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TOXIC EFFECT OF METHNOLIC EXTRACT OF *HOUTTUYNIA CORDATA* ON KIDNEY, BLOOD AND LIVER OF RATS

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ABSTRACT
The plant *Houttuynia cordata* Thunb (*H. cordata*) Family Saururuacea locally known as "Moshundari" is frequently used for medicinal and nutritional purpose in the Assam region of India. It is an erect, annual herbecious plant. It is posses variety of therapeutic and nutritional property such as antibacterial, anti-inflammatory, Anti allergic activity, Anticancer/Antitumor activity, Antidiabetic activity, Antioxidant activity, Antiviral activity, Immunomodulatory activity, Anti-obesity activity and Antidiarrhoeal activity but not yet toxicity study evaluated. On the basis of these activity the present study was initiated 28day sub-acute toxicity of 80%methnolic extract of *H. cordata* on experimental animal to estimate human susceptibility to its toxicity. The Organization for Economic Co-operation and Development (OECD) test guideline 407, for the testing of chemicals was used for toxic evaluation of *H. cordata* plant traditionally used by rural person of Assam. The plant extract did not show any change in the SGOT, SGPT, albumin, creatinine, bilirubin, total leucocytes count, deferential leukocytes count etc the plant extract did not induce any damage to the liver and kidney. Histopathology examination of liver, brain and kidney did not reveal major changes indicative of tissue damage owing to test drug administration. The results have demonstrated that the *H. cor*data plant extract at the dose level of 400mg/kg b.w. is safe when administered orally in rats. The findings support an assurance for the medicinal use the plant in folk medicine.

KEY WORDS: *H. cordata*, Sub-acute toxicity, 80%methanolic extract, OECD-407.

1. INTRODUCTION
Medicinal plants contain potentially useful chemicals that serve as basis for the manufacturing of modern medicine. Now a day frequently used herbal medicine and their clinical importance has received considerable attention. Some plants may produce adverse effect on long term used such as hepatotoxicity, nephrotoxicity etc. Toxicity is defined as any harmful effect of chemical or a drug on a target organism. It can refer to the effect on a cell, an organ, or the whole organism. All substances are potentially toxic but they are beneficial when used in appropriate level. The traditional medicine is the main resources for majority of the people for primary health care. The safety of these traditional medicines is still doubtful, though effectiveness of these plants has been validated through research and clinical studies.[1] This is because although plants generally contain some bioactive principles believed to be responsible for their therapeutic effects. They also contain some phytotoxins and other heavy metal contaminants whose toxicological actions have always been ignored. The plants are often considered as safe without considering the potential toxicity associated with the plant. Generally plant substances are absorbed from blood stream and detoxify by the liver. After detoxification by the liver these are excretes out from the body through the kidney. Therefore it is very much essential to analyze the effect of plant constituent on the liver and kidney function as well as their metabolism. The plant *Houttuynia cordata* Thunb (*H. cordata*) Family Saururuacea locally known as "Moshundari" is frequently used for medicinal and nutritional purpose in the Assam region of India. It is an erect, annual herbecious plant. It is posses variety of therapeutics and nutritional property such as antibacterial[2], antiinflammatory[3], antiallergic activity[4], anticancer/antitumor activity, antidiabetic activity[5], antioxidant activity[6], antiviral activity[7], immunomodulatory activity[8], anti-obesity activity[9] Antidiarrhoeal activity.[10] It also contains more than 20 amino acids, many micro elements including iron, manganese, magnesium, copper, zinc, potassium, calcium etc.[5], but not yet toxicity study evaluated. On the basis of these activity the present study was initiated

28 days sub-acute toxicity of 80%methnolic extract of *H. cordata* on experimental animal to estimate human susceptibility to its toxicity.

2. MATERIALS AND METHOD

2.1 Collection and authentication of plant material

The whole plant of *H. cordata* were collected from surrounding Dibrugarh University, Assam (India) in the month of Jan-Feb 2010 and were authenticated by Dr. Tariq Hussain Scientist, Taxonomy division at National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh (India). A voucher specimen (NBRI/CIF/145/2010) has been deposited in the institute for further reference.

