19).

The mean neutrophil counts during post-treatment period in the three groups varied between 20.0 ± 0.70 and 23.2 ± 1.01 % as compared to the pre-treatment neutrophil count of 21.2 ± 0.86 %. The variation in neutrophil counts between intervals or between groups was not statistically significant as compared to the pre-treatment neutrophil count (Table 20).

The mean monocyte counts during post-treatment period in the three groups varied between 2.0 ± 0.31 and 2.8 ± 0.37 % as compared to the pre-treatment monocyte of 2.4 ± 0.24 %. The variation in monocyte counts between intervals or between groups was not statistically significant as compared to the pre-treatment count (Table 21).

The mean eosinophil counts during post-treatment period in the three groups varied between 1.2 ± 0.37 and 1.6 ± 0.33 % as compared to the pre-treatment eosinophil count of 1.8 ± 0.37 %. The variation in eosinophil counts between intervals or between groups was not statistically significant as compared to the pre-treatment count (Table 22).

Table 19: Effect of feeding *Jatropha curcas* seed protein supplemented diet on lymphocytes count of Wistar rats

Group No.	Treatment	Mean Lymphocytes (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	74.6 ± 1.24 (5)	75.0 ± 0.54 (5)	75.4 ± 0.54 (5)	75.2 ± 0.59 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		74.0 ± 1.04 (5)	74.5 ± 0.64 (4)	75.0 ± 1.15 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		74.0 ± 1.14 (5)	73.7 ± 1.49 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

Table 20: Effect of feeding *Jatropha curcas* seed protein supplemented diet on neutrophils of Wistar rats

Group No.	Treatment	Mean Neutrophils (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	21.2 ± 0.86 (5)	21.0 ± 0.44 (5)	20.2 ± 1.15 (5)	20.8 ± 0.80 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		22.2 ± 0.80 (5)	21.5 ± 0.95 (4)	20.6 ± 1.76 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		23.2 ± 1.01 (5)	20.0 ± 0.70 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

Table 21: Effect of feeding *Jatropha curcas* seed protein supplemented diet on monocytes of Wistar rats

Group No.	Treatment	Mean Monocytes (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	2.4 ± 0.24 (5)	2.6 ± 0.40 (5)	2.8 ± 0.37 (5)	2.0 ± 0.31 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		2.2 ± 0.37 (5)	2.5 ± 0.55 (4)	2.6 ± 0.33 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		2.8 ± 0.37 (5)	2.5 ± 0.28 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

Table 22: Effect of feeding *Jatropha curcas* seed protein supplemented diet on eosinophils of Wistar rats

Group No.	Treatment	Mean Eosinophils (%) ± S.E.			
		Pre-treatment	Post-treatment (Days)		
			Seven	Fourteen	Twenty-one
I	Normal Feed	1.8 ± 0.37 (5)	1.4 ± 0.24 (5)	1.6 ± 0.24 (5)	1.2 ± 0.37 (5)
II	*Jatropha* Protein Supplementation (25% of CP)		1.6 ± 0.24 (5)	1.5 ± 0.28 (4)	1.6 ± 0.33 (3)
III	*Jatropha* Protein Supplementation (50% of CP)		1.4 ± 0.24 (5)	1.5 ± 0.64 (4)	--

Figures in parentheses refer to No. of observations
Variations between groups and intervals among the groups were insignificant

The data of all leucocyte parameters did not indicate significant effect of *Jatropha* seed protein supplementation either on total or differential leucocyte counts. However, Abdel-Gadir *et al.* (2003) reported leucopaenia in kids fed on *J. curcas* seeds at the rate of 1 or 0.25 g/kg/day for 7 to 21 days.

Pathological Changes

All the rats which succumbed to the toxicity were observed for gross pathological and histopathological changes in the visceral organs. The prominent pathological observations are as under.

Gross Pathology

All the visceral organs such as liver, lungs, heart and kidney showed varying degrees of congestion. The intestine were grossly engorged with severe haemorrhages/unclotted blood all through its length.

Histopathology

Liver: The liver sections showed proliferation of fibrous tissue and bile duct along with infiltration of mononuclear cells in portal areas and degenerative changes leading to necrotic changes in liver parenchyma. These changes were less prominent in rats of Group II (25 % JSPS; Fig. 15.A.) as compared those in Group III (50 % JSPS; Fig. 15.B). Further, the rats in Group III also revealed severe haemorrhages and dilatation of sinusoids (Fig. 15.B). Fatty degeneration and necrotic changes were also noted in goats fed on *J. curcas* seeds @ 0.25 to 10 gm/kg/day (Adam and Magzoub, 1975) and sheep fed on the seeds @ 0.05 to 1 g/kg/day (Ahmed and Adam, 1979 b).

Kidney: The changes in kidney in Group II rats were characterized by moderate haemorrhages and necrosis in the proximal and distal convoluted tubules (Fig. 16.A). There were extensive degenerative/necrotic changes in the proximal and distal convoluted tubules along with haemorrhages in some areas of kidney in Group III (Fig. 16.B). Makkar *et al.* (1998) also recorded gastroenteritis and nephritis accompanied by renal sub-cortical haemorrhages in animals due to presence of phorbol esters (anti-nutrients) in *J. curcas* seeds.

Intestine: The Group II rats exhibited mild desquamation of superficial epithelial cells in duodenum and jejunum, with no significant changes in ileum or rectum. However, the rats in Group III (50 % JSPS) revealed marked desquamation of epithelial cells lining the tips of duodenal villi (Fig. 17. A). The Group III rats also showed desquamation of superficial epithelium, congestion and degenerative changes in jejunum (Fig. 17.B). Marked intestinal pathology characterized by catarrhal enteritis, erosion of intestine, congestion or haemorrhages in small intestine was reported in mice fed on diets containing 40 to 50 % of *J. curcas* seeds (Adam, 1974). The anti-nutrients present in *Jatropha* seeds might have also contributed in altering the morphology of intestines. The report of altered height of intestinal villi among weanling pigs fed soya bean meal or cowpea (Makinde *et al.*, 1994) also supports the present observation. The retarded growth rate of the rats following JSPS might also be due to reduced digestive and absorptive capacities as a result of intestinal mucosal pathology, as also observed in weanling pigs fed on cowpea (Makinde *et al.*, 1996). Long-term oral administration of soya bean extracts was also reported to cause enteritis (Sobbhy *et al.*, 1995). The anti-nutrient trypsin inhibitor (Ge and Morgan, 1993) also present in *Jatropha* seeds might have also contributed to intestinal pathology in the rats. Similarly, soyabean antinutritional factors were also found to induce intestinal damage in chickens (Li *et al.*, 1999)

The degenerative changes in liver, kidney and intestines of rats observed in the present study may also be attributed to presence of curcin, toxalbumin of *J. curcas* seeds (Stirpe *et al.*, 1976). Abdel-Gadir *et al.* ((2003) reported lesions such as widespread haemorrhages and enterohepatonephrotoxicity in kids fed on *J. curcas* seeds 1 or 0.25 gm/kg/day (7 to 21 days).

