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Toxicity evaluation of Ethanolic Extract of *Astercantha longifolia* Seeds

Rajina PV[1] and Shini Dominic [2]*

1. Crescent College of Pharmaceutical Sciences, Kannur, Kerala, India 670358
2. Govt. Medical College Kozhikode, Kerala, India.673008.

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Abstract

Plan: *To verify the toxic effects of the ethanolic extract of Asteracantha longifolia seeds after single and repeated administration*
Prologue*: Usually traditional medicines are not subjected to routine toxicological studies. As a result many adverse effects caused by the chronic use of herbal medicines may be wrongly attributed to certain acute or chronic diseases Though Asteracantha longifolia seeds are traditionally used for the treatment of sexual debility, premature ejaculation and oligospermia, there is no evidence about its toxicity.*
Methodology: Acute toxicity of the plant extract was carried out as per the OECD guideline 425. *Sub acute toxicity study of 28 day duration was carried out in three different doses of* 100 mg/kg, 250 mg/kg and 500 mg/kg.*orally*.
Outcome*: No significant variation in hematologic and biochemical parameters and in relative organ weight with doses of 100, 250 and 500mg/kg body weight. Histopathology showed the presence of slight necrosis in seminiferous tubules at dose of 500mg/kg. It may be concluded that the alcoholic extract of Asteracantha longifolia seeds is safe for oral administration at low and moderate doses while high dose (500mg/kg) is not absolutely free from toxic effects.*
Key words**:** *Asteracantha longifolia, acute toxicity, Sub acute toxicity.*

1. Introduction

Herbs have been a part of civilization since ancient times.. Every part of world has its herbs which are peculiar to that particular area.[1] In India, plants and their importance have been highlighted from the vedic literature itself. Adharva Veda suggests that man learned the therapeutic value of plants by observing the behavior of wild animals and birds in disease.[2] This does not mean that all plants and the active ingredients extracted from them are safe .As the plants and herbs are mines of large number of bioactive phytochemicals and the crude preparations from plants harbor different types of active ingredients, the beneficial effects of some may be neutralized / reversed by others.[3].

Unlike the practice in Allopathy, these traditional medicines are not routinely subjected to toxicological studies before using them on a regular basis.

For Correspondence: shinidm@yahoo.com

Researcher ID: C-3510-2013

As a result chances are high that many adverse effects/ organ toxicities caused by the chronic use of herbal medicines may be wrongly attributed to certain acute or chronic diseases. So upon considering the immense significance and unparallel impact that the herbal medicines have on human life, the toxicological screening of widely used plant preparations are highly relevant.

Astercantha longifolia is a plant common in India. It is widely distributed throughout tropical and sub-tropical regions of India. Internally, the plant is used in vast range of diseases. In vata diseases like nervine debility, gout and rheumatoid arthritis, the seeds are used with great benefit. Traditionally seeds are used for the treatment of sexual debility, premature ejaculation, erectile failure and oligospermia.[4,5]

But, as there is no evidence about the toxicity of the seed in acute as well as on repeated use, the present study is aimed to see the toxic effect of ethanolic extract of the seeds after single and repeated administration

2. Materials and methods

2.1. Plant material

The seeds of *Asteracantha longifolia* collected from Thiruvananthapuram and authenticated by Dr.M.A.Shajahan, M.D (Ay), PhD, Professor& HOD, Govt. Ayurveda College & Hospital, Thiruvananthapuram were used for the present study. The seeds were powdered and extracted using 95% ethanol by continuous soxhlet extraction[6,7]A suspension of extract(percentage yield 13%) in 0.4% Carboxy methyl cellulose (CMC) was prepared and this suspension (0.5ml/100g body weight) was used for the oral administration.

2.2. Ethical clearance

The study protocol was approved by the Institutional Animal Ethics Committee, Medical College, Thiruvananthapuram (IAEC NO.04/06/2011/MCT) dated 20.08.2011

2.2.1. Animal selection, maintenance and care

Healthy albino rats (Wistar strain) of either sex weighing 170-200 gm were used for the study. They were obtained from animal house Medical College, Thiruvananthapuram. These animals were fed on standard pellet diet (manufactured by Nav Maharashtra Chakan Oil Mills Ltd; Pune and supplied by Sai Durga Feeds and Foods, Banglore) and water ad libitum.The animals were maintained under standard conditions of relative humidity, 12 hrs light-dark cycle, adequate ventilation and ambient room temperature.

