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Chronic toxicity studies: Effects of *Urtica urens* Linn. on hematological, biochemical and histo-pathological parameters in albino rabbits.

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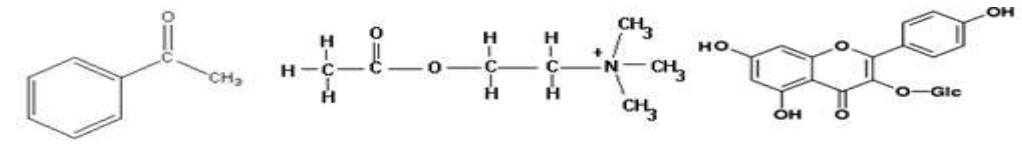
Abstract: *The purpose of this research work was to explore chronic toxicity data on Urticaurens Linn (U. urens). To achieve this purpose 25 mg/kg/day dose of U. urens was given orally to test group albino rabbits (both sexes; UM: male test group treated with U. urens; UF: female test group treated with U. urens) for threemonths. The blood was collected by cardiac puncture and evaluation of hematological, blood and urine biochemistry of male and female test groups were carried out by comparing with their standard control groups respectively. Histo-pathological examination of heart, liver, kidney and stomach tissues of male test group (UM) was carried out with reference to the male control group. Platelet count was found significantly raised ($p<0.01$) in both sexes test groups. Urea level was ($p<0.01$) lowered in female test group while ($p<0.01$) raised in male test group as compared to their respective control groups. Significant elevation and depression ($p<0.01$) of cardiac enzymes and lipid profile parameters were observed in both test groups on comparison with their control groups. SGOT and alkaline phosphatase levels were ($p<0.05$) lowered in female test group whereas; ($p<0.01$) depressed in male test group as matched with their control groups. Gamma GT was found ($p<0.01$) elevated in female test group and ($p<0.05$) lowered in male test group. No noteworthy changes were observed in urine parameters of male and female test groups except for the presence of blood in male test group urine. No significant histo-pathological changes were found in stomach, heart and liver tissues; while acute tubular necrosis was observed in male test group in comparison to male control group. Our results affirm the safe and effective use of U. urens if taken in prescribed dose and for recommended time period.*

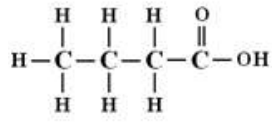
Keywords:*Biochemical evaluation,burning nettle,hematological parameters, histo-pathological evaluation*

I. Introduction

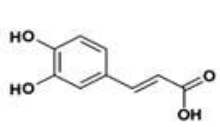
Urtica urens L. (Urticaceae) is commonly known as small nettle or burning nettle. Stinging hairs are the characteristic feature of this plant.It was incorporated in European Pharmacopeia 7th edition, under the heading of *Urticaefolium* monograph [1-2]. Traditionally, the herb was used for the treatment of diabetes mellitus, arthritis, rheumatism of joints and muscles.

In general,*Urtica urens* is used in allergic conditions, blood cleansing, inflammation reduction, relieving of pain, to stop hair fall, increase in urine flow, stop bleeding, dilating blood vessels, lowering blood pressure and healing wounds [3]. The medicinal uses of *U. urens* are attributed to its rich chemical constitution. Its known chemical constituents are formic acid, histamine, serotonin, choline, amino acids, lecithin, kaempferol, sterols and vitamins, scopoletin, sterols, fatty acids, isolectin and carbohydrates [4]. Sterols and phenolcarboxylic acids are the main active constituent [2]. Reported chemical constituents are shown below.