Testis: The testicular sections showed degenerative/necrotic changes in the seminiferous tubules (Fig. 18.A and B), where the changes were more marked in Group III. Further, the Group III rats also revealed a very few spermatids with elongated nuclei in the intact stage (Fig. 18.B). The rat testicular pathology observed in the present study may also be due to the anti-nutrients present in *Jatropha* seeds. This fact is supported by the reports of testicular pathology, in animals fed on legume feeds having anti-nutrients (Umapathy, 1993; Umpapathy *et al.*, 1993; Umpapathy *et al.*, 1994). The testicular pathology may be due to dietary protein deficiency adversely affecting the hypothalamo-pituitary axis and gonadotrophin production (Vawda and Mandlwana, 1990; Adam and Findlay, 1997).

No significant histopathological changes were observed in heart, lungs or ovaries in the *Jatropha* seed protein supplemented groups.

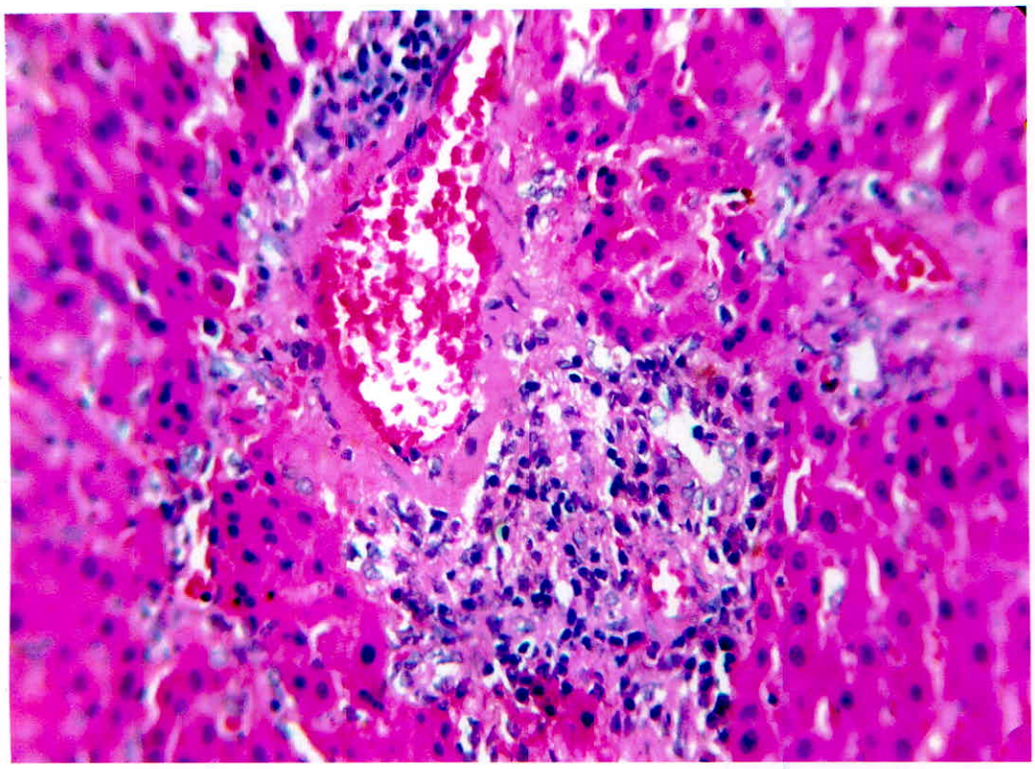


Fig.15A: Section of liver of rat (25% JSPS) showing mild degenerative and necrotic changes, proliferation of bile duct and fibrous tissue along with infiltration of mononuclear cells in portal areas (H&Ex 400)

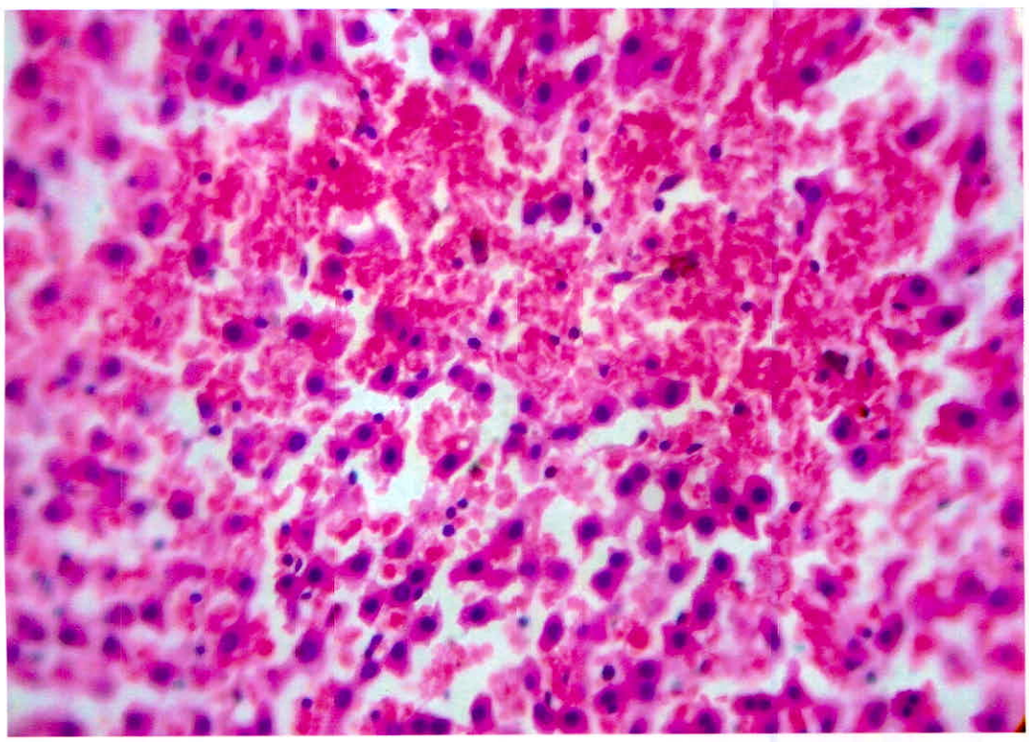


Fig.15B: Section of liver of rat (50% JSPS) showing degenerative and necrotic changes severe haemorrhages and dilatation of sinusoids (H&E x 400)