2.3. Acute toxicity study (OECD-425) [8, 9]

Acute toxicity of the plant extract was carried out as per the OECD guideline 425. A limit test was performed using healthy female albino rats weighing 175-200g. Prior to dosing, animals were fasted overnight and the dose for each animal was determined based on body weight. Initially the extract was administered to one animal in a single dose of 2000 mg/kg by gavage using a stomach tube. After the administration, food was withheld for a further 3-4 hours.

The animal was observed once during the first 30 minutes after dosing, then periodically, during the first 24 hours. As the animal was not died, 4 additional animals were given the same dose and observed similarly. All the survived animals were then kept for 14 days observation. The LD_{50} was calculated using the software program-AOT425statpgm.

2.4. Sub acute toxicity study: [10,11,12,13]

Results of acute toxicity studies in wistar female rats indicated that the LD_{50} was greater than 2000mg/kg body weight. On the basis of these results, the doses selected for the sub acute toxicity study were 100 mg/kg, 250 mg/kg and 500 mg/kg. The oral route was selected for use because oral route is considered to be the proposed therapeutic route.

The animals were divided in to 4 groups consisting of 3 male & 3 female rats of matched body weight and age in each group.

Group I: Normal control- 0.4% sodium Carboxymethyl cellulose (0.5ml/100g) orally, daily at 10 AM for 28 days
Group II: 100mg/kg of ethanolic extract of Asteracantha longifolia seeds in 0.4% CMC orally, daily at 10 AM for 28 days
Group III: 250 mg/kg of ethanolic extract of Asteracantha longifolia seeds in 0.4% CMC orally, daily at 10AM for 28 days
Group IV: 500mg/kg of ethanolic extract of Asteracantha longifolia seeds in 0.4% CMC orally, daily at 10AM for 28 days

2.5. Effect on body weight

Initial body weight of the animals were 170-200g.The body weight of the animals on each day was noted. A change in body weight in one week interval was noted for 4 weeks.

2.6. Food and water consumption

The food and water consumption of the animals were observed daily and a change in these per 100 gm body weight were noted on weekly basis.

After 28 days blood was collected by retro orbital bleeding under ether anesthesia into two tubes, one tube added with EDTA for hematological estimations and the other without EDTA for biochemical estimations. For biochemical examinations the blood was kept for about 30 minutes for clotting and centrifuged at 3000rpm for 10 min. Clear serum obtained as supernatant was used for the estimation.

2.7. Hematological parameters [14, 15, 16]

2.7.1. Hemoglobin estimation

Hemoglobin present in the sample is converted in to acid haematin by addition of 0.1N HCl to the blood and its hemoglobin content is determined by matching the solution against a non fading glass having a standard colour.The reading was noted in gram per 100ml by reading the lower meniscus of the diluting tube.

2.7.2. RBC count

RBC count was determined using Neubauer counting chamber and RBC pipette.The number of RBCs in a known volume of diluted blood is determined and from this the number of RBCs per cubic millimeter of undiluted blood is calculated by multiplying with the dilution factor.

2.7.3. WBC count

As the number of WBCs present in $1mm^3$ of blood is in thousands, the blood has to be diluted so that WBCs can be seen distinctly and separately and can be counted. Therefore a known volume of blood is diluted 20 times.

The WBCs are made more distinctly by the addition of stain in diluting fluid. The red cells are hemolysed and not visible. Count was determined using Neubauer counting chamber and WBC pipette

2.7.4. Clotting time

A drop of blood obtained by the tail vein puncture was drawn in to the capillary glass tube. Time of appearance of blood was noted. A small portion of capillary tube was broken at regular interval of 15 seconds, till a thread of fibrin of clotted blood appeared between the cut pieces of capillary tube. The time interval between appearance of blood and the appearance of fibrin thread is the clotting time.