2.2 Preparation of the extract: The whole plant of *H. cordata* was collected and washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. The dried plant material were reduced to coarse powder by mechanical grinder, the powdered drug was extracted with 80% methanol as solvent in soxhlet extractor for 18 h. The extract was filtered and concentrated under reduce pressure using rotavapor. The extract was freeze-dried and stored in deep freezer for further use. The solutions of "80%methanolic extract of *H. cordata*" (HCM) was prepared freshly each day for toxicity study.[11, 12]

2.3. Preliminary phytochemical screening: The methanol extract obtained was tested for the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar.[13, 14]

2.4. Experimental animals: The experiment was carried out on adult wistar albino rats, weighing between 140-180gm, of both sex. The animals were acclimatized for 5 days before experiment in standard laboratory conditions in cross ventilated animal house of the Institute at temperature 25±2 ^{0}C, relative humidity 45-55% and light and dark cycles of 12:12 hours, feed with standard pallet diet and water ad libitum. The experiment was approved (Reg.No.1413/PO/a/11/CPCSEA) by the institutional animal ethics committee of the organization. Each animal treated with 400mg/kg/day of extract.

2.5 Sub-acute toxicity evaluations: The method was performed according to the OECD guidelines for the testing of chemicals, Test guidelines 407, with slight modifications. Healthy male and female rats were randomly divided into two groups of 10 rats (5 males and 5 female) namely the Group-I control, Group- II test (HCM) groups. Each animal were testing for sub-acute toxicity treated as- Control group (Group I), received vehicle (0.5% CMC, 10ml/kg p.o.) once daily for 28 days, test group (Group II) received test sample (HCM, 400mg/kg p.o.) once daily for 28 days. The animals in each group were weighed on day zero and weekly thereafter and food consumption and water intake were monitored daily. Animals were observed for signs of abnormalities during the treatment period. At the end of the treatment, animals were fasted overnight, but allowed access to water ad libitum. Before sacrifice of animals, urine collections were done. At the end of study, animals were euthanized by chloroform for blood collection through retro-orbital puncture with and without anticoagulants for hematological and biochemical studies, thereafter animals were sacrificed by cervical dislocation for organ study, the following parameter were observed during the period and end of the experiment.[15, 16]

2.5.1 Body weight analysis: The body weight should be measured on day zero and weekly thereafter. Body weight is one of the most sensitive indicators for the condition of an animal if it is monitored frequently and carefully during a study. Marked body weight loss is usually a harbinger of ill health or death. Body weight loss can be due to decreased food consumption, water consumption, disease or specific toxic effects.

2.5.2 Urine analysis: Animals were kept in metabolic cage and urine samples were collected for 24 h of period before sacrifice of animals. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40 ^{0}C. The changes in urinalysis parameters due to the treatment of HCM were evaluated by measuring urine volume, appearance, pH, specific gravity, albumin protein and glucose.

2.5.3 Hematological analysis: At the end of study, Blood was obtained from all animal by puncturing retro-orbital plexus. The blood sample were collected and preserved with anticoagulant (EDTA) agent. The preserved blood was utilized for various hematological parameters.

2.5.4 Bio-chemical analysis: At the end of study, Blood was obtained from all animal by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45min. at room temperature for serum analysis. Serum was separated by centrifugation at 3000 rpm at 40 ^{0}C for 15min. and utilized for the estimation of various bio-chemical parameters.

2.5.5 Organ weight and histopathology analysis: At the end of study animals were sacrificed; various organs (Liver, brain and kidney) were weighed, excised and fixed in formalin solution (Fig.1). The fixed organs were cut to slices approximately 1-mm thick, and after tissue processing, paraffin sections (5-µm thickness) were prepared and stained with hematoxylin and eosin (H&E) for histopathological study.