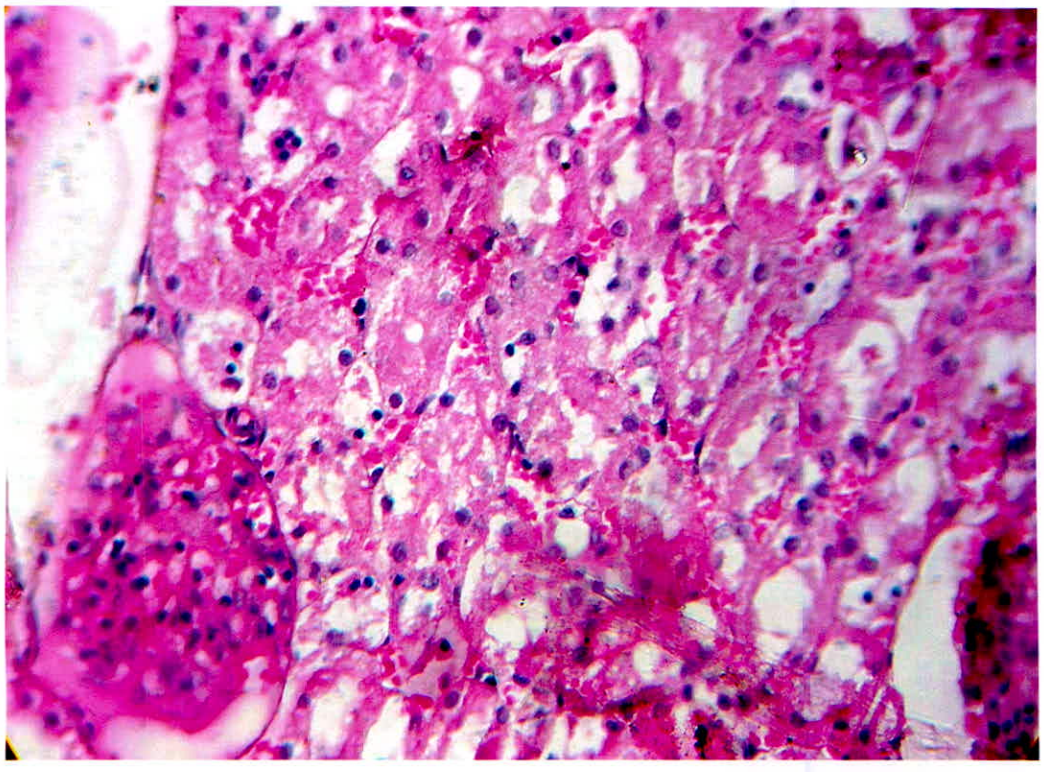


Fig.16A: Section of kidney of rat (25% JSPS) showing degenerative and necrotic changes in the proximal and distal convoluted tubules and moderate haemorrhages (H&E x 400)

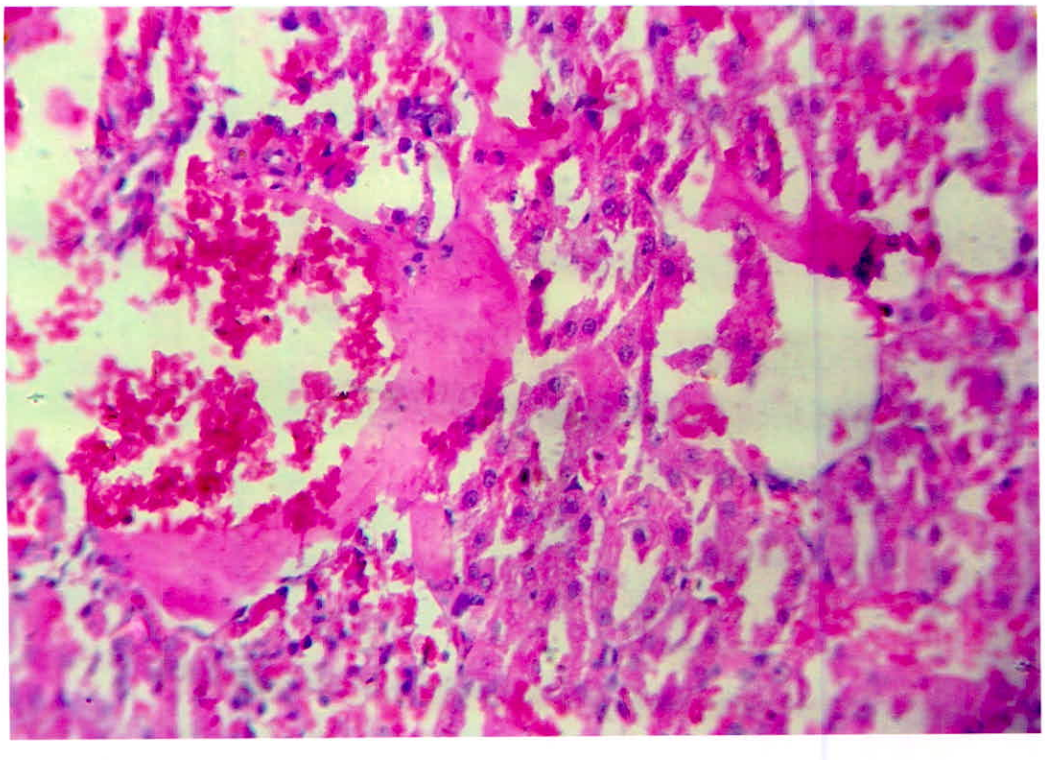


Fig.16B: Section of kidney of rat (50% JSPS) showing extensive degenerative and necrotic changes in the proximal and distal convoluted tubules and haemorrhage (H&E x 400)