2.7.5. Biochemical parameters [17, 18, 19]

The following Standardized diagnostic kits (Span Diagnostics Ltd., Surat, India) were used for the Spectrophotometric determination of biochemical parameters.

- *Alkaline Phosphatase kit*
- *Aspartate aminotransferase kit*
- *Alanine aminotransferase kit*
- *Glucose estimation kits*
- *Cholesterol estimation kit*
- *Urea estimation kit*

2.7.6. Estimation of Alanine Aminotransferase level (ALT):

Alanine Aminotransferase catalyses the transamination of L Alanine and α-ketoglutarate to form pyruvate and L –glutamate.Pyruvate so formed is coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown colored complex in alkaline medium and this can be measured colourimetrically at 505nmwith in 15 minutes

2.7.7. Estimation of Aspartate Aminotransferase level (AST):

Aspartate aminotransferase catalyses the transamination of L- Aspartate and α-ketoglutarate to oxaloacetate and L –glutamate. Oxaloacetate so formed is coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and this can be measured colourimetrically at 505nm within 15 minutes.

2.7.8. Estimation of Alanine Aminotransferase level (ALP):

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH10. Phenol so formed reacts in alkaline medium with 4 aminoantipyrine in presence of oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured colourimetrically at 510nm.

2.7.9. Estimation of urea level

Urea is hydrolyzed in presence of water and urease to produce ammonia and carbon dioxide. Under alkaline conditions, ammonia so formed, reacts with hypochlorite and phenolic chromogen to form colored indophenol, which is measured at 578nm. Sodium nitroprusside acts as a catalyst. The intensity of color is proportional to the concentration of urea in the sample.

2.7.9.1. Estimation of serum Creatinine

Creatinine in a protein free solution reacts with alkaline picrate with the development of' a red colour complex. The intensity of the developed colour is measured at 520 nm.

2.7.9.2. Estimation of glucose level

Glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with phenol and 4-aminoantipyrene to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505nm and is directly proportional to glucose concentration in the sample.

2.7.9.3. Estimation of cholesterol level

Cholesterol reacts with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives lavender coloured complex which is measured at 560nm.

2.8. Vital organ weight changes

At the end of the experiment animals were sacrificed by cervical dislocation. Liver, kidney, lungs, brain, spleen and testes were isolated and noted the weight.

2.9. Histopathological studies

The organs were preserved in 10%formalin,dehydrated with ascending grades of ethyl alcohol, embedded in paraffin wax, sliced on a rotary microtome, stained with haemotoxylin and eosin and histomorphological features were examined.

3. Results and Discussion

Acute toxicity study: The LD_{50} value of the alcoholic *extract of the seeds of Asteracantha longifolia* was found to be greater than 2000mg/kg of body weight. This study shows that the seed extract is safe or non toxic even in high acute doses

Sub acute toxicity study: In sub acute toxicity study the following parameters were evaluated in details.

Body weight change: Generally reduction in body weight and internal organ weights are simple indices of toxicity after exposure to toxic substances [20,21.] Table 1 shows the body weight of the control group and extract treated groups on weekly basis for 28 days.

The initial body weight of the control rats (Group I) were 179±2.4 gm and the initial weight of extract treated groups at doses of 100mg/kg (Group II), 250mg/kg (Group III) and 500mg/kg (Group IV) were 186.7±4.4, 195.8±5.2& 192.8±3.74 g respectively. The result of the study showed a significant increase in body weight in the groups II & III. The increase of body weight in the control groups treated with 0.4% CMC was not significant. The increase in body weight in Group II was significant at 21 ($P<0.01$) & 28days ($P<0.001$) of treatment compared to its initial body weight. Group III animals also showed a significant increase in body weight on 21($P<0.001$) and 28 ($P<0.001$) days of treatment. The group IV animals, i.e., rats treated with 500mg/kg extract showed no significant gain in body weight, may be due to the slight toxic effect of the extract at high doses.

Food and water consumption

Table 2 and Table 3 show the food and water consumption of the normal rats and extract treated rats on weekly basis for 28 days. The food intake was expressed as g/100g body weight of the rat and the water intake was expressed as ml/100g body weight of the rat.