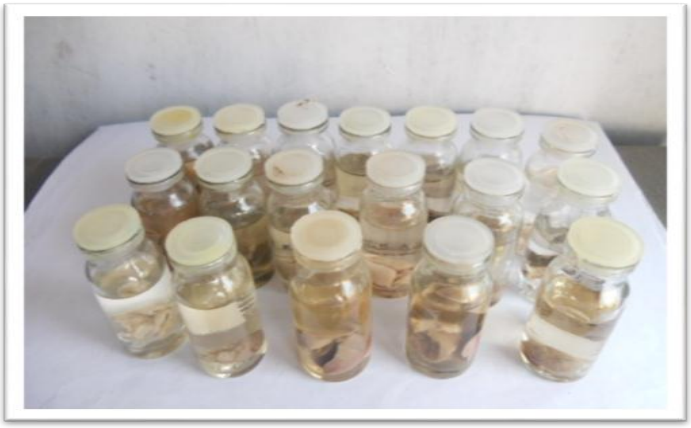

Fig. 1: ***All organs in formalin solution***

2.6 Statistical analysis: All the values are expressed as mean ±standard error of mean (S.E.M.) and analyzed for ANOVA and post-hoc Tukey-Kramer Multiple Comparisons Test by employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at P <0.05 levels.

3 RESULTS

3.1 Phytochemical screening: The 80%methanolic extract was tested and showed presence of alkaloids, flavonoids, terpenoids, sterols, tannins carbohydrates and amino acids.

3.2 Effect of Extract on General Behavior/Mortality: In the sub-acute toxicity study, both the male and female rats administered with 400mg/kg b.w. HCM extract has did not show symptoms of toxicity. No morbidity/ mortality (Tables 1 and 2) were observed in treated group of rats throughout the observation period (28day) of experiment.

Table 1: Effect of extract on general behavior of animals

Parameters	Observations
Skin	Normal
Color & consistency of the faces	Normal
Condition of fur	Normal
Abdominal distension	Nil
Pupil diameter	Normal
Eyes dullness	Nil
Eyes opacities	Nil
Condition of teeth	Normal
Breathing abnormalities	Nil
Gait (manner of moving)	Normal

Table 2: Effect of extract on mortality of animals

Groups	No. of rats treated	% Mortality (up to 28 days)	
		Male	Female
G-I (Control)	5 Males + 5 Females	NIL	NIL
G-II (HCM)	5 Males + 5 Females	NIL	NIL

3.3 Effects of Extract on the Body Weight of Animals All rats showed significant increase in body weight compared to their initial weights on day 0. However, no significant change in mean body weight was observed on days from initial to end of study between the control group and test groups. The body weight gains (Fig. 2) of both, the female and male rats treated with HCM at dose of 400mg/kg b.w. are presented in (Table 3).

3.4 Effects of Extract on the Urine Analysis: At test dose of HCM, volume and pH of urine in test groups was slightly increased (Fig. 3); it indicates the excretion of samples through kidney and presence of basic compound in urine. These changes may not be indicative of the toxicological sign of kidneys (Table 4).

3.5 Effects of extract on Hematological analysis: The test dose of treated plants material extract at 400mg/kg dose showed nosignificant variations (Fig. 4 and 5) in the values obtained for red blood cell count (RBC), white blood cells count (WBC), hemoglobin content (Hb), hematocrit, differential leukocytes counts (DLC), and platelets counts among all the groups (Tables 5, and 6).

3.6 Effects of Extract on Bio-Chemical Analysis: The biochemical parameters of the control and test groups are

presented in Table 7. The following biochemical parameters in serum were evaluated at the end of the test period: Blood Glucose, Total Cholesterol, Total protein, albumin, SGOT, SGPT, Total Bilirubin, Creatinine and urea. There were no significant differences (Fig. 6) recorded in any of the analyzed biochemical parameters.

3.7 Effects of Extract on Organ Weight and Histopathology: At the end of study, Animals were sacrificed; various organs were excised and their wet weight was measured (Table 8). The treated animals did not show significant changes in color, texture and weight (Fig. 7) when compared to control group. The histological study of various organs of test groups compared to control group and found no change in cellular parts (Fig. 8).

Table 3: Analysis of extract on body weight of animals

Body Wt. (g)	Groups			
	Control		HCM	
	Male	Female	Male	Female
Day 0	146.15±5.62	140.6±6.19	164.6±6.56[a]	153.87±5.32[b]
Day 7	146.9±5.24	141.2±3.54	165.14±2.18[a]	155.32±4.54[b]
Day 14	147.7±3.49	142.4±3.78	166.12±7.65[a]	156.7±5.48[b]
Day 21	148.3±4.31	143.0±5.16	166.7±4.19[b]	157.39±8.20[a]
Day 28	148.6±4.26	143.4±3.22	167.48±4.20[c]	157.66±6.79[a]