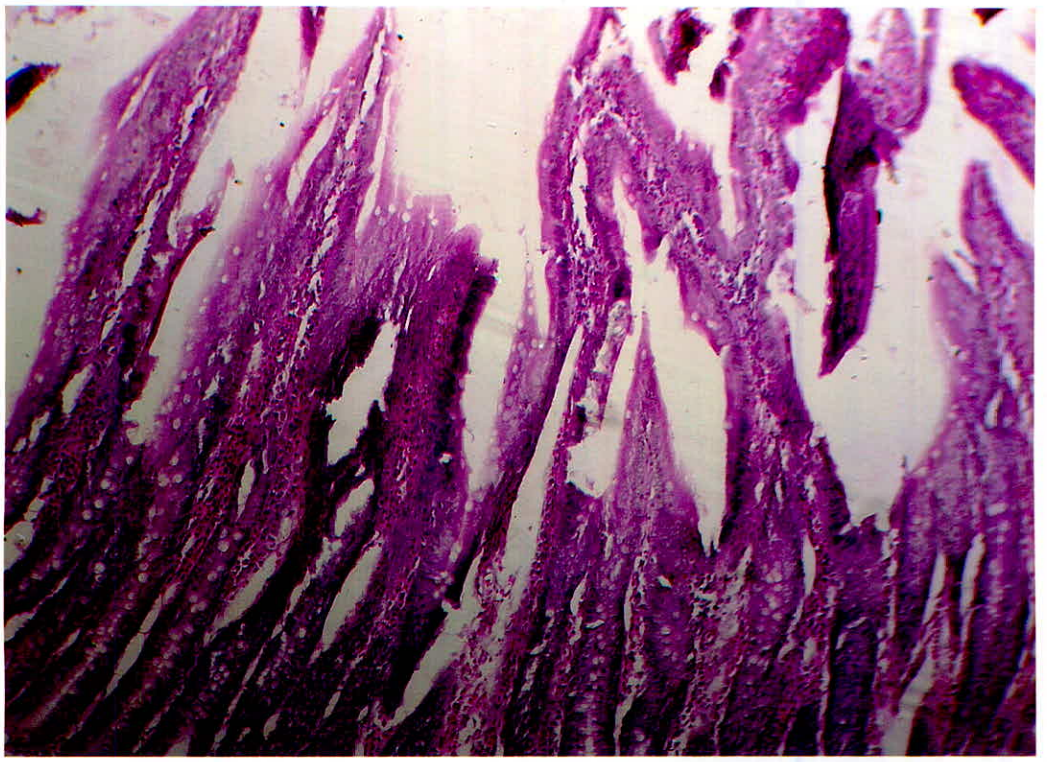


Fig.17A: Section of duodenum of rat (50% JSPS) showing desquamation of epithelial cells lining tip of villi (H & E x 100)

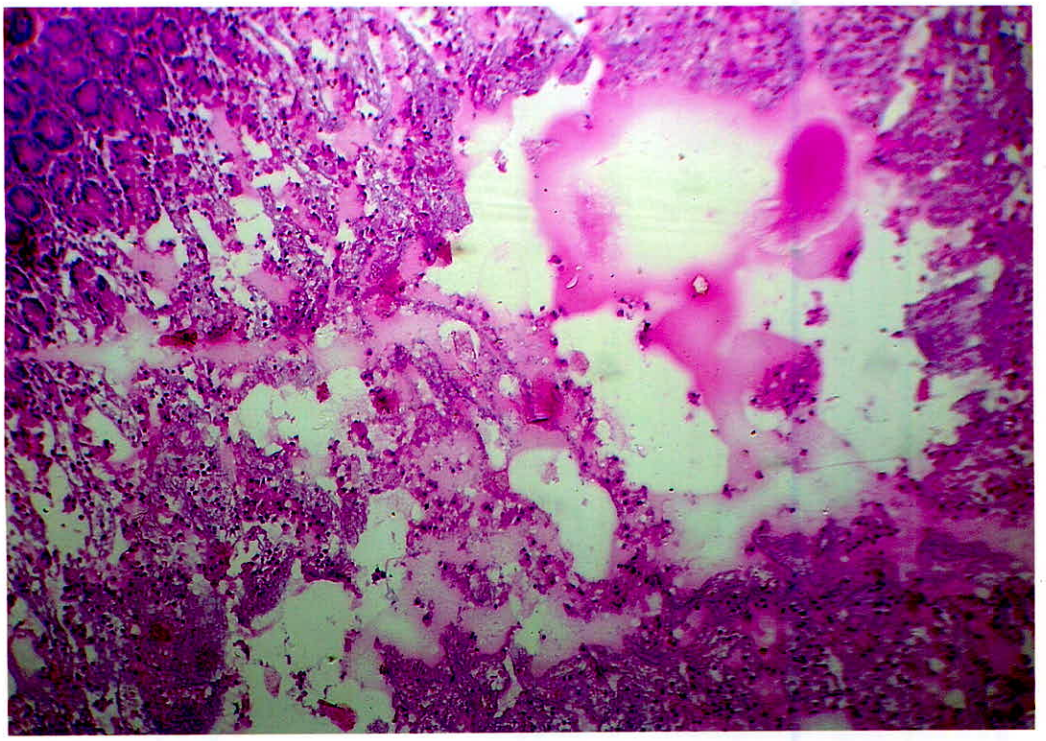


Fig.17B: Section of jejunum of rat (50% JSPS) showing desquamation of epithelial cells (H&E x 100)

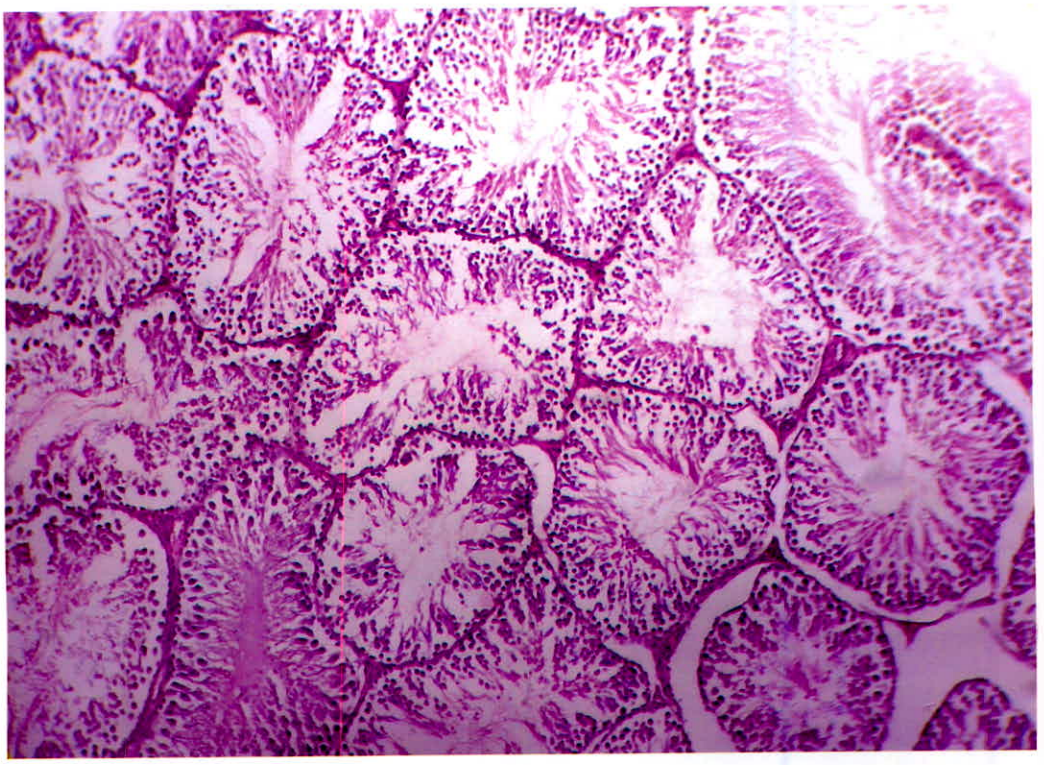


Fig.18A:Section of testis of rat (25% JSPS) showing mild degenerative and necrotic changes in the seminiferous tubules (H&E x 100)