There is significant increase in food intake in Group II (100mg/kg) ($P<0.01$) and Group III (250mg/kg) ($P<0.01$) animals at 28 days of treatment compared to that in Group 1 (control) animals. Group IV animals showed a significant ($P<0.01$) reduction in food intake compared to the control group. The loss of weight exhibited by the animals treated with high dose may result from the reduced food intake. This shows that extract may have a toxic effect at high dose (500mg/kg).

Hematological parameters

Hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals.Table.4 shows haematological status after 28 days oral administration of the extract. The result showed no significant difference in hematological parameters between the extract treated and vehicle treated animals.

Biochemical parameters

Table. 5 shows the level of biochemical parameters in rats treated with various doses of ethanolic extract *of Asteracantha longifolia* seed orally for 28 days. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are markers of liver function. The tansaminases AST and ALT are found in many body tissues, with the highest concentration being in hepatocytes and muscle cells.

These enzymes are released in significant amounts in to the blood stream only when hepatic injuries occur.ALT is more hepato-specific than AST because it is more sensitive to hepatic damage. Alkaline Phosphatase are found in the canalicular plasma membrane of hepatocytes, in bone, where they reflect bone building osteoblastic activity. Pathological increases in serum ALP activity may arise in bone disorders serum creatinine concentration is largely determined by its rate of production and rate of renal excretion. Creatinine undergoes complete glomerular filtration with little reabsorption by renal tubules. A reduction in serum creatinine level is observed in cases of muscle wasting as seen in malnutrition. It is frequently used to evaluate renal function.

Catabolism of dietary and endogenous amino acids in the body produces large amounts of ammonia. Ammonia is toxic and its concentration is kept very low by conversion in liver in to urea. Urea is eliminated in urine and represents the major route of nitrogen excretion. Urea levels vary widely with diet, rate of protein metabolism, liver production and glomerular filtration rate (GFR). A very high urea level is usually due to renal disease or decreased renal blood flow. Production is decreased in situations where there is a low protein intake and in some patients with liver disease. Thus non-renal and renal influences should be considered when evaluating changes in serum urea concentrations. The blood urea nitrogen (BUN) is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. BUN is an indication of renal health. If GFR and blood volume decrease BUN will increase.

The raised level of glucose in serum will suggest clinically the diagnosis of diabetes mellitus and raised level of cholesterol increases the risk of heart disease.

The results of the present study showed that there is no significant difference in the level of biochemical parameters such as AST, ALT, ALP, Creatinine, urea, glucose and cholesterol between extract treated groups and vehicle treated groups

Effect of plant extract on organ weight

In toxicology studies organ weight changes is an important endpoint for detecting harmful effects of chemicals. Organ weight changes are often associated with treatment related effects.[22]

Table.6 shows the relative weight in gram per 100g body weight of the vital organs such as Brain, Heart, Kidney, Lungs, Liver and Spleen of control group and the treated groups. The data shows that there is no significant difference in relative organ weight between the control and the extract treated group.

Histopathology of vital organs [23]

Histopathological studies showed the following characters:

Liver: Showed a normal histology in vehicle and all the extract treated groups. Central vein was visible and hepatocytes were visible with distinct purple colour.(Figure I)

Lungs: Showed normal histology in vehicle and all the extract treated groups. Lung is composed of thin-walled alveoli. Alveoli are blind sacs having very thin walls. .(Figure II)

Kidney: Showed a normal histology in vehicle and all the extract treated groups with normal glomerulus and renal tubules. (FigureIII)

Heart: Showed a normal histology in vehicle and all the extract treated animals. Cardiac muscle with centrally located nucleus and intercalated discs are seen. Intercalated discs are specialized junctions between cardiac cells. .(Figure IV)

Brain: Showed a normal histology in vehicle and all the extract treated groups. Histology showed normal neurons arranged in groups separated by bundle of fibers. . (Figure V)

Spleen: Showed a normal histology in vehicle and all the extract treated groups. Histological features of spleen include the white pulp and red pulp. White pulp is made up of lymphocytes that surround arterioles. The part of the splenic tissue which is infiltrated with blood is the red pulp. .(Figure VI)