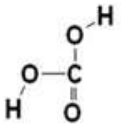




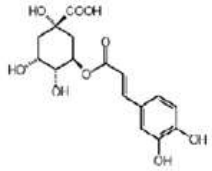
Butyric acid



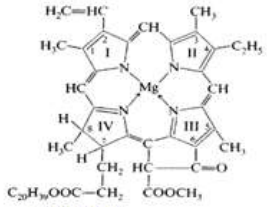
Caffeic acid



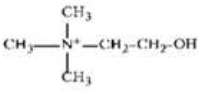
Carbonic acid



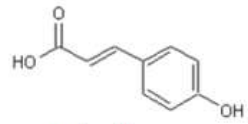
Chlorogenic acid



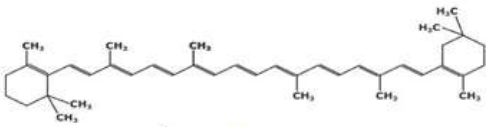
Chlorophyll



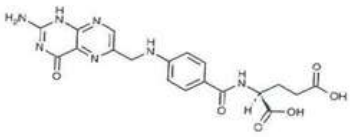
Choline



ρ-coumaric acid

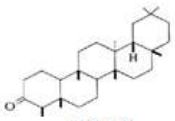


β-carotene

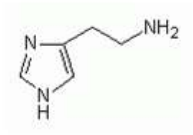


Folacin

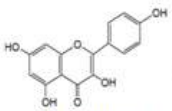
Formic acid



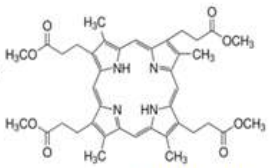
Friedelin



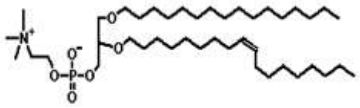
Histamine



Kaempherol

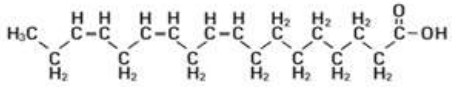


Coproporphyrin

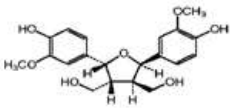


Linoleic acid

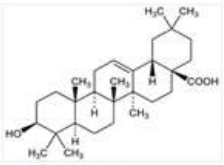
Lecithin



Linolenic acid

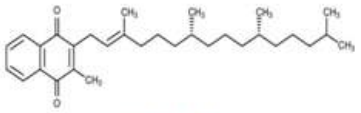


Neoolivil

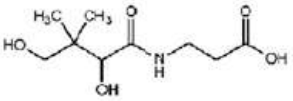


Oleanolic acid

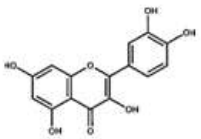
Palmitic acid



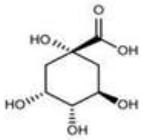
Vitamin K_1



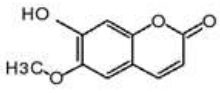
Pantothenic acid



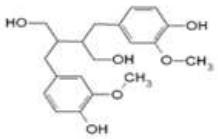
Quercetin



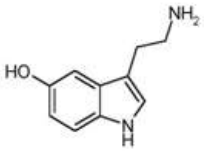
Quinic acid



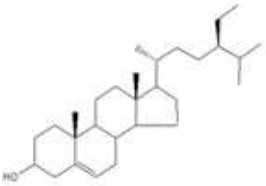
Scopoletin



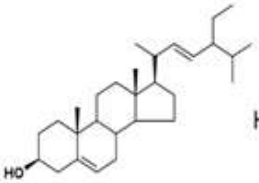
Secoisolariciresinol



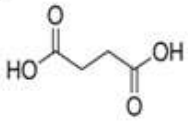
Serotonin



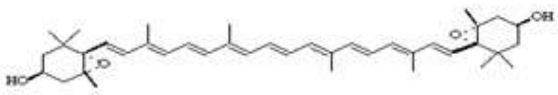
Sitosterol



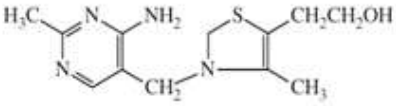
Stigmasterol



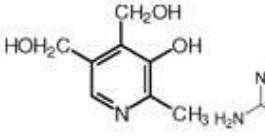
Succinic acid



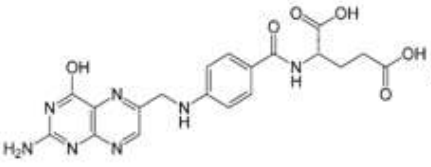
Thiamine



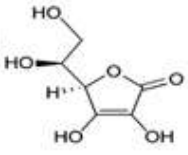
Violaxanthin



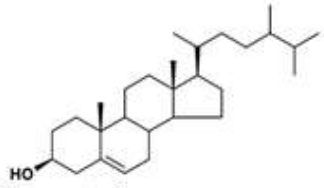
Vitamin B_6



Folic acid



Vitamin C



Campesterol

The purpose of this research work was to carry out chronic toxicity studies by administering low dose of *U. urens* L. (25 mg/kg/ml) for the period of three months in both sexes of rabbits (UM and UF) and then determining their hematological, blood biochemistry and urine biochemistry parameters with reference to their respective control groups. While histo-pathological studies were carried out only on male control and treated (UM) groups after taking out blood samples for above stated tests.

II. Materials And Method

2.1 Chemicals

Ethanol, acetic acid, formalin, diagnostic kits, xylene, paraffin wax, eosin, hematoxylin and canada balsam were purchased from Merck, Germany. All the chemicals were of analytical grade.

2.2 Plant Extract

Urtica urens mother tincture (Lot # 3030909) was purchased from Willmar Schwabe, Germany suppliers. Extracts was concentrated by rota-evaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40°C. The extracts obtained were stored in cool, dry place for further studies.

2.3 Experimental Animals