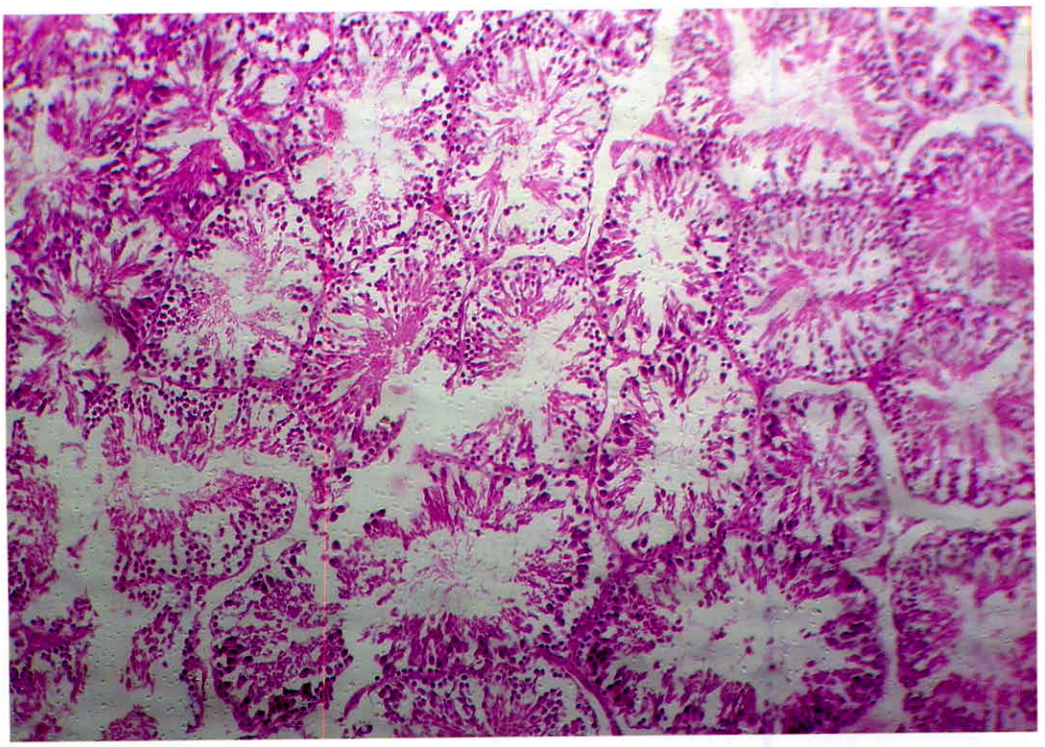


Fig.18B: Section of testis of rat (50% JSPS) showing severe degenerative and necrotic changes in the seminiferous tubules. Note a few spermatids with elongated nuclei in intact stage (H&E x 100)

Chapter-V

SUMMARY, CONCLUSION & SUGGESTIONS FOR FUTURE RESEARCH WORK

CHAPTER – V

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FUTURE RESEARCH WORK

Jatropha curcas (Euphorbiaceae), locally known as '*Ratanjyot*' is a multipurpose shrub with varied medicinal uses and is of significant economic importance. In addition to being the source of bio-diesel, its seeds are also considered highly nutritious and could be exploited as a rich and economical protein supplement in animal feeds. However, the inherent phytotoxins present in the seed is the hindrance. There are reports of existence of nontoxic varieties of the plant and variation in toxicity potential of the plant in different geographical regions. The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, Investigations were undertaken to evaluate the acute and subacute oral toxicity of the seeds of locally grown *J. curcas*.

The acute oral toxicity was assessed by using *J. curcas* seed powder. The whole as well as the defatted/de-oiled whole seed powder was processed to obtain cold and hot aqueous extracts. The extracts were not toxic to the Wistar rats at oral doses as high as 3000 mg/kg. The rats administered the cold extracts @ 7000 mg/kg showed circling movement, depression and blood-tinged loose feces and became normal after 2 to 3 days of treatment. The hot extract of the whole seeds at the same dose level produced only with depression, but not the blood-tinged diarrhea. Since there was no mortality at the highest possible dose of the prepared extracts, the acute oral LD_{50} in rats was presumed to be more than 7000 mg/kg. Acute oral dosing of the extracts also had no adverse effect on the body weight of the animals.

Subacute toxicity was conducted in rats by daily feeding the basal diet (Group I), and the diet where the crude protein requirement was supplemented at 25 % (Group II) and 50 % (Group III) levels through *Jatropha* seed powder. The adverse

effects of *Jatropha* seed protein supplementation (JSPS) were evaluated by observing the toxicity signs and symptoms, growth rate, feed intake, alterations in biochemical and haematological profiles and pathological changes.

The rats which received the *Jatropha* seed protein supplementation (Groups II and III) were dull and depressed from 3rd day of treatment and showed passage of blood-tinged loose feces from 6th post-treatment day onwards. The rats in both the groups became weak and gradual loss of condition. Two rats of Group II (25 % JSPS) and four rats of Group III (50 % JSPS) died during 12th to 13th day of the trial. Further, two rats of Group III and one rat of Group II succumbed to the toxicity on 16th and 20th days of treatment, respectively. The rats on 25 % JSPS lost 5.6 to 11 gm in their weight on 7th to 21st post-treatment day and the rats on 50 % JSPS also showed progressive decline in their body weight (8.3 to 14.7 gm) in comparison to progressive increase in body weight by the control rats on 7th to 14th day (3.1 to 5.4 gm) and 21st day (11 gm) of the trial as against their respective pre-treatment body weight (94.0 ± 4.52 to 96.3 ± 6.52 gm). Similarly, the feed intake of rats with JSPS progressively decreased as against the gradual increase by the control group rats.

The biochemical profile of rats fed on diet with JSPS at both the levels revealed significant reduction in plasma glucose and total protein and increase in plasma creatinine, transaminases (PGPT and PGOT) and alkaline phosphatase. Haematological examination of the rats under JSPS also showed significant changes in the haemogram characterized by reduction in total red blood cell count, packed cell volume and haemoglobin. The JSPS was also found to induce macrocytic-hypochromic anaemia in the rats. However, no significant changes were noted in total or differential leucocyte counts.