Testis: The testis showed a normal histology in all the groups except the 500mg/kg extract treated group. This group showed a slight necrosis in seminiferoustubules. (Figure VII)

Conclusion

Asteracantha longifolia is a plant common in India. Seeds are used traditionally for sexual debility, premature ejaculation, erectile failure and oligospermia. Scientific studies have been done to verify its pharmacological and phytochemical aspects. But though it is a widely used plant there is no information about its toxicity upon acute and chronic administration.. Here the present study is conducted to verify its toxicity. Acute toxicity study of the extract as per the latest OECD guidelines revealed that its LD50 is more than 2000mg/kg body weight in albino rats.

Subacute toxicity study of 28 day duration result showed:

1. Increase in body weight and food intake with doses 100mg/kg and 250mg/kg and no gain in body weight and food intake with 500mg/kg of the extract. 2. No significant variation in haematologic and biochemical parameters and in relative organ weight when extract was given in doses of 100, 250 and 500mg/kg body weight. 3. Histopathology showed the presence of slight necrosis in seminiferous tubules at dose of 500mg/kg.

From the results of subacute toxicity studies it may be concluded that the alcoholic extract of *Asteracantha longifolia* seeds is safe for oral administration especially at low and moderate doses. High dose (500mg/kg) is not absolutely free from toxic effects. Further studies are needed to verify these effects.

Acknowledgement

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Table 1.Effect of Plant Extract on Body Weight Changes in Albino Rats

Groups	*Treatment*	*Dose*	*Initial body weight*	*Body weight of the animals in grams on days*			
				7	14	21	28
I	control(0.4%CMC)	0.5 ml/100g	179± 2.4	181±1.7	182.5±1.7	185±2.2	187±1.7$^{\#}$
II	Extract in 0.4%CMC	100mg/kg	186.7±4.41	197.5±4.425	206.7±5.110	213.3±4.944**	220±4.65***
III	Extract in 0.4%CMC	250mg/kg	195.8±5.23	209.2±7.5	216.7±7.26	226.7±2.78$^{\wedge\wedge\wedge}$	233.3±3.3$^{\wedge\wedge\wedge}$
IV	Extract in 0.4%CMC	500mg/kg	192.8±3.745	187.2±2.713	189±3.27	193±4.655$^{\#}$	190±4$^{\#}$

Data were analyzed by one way ANOVA followed by Turkey's multiple comparison tests and student's t test.
Values are expressed as mean ±SEM (n=6 rats) P values: * < 0.05, ** < 0.01, *** < 0.001 Vs initial weight of Group II
P values: ^ <0.05, ^^ < 0.01, ^^^ < 0.001 Vs initial weight of Group III # - Not significant compared to initial weight of the Group.

Table 2.Effect of Extract on Food Intake of Albino Rats

Groups	*Treatment*	*Dose*	*Food intake in g/ 100g body weight on days*				
			1	7	14	21	28
I	Normal control (0.4%CMC)	0.5 ml/100g	11.86 ±0.20	12.41±0.11	12.75 ±0.08	12±0.21	12±0.17$^{\#}$
II	Extract in 0.4%CMC	100mg/kg	11.23±0.40	12.21±30	12.49±0.10	12.94±0.38	13.93±0.36**
III	Extract in 0.4% CMC	250mg/kg	12.3±0.28	12.28±0.33	12.43±0.23	12.69±0.57	13.97±0.41**
IV	Extract in 0.4% CMC	500mg/kg	11.35±0.43	10.9±0.36$^{\wedge\wedge}$	10.59±0.19$^{\wedge\wedge}$	9.8±0.37$^{\wedge\wedge}$	10.97±0.34$^{\wedge\wedge}$

Data were analyzed by one way ANOVA followed by Turkey's multiple comparison test and student's t test.
Values are expressed as mean ±SEM (n=6 rats)
P values: * < 0.05, ** < 0.01, *** < 0.001 Vs 28days food intake of Group I $^{\wedge\wedge}$ P <0.01 Compared to food intake of Group I