Twenty-four male and female rabbits weighing between 1.0 and 1.2 kg were purchased from animal house of Dow University of Health Sciences, (DUHS) Karachi and kept in animal house for a period 15 days to acclamized in separate cages. They were fed commercial feed and water *ad libitum*. Their weights were checked on random basis. Blood (6 ml) was collected from rabbits for evaluation of hematological and biochemical parameters by cardiac puncture at the end of three month.Blood samples collected into clean non-heparinized bottles were allowed to clot and serum was separated from the clot and centrifuged according to groups into clean bottles for the biochemical analysis.After the collection of blood samples as well as urine sample for urine analysis; histopathology was carried out by carefully dissecting out liver, kidney, heart and stomach organs of male rabbits. Animal studies were carried out according to the NIH guide for the care and use of laboratory animals [5].

2.4 Animal Grouping And Drug Dosing

Four groups were made (male control – 6 rabbits), (female control – 6 rabbits), (male test (UM) – 6 rabbits) and (female test (UF) – 6 rabbits). Male and female control groups were given distil water, while test groups UM and UF were given 25mg/kg *U. urens.* All the administrations were given orally. The treatment continued for 90 days. Blood (6 ml) was collected by cardiac puncture with 10 ml sterile syringe using 1mg/1ml EDTA as anti-coagulant for the determination of blood and biochemical parameters. Voided urine samples were collected for each group animals. After collecting blood samples, male control and treated (UM) groups' animals were sacrificed and their heart, stomach, liver and kidneys were extracted out for carrying out their histo-pathology.

2.5 Hematological Evaluation

Hematological examination of the collected blood samples was performed according to standard procedures listed as follow. Total erythrocyte counts were counted using a Neubar chamber under a light microscope at 40 x 10 magnifications. Blood samples were diluted to 200 times by Hayem's reagent before counting. Blood hemoglobin concentration was determined using a Sahli'shemometer. Micro wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the (PCV). Total leucocyte counts were detected using a Neubar chamber under a light microscope at 10 x 10 magnification after diluting blood samples to 10 times with Turk's solution. Mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for particular blood samples were also calculated[6-10].

2.6 Biochemical Evaluation

Serum samples were obtained by centrifugation of blood at 1300 x g for 15 min. The Menariniclassic chemistry analyzer was used to determine the calcium (Ca), phosphorus (P), blood urea, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine phosphokinase (CPK), cholesterol, glucose, amylase, and gamma-glutamyltransferase (GGT). The globulin concentration was determined by subtracting the albumin concentration from the total protein concentration [11-12].