Gross necropsy of the rats that succumbed to the toxicity of JSPS showed congested visceral organs in general, where the intestines were grossly engorged with haemorrhages. Histopathological changes observed were characterized by degenerative changes extending to necrotic lesions in liver parenchyma and in the proximal and distal convoluted tubules in kidney, congestion and desquamation of superficial epithelium in intestines (duodenum and jejunum), and degenerative changes in seminiferous tubules along with few spermatids with elongated nuclei.

CONCLUSION

The adverse effects of feeding diets with JSPS in rats have been attributed to be due to presence of the phytotoxin curcin and a variety of anti-nutritional factors. From the present investigations it may be concluded feeding *J. curcas* seeds as such or adding to diets as a source of crude protein is harmful to the animals. However, the seed protein can be made edible after detoxification and/or removal or inactivation of anti-nutrients.

SUGGESTIONS FOR FUTURE RESEARCH WORK

Research on of *J. curcas* must be in the direction of devising methods to detoxify the seeds or make them free from the anti-nutrients so that the seeds can be utilized as a rich source of protein in livestock feeds.

REFERENCES

REFERENCES

Abdu-Aguye, I., Sannusi, A., Alafiya-Tayo, R.A. and Bhusnurmath, S.R. 1986. Acute Toxicity Studies with *Jatropha curcas L.* Human Toxicology 5(4): 269-274.

Abdel-Gadir,W.S., Onsa, T.O., Ali, W.E.M., El-Badwi, S.M.A. and Adam, S.E.I. 2003. Comparative toxicity of *Croton macrostachys, Jatropha curcas* and *Piper abyssinica* seeds in Nubian goats. Small Ruminant Res. 48(1): 61-67.

Abd-Elhamid, H.F. 2004.Investigation of induced biochemical and histopathological parameters of acetonitril extract of *Jatropha curcas* in albino rats. J. Egypt's Soc. Parasitol. 34(2): 397-406.

Adam, S.E. 1974. Toxic effect of *J. Curcas* in mice. Toxicology I (1):67-76.

Adam, C.L. and Findlay, P.A. 1997. Effect of nutrition on testicular growth and plasma concentrations of gonadotrophins, testosterone and insulin-like growth factor I (IGF-I) in pubertal male Soay sheep. J. Reprod. Fert. 111 (1): 121-125.

Adam, S.E.I. and Magzoub, M. 1975. Toxicity of *Jatropha curcas* (Euphorbiaceae) for goats. Toxicology, 4(3): 347-354.

Adedapo, A.A., Abatan, M.O. and Olorunsogo, O.O. 2004. Toxic effects of some plants in the genus *Euphorbia* on haematological and biochemical parameters of rats. Vet. Arhiv. 74 (1): 53-64.

Adeyemi, O.A., Balogun, M.O. and Fasina, O.E. 2001. Response of finishing broilers to graded levels of boiled *Jatropha* seeds. Indian J. Anim. Sci. 71:800-803.

Adolf, W., Opferkuch, H.J. and Hecker, E. 1984. Irritant phorbol derivatives from four *Jatropha Sp.* Phytochem. 23(1):129-132.

Ahmed, O.M.M. and Adam, S.E.I. 1979a. Effects of *Jatropha curcas* on calves. Vet. Pathol. 16(4): 476-482.

Ahmed, O.M.M. and Adam, S.E.I. 1979b. Toxicity of *Jatropha curcas* in sheep and goats. Res. Vet. Sci. 27(1): 89-96.

Aponte, C. 1978. Estudio de *Jatropha Curcas L.* Como recurso biotico. Diploma Thesis. University Veracruz, Mexico.

Aregheore, E.M., Becker, K. and Makkar, H.P.S. 2003. Detoxication of a toxic variety of Jatropha curcas using heat and chemical treatments, and preliminary nutritional evaluation with rats. S. Pac. J. Nat. Sci. 21 (1): 50-56.

Arora, S.K. and Luthra, Y.P. 1974. The *in vitro* digestibility of promising Indian varieties of soybean and its relation with tannin content. Indian J. Nutr. Dietet. 11: 233-234.

Balmar, R., Nava-Montero, R., Sandoval-Castro, C and McNab, J.N. 1999. Jack bean (*Canvalia ensiformis* L. DC) in poultry diets: Antinutritional factors and detoxification studies. A review. World's Poultry Sci. J. 55 (1): 37-59.

Barri, M.E.S., Onsa, T.O., Elawad, A.A., Elsayed, N.Y., Wasfi, I.A., Abdul Bari, E.M., and Adam, S.E.I. 1983. Toxicity of five Sudanese plants to young ruminants. J. Comp. Pathol. 93: 559-575.

Barroga, C.F., Laruena, A.C. and Nerdoza, E.M. 1985. Polyphenols in mung bean (*Vigna radiata* L) Wilczek: Determination and revomal. J. Ag. Food Chem. 33 910): 1006-1011.

Becker, K. and Makkar, H.P.S. 1998. Effects of phorbol esters in carp (*Cyprinus carpio* L.). Vet. Human Toxicol. 40(2): 82-86.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian Medicinal plants. 1st Edition, I, Council of Scientific and Industrial Research, New Delhi, India, p 145.

Chen, M.J., Hou, L.L. and Zhang, G.W. 1988. The diterpenoids from *Jatropha Curcas* L. Acta Botanica Sin. 30(3):308-311.

Culling, C.F.A. 1974. Hand Book of Histopathological and Histochemical Techniques. 3rd edition, Butter Worth and Company Limited, London, pp. 219-221.

Denman, B., Hamdi, E., Fox, F. and Frajola, W. 1983. Carbon tetrachloride toxicity. 3. Hepatostructural and enzymatic change. Arch. Environ. Hlth. 7: 630-646.

Dutra, F.V., Calixto, J. B., Medeiros, Y.S. and Brum, R.1996.Jatrophone inhibits platelet rich plasma aggregation from man, rat and guinea pig induced by different agents. Phytother. Res. 10 (3): 271-273.

El-Badwi, S.M.A., Adam, S.E.I. and Hapke, H.J. 1995. Comparative toxicity of Ricinus communis and *Jatropha curcas* in Brown Hisex chicks. Deutsche-Tierarztliche-Wochenschrift 102(2): 75-77.

El-Badwi, S.M.A., Mousa, H.M., Adams, S.E.I. and Hapke, H.J. 1992. Response of Brown Hisex chicks to low levels of *Jatropha curcas*, *Ricinus communis* or their mixture. Vet. Human Toxicol. 34(4): 304-306.

El-Sayed, M.M., Ramadan, M.E., Ali, H.M. and El-Morsi, E.A. 1997. Biochemical studies on alpha-amylase inhibitors in some legume seeds. Annals of Agric. Sci. 35 (1): 357-374.