Table3. Effect of Extract on Water Intake of Albino Rats

Groups	*Treatment*	*Dose*	*Water intake in ml/100g body weight on days*				
			1	7	14	21	28
I	Normal control (0.4%CMC)	0.5 ml/100g	14.23±0.59	14±0.70	13.6±0.37	14.5±0.46	14±0.36
II	Extract in 0.4%CMC	100mg/kg	14±0.27	15±0.28	16±0.17	15±0.24	15±0.36$^{\#}$
III	Extract in 0.4% CMC	250mg/kg	15±0.28	15±0.26	15±0.059	16±0.20	15±0.11$^{\#}$
IV	Extract in 0.4% CMC	500mg/kg	15±0.20	15±0.21	15±0.13	14±0.19	15±0.19$^{\#}$

Data were analyzed by one way ANOVA followed by Turkey's multiple comparison test and student's t test.
Values are expressed as mean ±SEM (n=6 rats)
Comparisons were made between group I with group II, III and IV
#-non significant

Table 4.Effect of the Plant Extract on Hematological Parameters in Albino Rats

Groups	*Treatment*	*Dose*	*RBC (10^6 cells /Cu.Mm)*	*WBC (Cells/Cu.Mm)*	*Haemoglobin (gm %)*	*Clotting time (seconds)*
I	Normal control (0.4%CMC)	0.5 ml/100g	8.53±0.16	8408±346	15±0.075	121±0.034
II	Extract in 0.4%CMC	100mg/kg	9.43±0.13$^{\#}$	9808±680$^{\#}$	16±0.19$^{\#}$	122±065$^{\#}$
III	Extract in 0.4% CMC	250mg/kg	8.26±0.23$^{\#}$	10150±784$^{\#}$	14±0.69$^{\#}$	121±0.92$^{\#}$
IV	Extract in 0.4% CMC	500mg/kg	8.69±0.30$^{\#}$	9667±233$^{\#}$	15±0.24$^{\#}$	120±.038$^{\#}$

Data were analyzed by one way ANOVA followed by Turkey's multiple comparison test and student's t test. , Values are expressed as mean ±SEM (n=6 rats) Comparisons were made between group I with group II, III and IV #-non significant

Table5.Effect of Plant Extract on the Biochemical Parameters of Albino Rats

Groups	*Treatment*	*Dose*	*ALT (U/L)*	*AST (U/L)*	*AP (U/L)*	*Glucose (mg %)*	*Cholesterol (mg %)*	*Creatinine (mg %)*	*Urea (mg %)*	*BUN mg %)*
I	Normal control (0.4%CMC)	0.5ml/kg	86±5.1	174±16	235.6±14.23	78±1.2	69±0.25	0.22±0.05	34.2±0.99	16±0.46
II	Extract in 0.4%CMC	100mg/kg	81±4$^{\#}$	210±16$^{\#}$	226.8±6.6$^{\#}$	80±2.2$^{\#}$	70±1.1$^{\#}$	0.21±0.051$^{\#}$	32.±1.25$^{\#}$	15±0.58$^{\#}$
III	Extract in 0.4% CMC	250mg/kg	83±2.1$^{\#}$	179±12$^{\#}$	245.3±6.5$^{\#}$	78±2.4$^{\#}$	68±1.8$^{\#}$	0.20±0.084$^{\#}$	34.4±1.09$^{\#}$	16±0.52$^{\#}$
IV	Extract in 0.4% CMC	500mg/kg	76±5.4$^{\#}$	157±8$^{\#}$	239.8±12.6$^{\#}$	91±2.3$^{\#}$	72±0.75$^{\#}$	0.20±0.043$^{\#}$	32.7±1.59$^{\#}$	15±0.74$^{\#}$

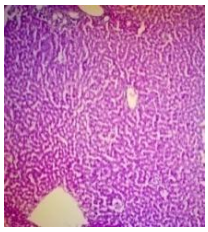
Data were analyzed by one way ANOVA followed by Turkey's multiple comparison tests and student's t test.
Values are expressed as mean ±SEM (n=6 rats) Comparisons were made between group I with group II, III and IV , #-non significant