All values are mean ± SEM, n = 5
Within row showed significant difference [a]p <0.05, [b]p <0.01, [c]p <0.001

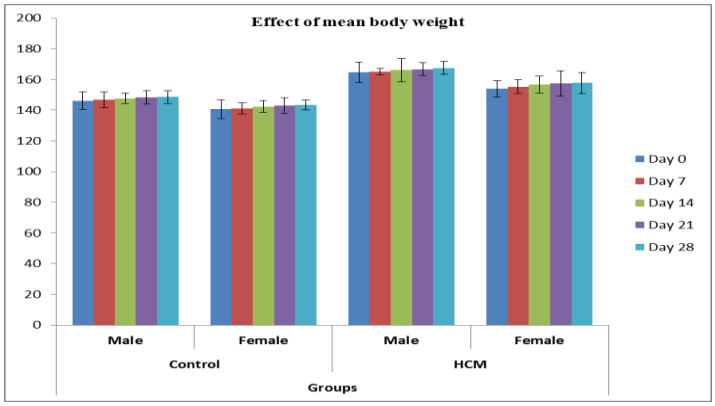

Fig. 2: ***Effect of extract on body weight of animals***

Table 4: Effects of extract on the renal parameters

Urinary parameters (24-hr urine)	Groups			
	Control		HCM	
	Male	Female	Male	Female
Appearance	**Light yellow**	**Light yellow**	**Yellow**	**Yellow**
pH	7.6±0.32	7.8±0.68	8.2±0.88	8.5±0.48
Urine volume (ml)	5.34±0.22	5.27±0.38	6.38±0.72	6.27±0.48
Specific gravity	1.012± 0.004	1.014± 0.002	1.015± 0.002	1.016± 0.004
Albumin	Ab	Ab	Ab	Ab
Glucose	Ab	Ab	Ab	Ab

All values are mean ± SEM, n = 5, Ab – Absent

Table 5: Effects of extract on Hematological parameters

Hematological parameters	Groups			
	Control		HCM	
	Male	Female	Male	Female
Hb (g/dl)	14.8±1.31	14.5±1.12	14.7±0.54	14.3±1.29
RBC (millions/mm^3)	6.8±1.13	6.6±1.23	6.9±1.18	6.7±1.16
WBC (Thousands/mm^3)	5.25±0.71	4.7±1.39	5.2±1.96	4.9±1.27
Platelet (x 10^3/µl)	428±4.39	396.2±5.79	426±7.21	402.8±5.42
Hematocrit (%)	46.7±4.83	43.2±2.41	44.8±2.71	41.6±2.24