Finco, D.R. 1997. Kidney function. In Clinical Biochemistry of Domestic Animals, 5th Edition, Editors: Kaneko, J.J., Harvey, J.W. and Bruss, M.L., Harcourt Brace and Company Asia PTE. Limited, Singapore, pp. 441- 484.

Fojas, F.R., Garcia, L.L., Venzon, E.L., Sison, F.M., Fojas, A.J. and Llave, I. 1986. Pharmacological studies on *Jatropha Curcas* as a possible source of anti-arrythmic (beta-blocker) agent. Philippines Agriculture 73 (3/4):343-348.

Ge, Y.C. amd Morgan, R.G.H. 1993. The effect of trypsin inhibitor on the pancreas and small intestine of mice. British J. Nutr. 70 (1): 333-345.

Goonasekera, M.M., Gunawardana, V.K., Jayasena, K., Mohammed, S.G. and Balasubramaniam, S. 1995. Pregnancy terminating effect of *Jatropha curcas* in rats. J. Ethnopharmacol. 47(3): 117-123.

Gandhi, V.M., Cherian, K.M. and Mulky, M.J. 1995.Toxicological studies on ratanjyot oil. Food Chem. Toxicol. 33(1): 39-42.

Glik, Z and Joslyn, M.A. 1970. Food intake, growth depression and metabolic effects of tannic acid in the rat. J. Nutr. 100: 509-511.

Grant, G., Dorward, P.M., Buchan. W.C., Armour, J.C. and Pusztai, A. 1995. Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: Effects on body composition and organ weights. Br. J. Nutr. 73 (1) 17-29.

Jain, N.C. 1986. Schalm's Veterinary Hematology, IV Edition, Lea and Febiger, Philadelphia. Jain, N.C. 1990. Scahalm's Veterinary Hematology, 4th edition, K. M. Verghese co., New Delhi, pp. 20-86.

Joubert, P. H., Brown, J.M.M., Hay, I.T. and Sebata, P.D.B. 1984. Acute poisoning with *Jatropha curcas* purging nut tree in children. S. African. Med. J. 65(18):729-730.

Karmegam, N., Sakthivadivel, M., Anuradha , V. and Thilagavathy , D. 1997. Indigenous plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. Bioresource Tech. 59(2-3): 137-140.

Kirtikar, K. R. and Basu, B. D. 1975. Indian Medicinal plants. Vol..III, Bishon Singh and Mahendra Singh, Delhi, pp:2240-2247.

Kulkarni, M.L., Sreekar, H., Keshavamurthy, K.S. and Nivedita, S. 2005. *Jatropha Curcas* – Poisoning. Indian J. Pediatr. 72 (1): 75-76.

Libarlino, A.A.A., Bambirra, E.A., Morae-santos, T. and Veiria, E.C. 1988. *Jatropha Curcas* seeds: Chemical analysis and toxicity. Arq. Biological Techol. 31(4): 539-550.

Lampe and Fagerstrom 1968. Plant toxicity and Dermatitis – A Manual for Physicians. The Williams and Wilkins Company, Baltimore, pp.

Li, G. X., Zhang, X.Y., He, Y., Yang, G.J. and Huo, G.C. 1999. Studies on the effects of soybean antinutritional factors on the thymus, pancreas and small intestines of chickens. Acta Vet. Et. Zootech. Sinicia, 30 (z): 121-126.

Lin, J., Yan, F, Tang, L and Chen, F. 2003. Antitumor effects of curcin from seeds of *Jatropha curcas*. Acta Pharmacol Sin. 24 (3): 241-246.

Liner, I.E. 1982. Toxic factors in edible legumes and their elimination. Am. J. Clin. Nutr. 11: 281-284.

Li-Shaoyan, Bauer, W.C. and Caine, W.R. 1998. Response of nutrient digestibilities to feeding diets with low and high levels of trypsin inhibitors in growing pigs. J. Sci. Food and Agric. 76 (3): 357-363.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edition, McGraw Hill Book Company INC. New York.

Makinde, M.O., Umapathy, E., Akingbemi, B.T. and Mandisodza, K.T. 1996. Effect of feeding of different levels of cowpea (*Vigna unguiculata*) on gut morphology and faecal composition in weanling pigs. South African J. Ani. Sci. 26 (2): 42-46.

Makinde, M.O., Umapathy, E., Akingbemi, B.T., Mandisodza, K.T., Skadhauge, E. and Poomvises, P. 1994. The effect of legume diets on gut morphology and faecal characteristics in weanling pigs. Proceedings of 13th International Pig Veterinary Society Congress, Bangkok, Thailand. P. Ingkaninun (Ed), pp. 26-30.

Makkar, H.P.S., Aderibigbe, A.O. and Becker, K. 1998. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility. Protein degradability and toxic factors. Food Chem. 62(2): 207-215.

Makonnen, E., Rostom, A.A.H., Assefa, G. and Zerihun, L. 1997. Antifertility effect of *Jatropha curcas* L. seed in guinea pigs. EthiopianJ.Health Develop. 11(2): 145-148.

Melaine, R and Andreas, R. 2000. Toxic activities of the plant Jatropha curcas against intermediate snail hosts and larvae of schistosomes. Trop. Med. Int. Health, 5 (6): 423-430.

Mole, S., Rogler, J.C. and Butler, L.G. 1993. Growth reduction by dietary tannins: Different effects due to different tannins. Biochem. System. Ecol. 21 (6-7): 667-677.

Mori, Y., Aoyama, T., Fukui, K., Kurokawa, N., Komiya, H. and Ikeda, Y. 1998. Effect of soy protein isolate on body weight reduction by energy restriction in OLETF rats. Soy Protein Res. 1: 96-101.

Naengchomnong, W., Thebtarananth, Y., Wiriachitra, P. and Clardy, J. 1986. Isolation and structure determination of four novel diterpenes. Tetrahedron Letters 27(22):2439-2442.

Nadkarni, K.M. 1998. Indian plants and Drugs with their medicinal properties and uses. Asiatic Publishing house, New Delhi, pp: 207-208.

Okuyama, E., Okamoto, Y., Yamazaki, M. and Satake, M. 1996. Pharmacologically active components of a Peruvian medicinal plant, huanapro (*Jatropha cilliata)* Chem. Pharmaceut. Bull. 44 (2): 333-336.

Oluwole, F.S. and Bolarinwa, A.F. 1997. .*Jatropha curcas* extract causes anemia in rat. Phytother. Res. 11(7):538-539.

Owen, L.N. 1991. Identifying and treating cancer in geriatric dogs. Vet. Med. 86 (1): 55-66.