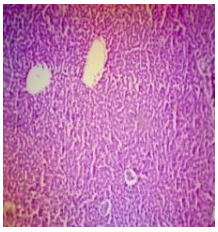
Table 6. Effect of Plant Extract on Relative Organ Weight of Albino Rats

Groups	*Treatment*	*Dose*	*Brain (g/100g)*	*Heart (g/100g)*	*Kidney (g/100g)*	*Lungs (g/100g)*	*Liver (g/100g)*	*Spleen (g/100g)*
I.	Normal control(0.4%CMC)	0.5ml/kg	1.1±0.021	0.35±0.006	0.79±0.0073	0.73±0.034	2.9±0.42	0.31±0.023
II.	Extract in 0.4%CMC	100mg/kg	1.1±0.058$^{\#}$	0.35±0.014$^{\#}$	0.83±0.027$^{\#}$	0.78±0.03$^{\#}$	3.0±0.15$^{\#}$	0.34±0.025$^{\#}$
I.	Extract in 0.4% CMC	250mg/kg	0.96±0.04$^{\#}$	0.34±0.017$^{\#}$	0.77±0.031$^{\#}$	0.75±0.055$^{\#}$	3.4±0.17$^{\#}$	0.27±0.028$^{\#}$
II.	Extract in 0.4% CMC	500mg/kg	0.99±0.03$^{\#}$	0.33±0.018$^{\#}$	0.83±0.040$^{\#}$	0.74±0.047$^{\#}$	3.9±0.32	0.24±0.022$^{\#}$

Data were analyzed by one way ANOVA followed by Turkey's multiple comparison test and student's t test. Values are expressed as mean ±SEM (n=6 rats), Comparisons were made between group I with group II, III and IV, #-non significant

Figure 1. Histopathology of liver


Control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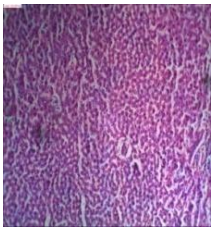
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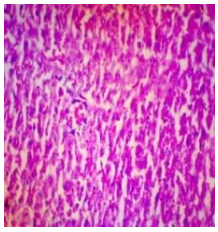
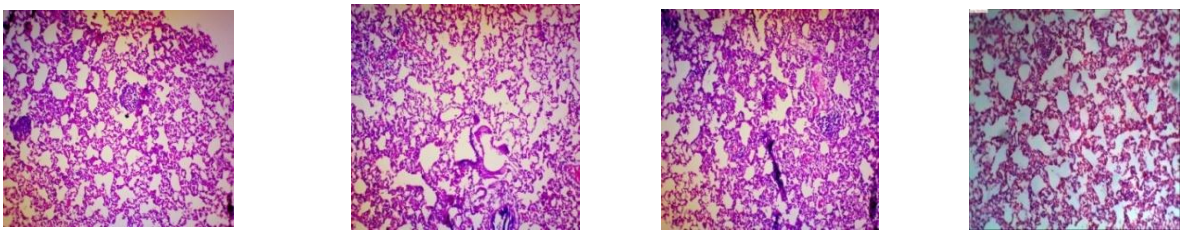
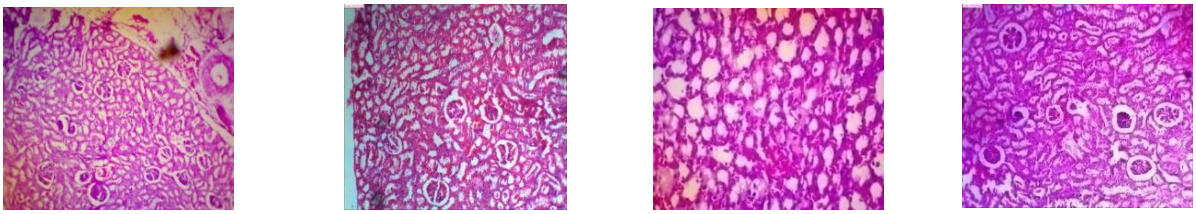