All values are mean ± SEM, n = 5

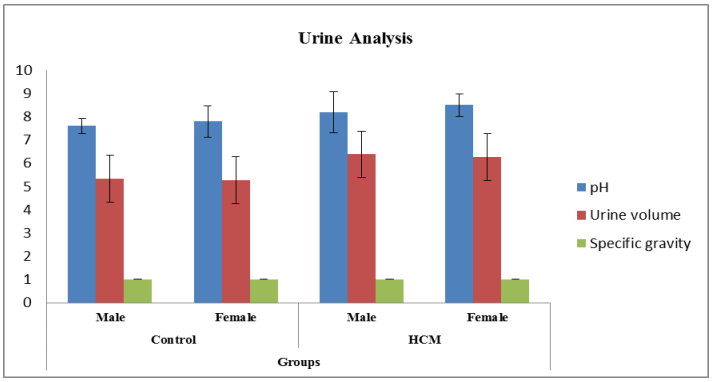

Fig. 3: ***Effect of extract on renal parameters of animals***

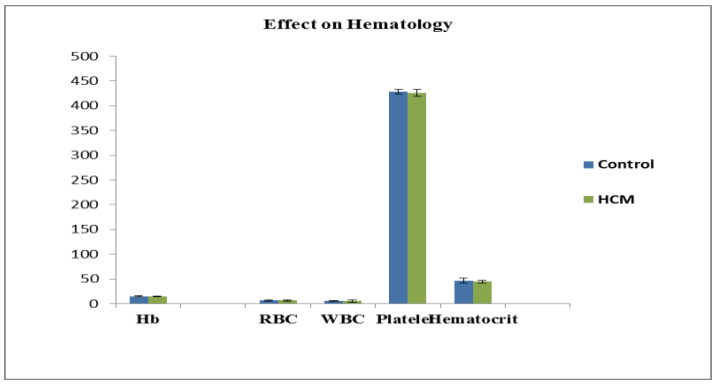

Fig. 4: ***Effect of extract on hematological parameters***

Table 6: Effects of extract on differential leucocytes count

Particulars (%)	Groups			
	Control		HCM	
	Male	Female	Male	Female
Neutrophils	23.1±1.42	23.2±1.27	20.6±1.61	22.6±1.48
Basophils	0	0	0	0
Eosinophils	1.6±0.14	1.3±0.07	1.4±0.13	1.2±0.18
Lymphocytes	73.2±3.27	73.5±2.41	75.7±2.87	73.9±2.47
Monocytes	2.1 ±0.26	2.0±0.19	2.3±0.28	2.3±0.13

All values are mean ± SEM, n = 5

Table 7: Effects of extract on Biochemical parameters

Biochemical parameters	Groups			
	Control		HCM	
	Male	Female	Male	Female
Blood Glucose (mg/dl)	91.5±3.21	98.1±4.48	92.3±3.29	100.6±4.22
T-Cholesterol (mg/dl)	112.42±3.98	110.9±4.68	111.2±4.42	112.6±4.33
Total protein (g/dl)	4.2±0.12	4.4±0.17	4.2±0.11	4.6±0.14
Albumin (g/dl)	2.9±0.14	2.8±0.17	2.6±0.12[a]	2.7±0.15
Total Bilirubin (mg/dl)	0.4±0.06	0.5±0.02	0.3±0.01	0.4±0.05
SGOT (IU/l)	54.2±2.31	52.4±2.18	53.25±2.68	55.76±2.61
SGPT (IU/l)	22.4±2.61	22.8±2.45	21.17±2.86	24.8±3.11
Creatinine (mg/dl)	0.5±0.08	0.3±0.03	0.4±0.06	0.2±0.02
Urea (mg/dl)	22.0±2.23	23.1±2.17	21.3±2.43	23.3±2.23

All values are mean ± SEM, n = 5
Within row showed significant difference [a]p <0.05