Pusztai, A., Grant, G., Brown, D.J., Stewart, J.C., Bardooz, S., Gatehouse, A.M.R. and Hilder, V. 1992. Nutritional evaluation of the trypsin inhibitor (EC 3.4.21.4) from cowpea (*Vigna unguiculata* Walp) Br. J. Nutr. 68 (3): 783-791.

Pusztai, A., Ewen, S.W.B., Grant, G., Brown, D.S., Stewart, J.C., Peumans, W.J., Bardocz, S. and Van-Damme, E.J.M. 1993. Antinutritive effects of wheat-germ agglutinin and other 5ON-acetylglucosamine-specific lectins. Br. J. Nutr. 70 (1): 313-321.

Rastogi, R.P. and Mehrotra, B.N. 1993. Compendium of Indian Medicinal Plants. Vol-II, CDRI (Lucknow) and Publication and Information Directorate, New-Delhi, pp. 397-398.

Ruppel, A. and Doenhoff, M.J. 1998. Vector biology and the control of parasitic diseases. Parasitol. Today. 14 (8): 299-300.

Salunkhe, D.K. 1982. Legumes in human nutrition. Current Sci. 51:387-390.

Salunkhe, D.K., Jafhav, S.J., Kadam, S.S. and Chavan, J.K. 1982. Chemical, biochemical and biological significance of polyphenols in cereals and legumes. Crit. Rev. Fd. Sci. Nutr. 17: 277-283.

Satwadhar,P.N., Kadam, S.S. and Salunkhe, D.K. 1981. Effect of germination and cooking on polyphenols and *in vitro* protein digestibility of horse gram and moth beans. Plant Fds. Hum. Nutr. 31: 71-74.

Schmeda, H.G., Razmilic, I., Sauvain, M., Moretti, C., Munoz, V., Ruiz, E., Balanza, E. and Fournet, A. 1996. Antiprotozoal activity of jatrogrossidione from *Jatropha.* Phytother. Res. 10 (5): 375-378.

Singh, D. and Singh, A. 2002. Piscicidal effect of some common plants of India commonly used in freshwater bodies against target animals. Chemosphere 49(1): 45-49.

Singh, N. and Bhargava, A.1997. Bioefficacy of *Jatropha curcas* against *Ailanthus fabricilella* Sed. World weeds 4(1-2): 25-27.

Sobbhy, H.M., Oraby, H.A., Essawy, G.S., Shehab, G.J. and Hosny, G.A. 1995. Pharmacological, cytogenetic and pathological effect of long-term administration of soya bean extract in rats. Egyptian J. Comp. Pathol and Clin. Pathol. 8 (2): 87-100.

Stirpe, F., Pession-Brizzi, A., Lorenzoni, E., Montannaro, L., Strocchi, P. and Sperti, S. 1976. Studies on the protiens from the seeds of *Croton tiglium* and of *Jatropha curcas.* Toxic properties and inhibition of protein synthesis in vitro. Biochem. J. 156(1): 1-6.

Talwar, G. P. 1983. A Hand Book of Practical Immunology. Vikas Publishing House, Pvt. Ltd., New Delhi, pp. 40-43.

Tennant, B.C. 1997. Hepatic function. In Clinical Biochemistry of Domestic Animals, 5th Edition, Editors: Kaneko, J.J., Harvey, J.W. and Bruss, M.L., Harcourt Brace and Company Asia PTE. Limited, Singapore, pp. 327-352.

Umapathy, E. 1993. Antifertility effects of cowpea on male rats. Central African J. Med. 39 (3): 52-56.

Umapathy, E., Msamati, B.C. and Torode, M.J. 1993. Electron microscopic observations on the luminal contents of rat epididymis induced by cowpeas. Central African J. Med. 39 (3): 52-56.

Umapathy, E., Msamati, B.C. and Torode, M.J. 1994. Ultrastructural changes in rat epididymides induced by cowpeas. Arch. Androl. 32: 121-133.

Vawda, A.I. and Mandlwana, J.G. 1990. The effects of dietary protein deficiency on rat testicular function. Andrologia, 22 (6): 575-583.

Watt, J.M. and Breyer, B..M.G. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition, E.and S. Livingstone Ltd., Edinburgh and London, p. 1457.

ABSTRACT

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Jatropha curcas (Euphorbiaceae), popularly known as '*Ratanjyot*' has varied medicinal values and is of great economic importance, being the source of bio-diesel. Its seeds are also considered highly nutritious and could be exploited as a rich source of protein supplement in animal feeds. However, the seeds are not edible as such, because of presence of phytotoxins and/or anti-nutrients. There also exist nontoxic varieties of *J. curcas*. The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, Investigations were undertaken to the toxicity of the seeds in laboratory animals.

The acute oral toxicity was assessed by using *J. curcas* seed powder. The whole as well as the defatted/de-oiled whole seed powder was processed to obtain cold and hot aqueous extracts. The extracts were not toxic to the Wistar rats at oral doses as high as 3000 mg/kg. However, the extracts at 7000 mg/kg oral doses produced only mild toxicity characterized by depression and passing of blood-tinged feces. The acute oral LD_{50} in rats was presumed to be more than 7000 mg/kg. Acute oral dosing of the extracts also had no adverse effect on the body weight of the animals.

Subacute toxicity of *Jatropha* seeds was evaluated in rats by daily feeding the basal diet supplemented at 25 and 50 % of crude protein through *Jatropha* seed powder. The rats which received the *Jatropha* seed protein supplementation (JSPS) were dull and depressed from 3rd day of treatment and showed passage of blood-tinged loose feces from 6th post-treatment day onwards. The rats became weak and gradual loss of condition, followed by mortality at both the levels of supplementation within 21 days of feeding. The rats on JSPS also showed progressive decline in their body weight. Similarly, the feed intake of rats with JSPS progressively decreased. The biochemical profile of rats fed on diet with JSPS at both the levels revealed significant reduction in plasma glucose and total protein and increase in plasma creatinine levels and increase in the activities of plasma transaminases and alkaline phosphatase. Haemogram of the rats under JSPS showed significant reduction in total red blood cell count, packed cell volume and haemoglobin accompanied by macrocytic-hypochromic anaemia. The rats that succumbed to the toxicity of JSPS showed congested visceral organs in general and histopathological changes such degenerative changes (necrotic lesions) in liver parenchyma and in the proximal and distal convoluted tubules in kidney, congestion and desquamation of superficial epithelium in intestines, and degenerative changes in seminiferous tubules.

From the present investigations it may be concluded that feeding *J. curcas* seed protein supplemented diets is harmful to the animals. However, the seed protein may be made edible after detoxification and/or removal or inactivation of anti-nutrients.