500 mg/kg extract

Figure 2. Histopathology of lungs



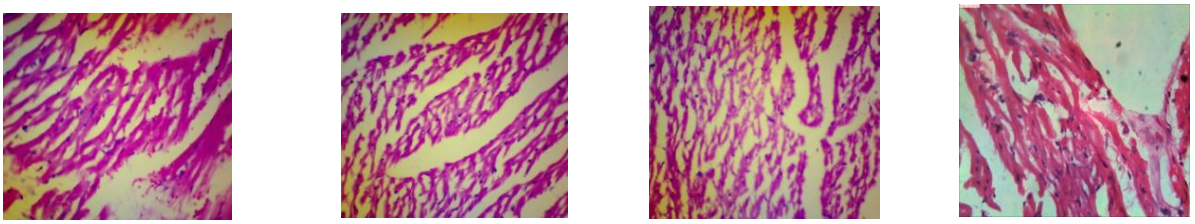
Control 100 mg/kg extract: 250 mg/kg extract: 500 mg/kg extract

Figure 3. Histopathology of kidney



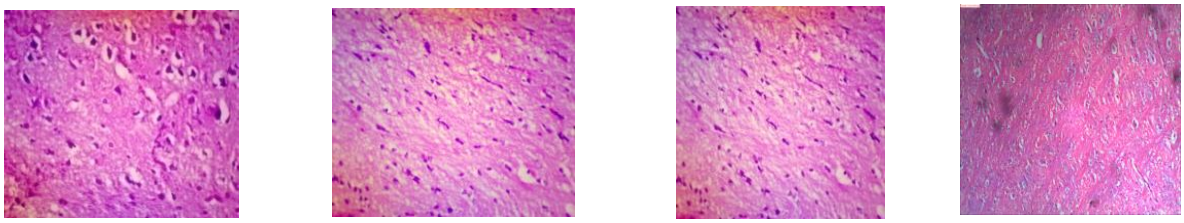
Control 100 mg/kg extract: 250 mg/kg extract: 500 mg/kg extract

Figure 4. Histopathology of heart



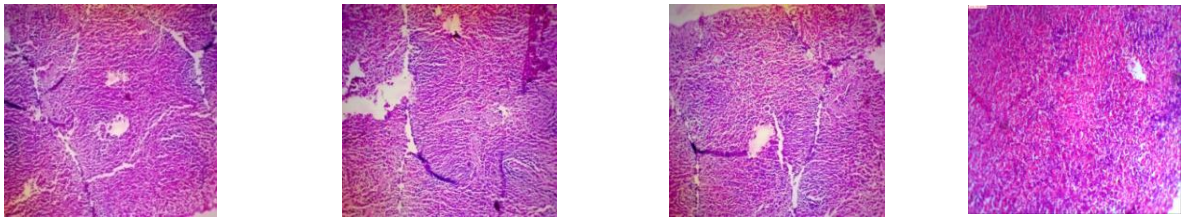
Control 100 mg/kg extract: 250 mg/kg extract: 500 mg/kg extract

Figure 5. Histopathology of brain



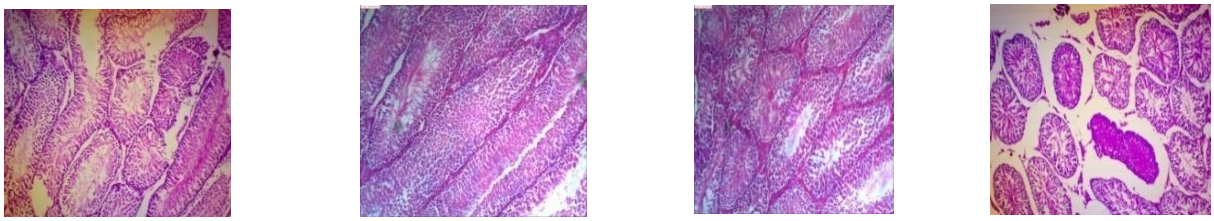
Control: 100 mg/kg extract: 250 mg/kg extract: 500 mg/kg extract

Figure 6. Histopathology of spleen



Control 100 mg/kg extract: 250 mg/kg extract: 500 mg/kg extract

Figure 7. Histopathology of testis



Control | 100mg/kg extract | 250mg/kg extract | 500mg/kg extract Necrotic tubules

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