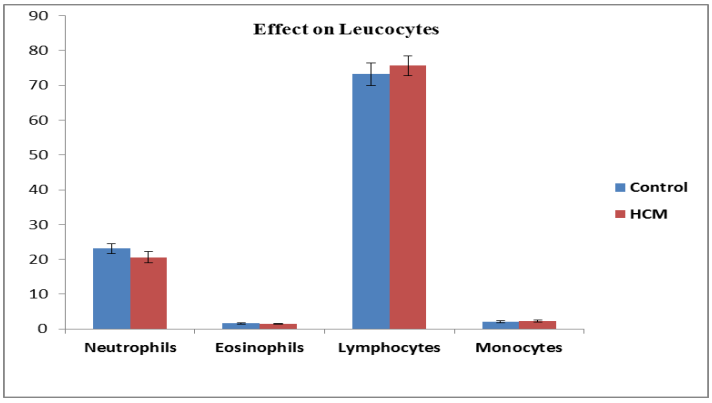

Fig. 5: ***Effect of extract on differential leucocytes count of animals***

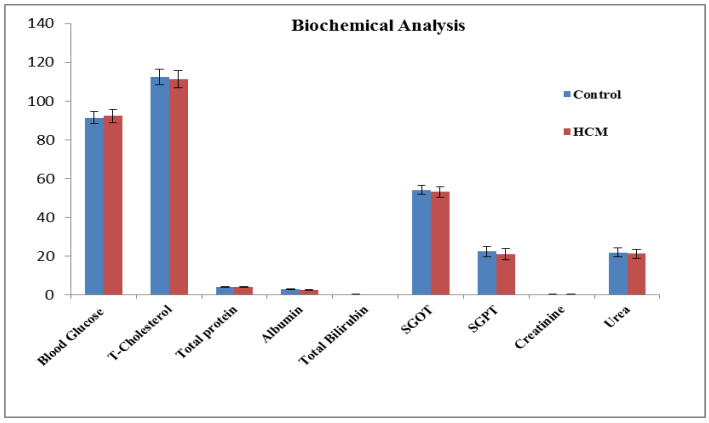

Fig. 6: ***Effect of extract on various biochemical parameters of animals***

Table 8: Effects of extract on organ weight

Organ Weights (gm)	Groups			
	Control		HCM	
	Male	Female	Male	Female
Liver	5.5±0.26	5.34±0.18	5.66±0.42	5.58±0.58
Kidney	0.86±0.16	0.83±0.12	1.09±0.08	1.04±0.02
Brain	1.2±0.42	1.1±0.22	1.3±0.39	1.25±0.21
Spleen	0.65±0.02	0.63±0.05	0.78±0.03[b]	0.74±0.02[a]
Heart	0.41±0.03	0.39±0.04	0.61±0.02[c]	0.6±0.03[b]

All values are mean ± SEM, n = 5

Within row showed significant difference [a]p <0.05, [b]p <0.01, [c]p <0.001

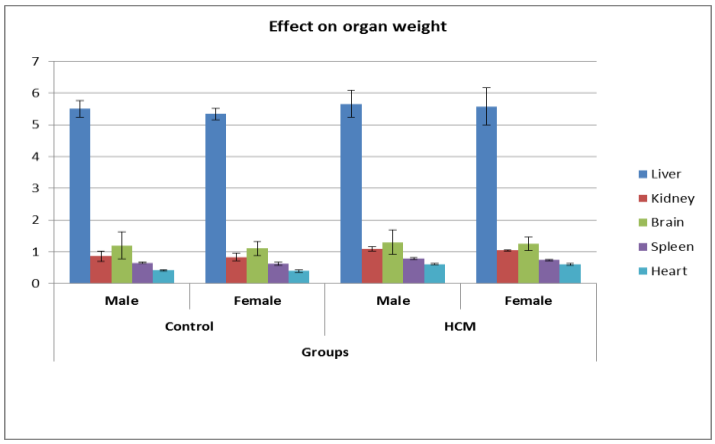

Fig. 7: ***Effect of extract on organ weight of animals***

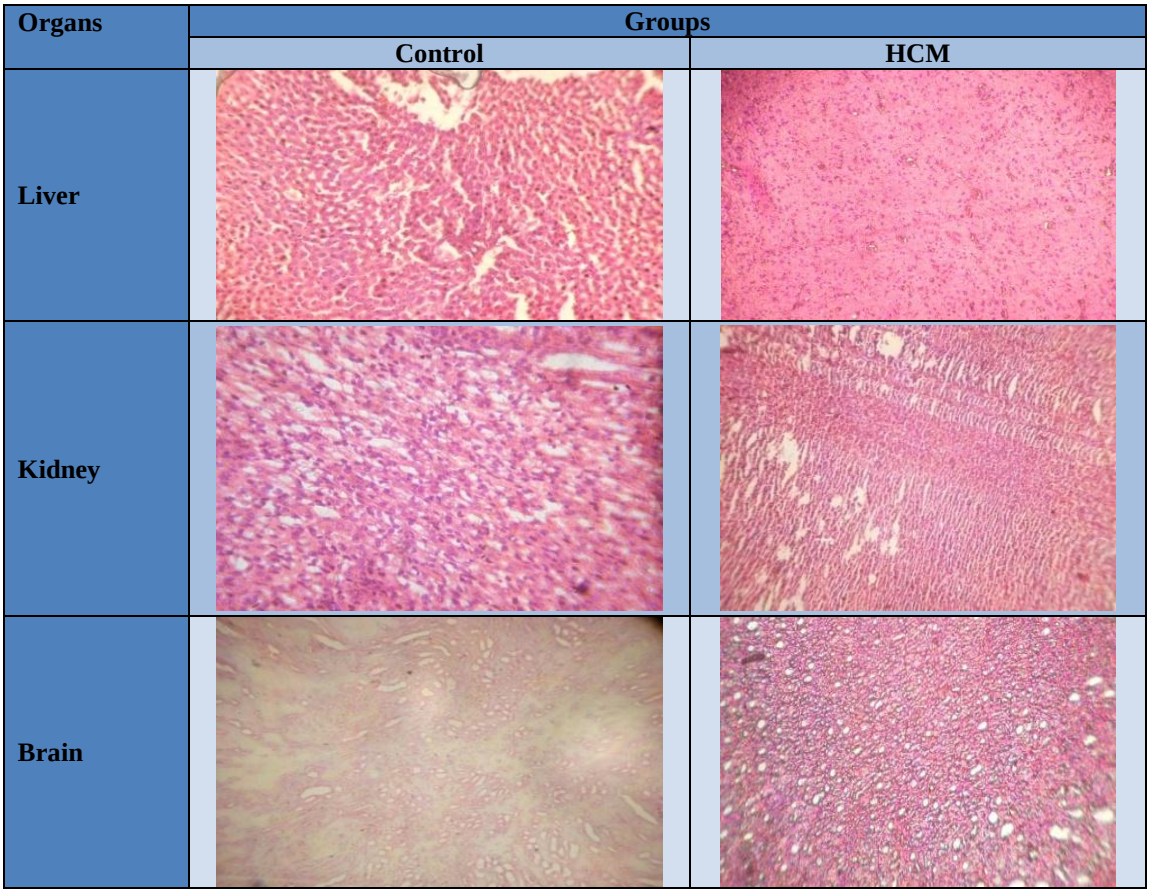

Fig. 8: ***Effect of extract on various organs (Histopathology) of animals***

4 DISCUSSIONS

In the assessment of liver damage by drugs or any other hepatotoxin, the determination of enzyme levels such as serum glutamic pyruvic transaminase (SGPT) also known as alanine aminotransferase (ALT) and serum glutamic oxaloacetic transaminase (SGOT) also known as aspartate aminotransferase (AST), is largely used. These liver enzymes form a major constituent of the liver cells. They are present in lesser concentration in the muscle cells. When the liver cells get damaged or injured, these enzymes seep into the blood stream, raising their blood levels. Hence raised blood levels of SGOT and SGPT signifies liver disease or injury. SGPT and SGOT are normally present in a number of tissues such as heart, liver, muscle, brain and kidney. SGPT is normally present in large concentrations in the liver. Hence, due to liver damage its level in the blood rises, thereby, serving as a specific indicator for liver injury. SGOT is not highly specific liver tissue damage indicator as it can be elevated in conditions other than liver damage.[17] The Liver and kidney play a key role in metabolic processes. They are target organs for toxic chemicals due to their essential functions in bodily detoxification and excretion processes. Thus, they are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity. Toxicity-related changes in the weights of these vital organs are often accompanied by corresponding histopathological findings.[18] Therefore, it could safely be claimed that the liver and the kidneys could serve as the primary target organs in investigations related to the sub-acute oral toxicity of herbal extract. Blood is an important index of physiological and pathological status in man and animals. The parameters usually measured are hemoglobin, white blood cell count, platelets count etc. The normal range of these parameters can be altered by the ingestion of some toxic plants.[19] Blood cells are produced in the bone marrow. In the haematological studies (Table 5), there was no significant difference between test and control animals. Hence, the extract may not have deleterious effects on bone marrow function. Serum ALP level on the other hand, is related to the function of hepatic cell and increased level is due to increased synthesis of the enzyme, in the presence of increasing biliary pressure. Albumin is synthesized in the liver decreased levels are implicated in malfunction in hepatic synthetic ability whiles excessive levels are usually due to dehydration.[20] The *H. cordata* plant extract did not induce any damage to the liver and kidney. Histopathology examinations of vital organs did not reveal major changes indicative of tissue damage owing to test drug administration (Fig. 8), no significant treatment related effect was observed when compared with control. It might be due to the absence of cyanogenic glycoside in the plant extract that was responsible for histopathological changes.[21] The results have demonstrated that the *H. cor*data plant extract at the dose level of 400mg/kg b.w. was safe when

administered orally in rats. The findings support an assurance for the medicinal use for these plants in folk medicine.

5 CONCLUSIONS

During sub-acute toxicity study no mortalities were observed and rats did not show any signs of toxicity throughout the study period of 28 days. There was a significant ($P<0.05$) increase in body weight. After 28 days treatment, there were no treatment-related changes in biochemical & hematological parameters between control and treated groups. This is indicating that the plant *H. cordaat* was not toxic in experimental animals. The sub acute toxicity study indicates that the selected plant material was safe as medicinal use.

6 ACKNOWLEDGMENT

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