2.7 Urine Analysis

Voided sample of urine was collected byplacing a clean, empty box in the site where the animals usually urinates[13].

2.8 Histo-Pathological Analysis

After blood collection, the liver, kidney heart and stomach of the male control and test group (UM) were carefully dissected from the abdominal region and were immediately fixed in 10% neutral buffered formalin. Fixed samples were trimmed and processed for paraffin embedding. Sections (5–7 μm) were cut and the tissues were dehydrated with alcohol of graded concentrations and allowed to dry. The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded[14-16].

2.9 Statistical Analysis

All the results were presented as a mean plus or minus standard error of mean (M ± SEM). Differences between control and treatment groups were analyzed by student *t-test*[17].

III. Results

3.1 Effects of *U. urens* on complete blood countof treated male (UM) and female (UF) rabbits.

Hemoglobin and hematocrit levels were $p<0.05$ lowered, while platelet count was found raised ($p<0.01$) in female test group (UF) as compared to the female control group. In male rabbits (UM), MCV level was observed raised ($p<0.05$) and platelet count was found to be raised $p<0.01$ (Table 1).

Table 1: Chronic toxicity test: Effect of *U. urens* extract on complete blood count of rabbits.

Blood Parameter	Control Female	Test Female (UF)	Control Male	Test Male (UM)	Reference Range
Hemoglobin	12.15±0.0836	9.218±0.022*	10.05±0.0836	10.308±0.063	10.75±0.689
RBC (Erythrocyte Count)	5.895±0.00836	4.485±0.0083	5.485±0.00836	5.316±0.0096	3.916±0.277
Hematocrit (HCT/PVC)	42.835±0.0739	31.79±0.063*	34.2±0.0632	35.89±0.063	38.67±1.932
MCV	72.416±0.0658	70.8±0.063	62.5±0.836	67.63±0.048*	89±3.183
MCH	20.835±0.0739	20.34±0.077	18.15±0.0836	19.425±0.0418	30.167±1.180
MCHC	28.783±0.0658	28.85±0.0836	29.05±0.0836	28.775±0.068	32.5±0.836
Total Leucocyte Count (WBC)	6.05±0.0836	8.675±0.0418	5.5±0.0632	5.315±0.0083	11±1.673
Platelet Count	353.5±0.836	404.11±0.118**	140.5±0.836	504.08±0.11**	275±41.83

UF = Female rabbit treated with drug; UM = Male rabbit treated with drug

All values are mean ± SEM; n = 6; * = Significant ($p<0.05$), ** = Highly significant ($p<0.01$).

3.2 Effects of *U. urens* on kidney function parameters of treated male (UM) and female (UF) rabbits.

Urea level was observed significantly lower ($p<0.01$) in female treated group (UF) while creatinine, phosphorus and A/G ratio were also lower ($p<0.05$). Uric acid level was found elevated ($p<0.05$) in female test group as compared to its respective control group. Urea level was found elevated ($p<0.01$) while creatinine level was lowered ($p<0.05$) in male treated group (UM) as compared with its respective control group (Table 2).

Table 2: Chronic toxicity test: Effect of *U. urens* extract on kidney function parameters of rabbits.

Biochemical Parameters	Control C (female)	Test Animal (UF)	Control C (male)	Test Animal (UM)	Reference Range
Urea	72.5±0.83	48.5±0.836**	23.5±0.83	48±1.058**	29.167±6.39
Creatinine	0.85±0.008	0.525±0.0083*	0.85±0.0083	0.643±0.0083*	0.8167±0.127
Calcium (serum)	14.59±0.063	14.867±0.24	14.17±0.0083	14.643±0.02	10.03±0.318
Phosphorus	3.825±0.068	0.988±0.023*	6.195±0.0083	4.845±0.0195	3.53±0.318
Uric acid	0.0175±0.004	0.08±0.0058*	0.165±0.0083	0.105±0.01	3.916±0.639
Total proteins	8±0.02	6.495±0.0083	7.495±0.0083	7.925±0.0083	7.467±0.347
Albumin	5.83±0.013	4.306±0.0096	4.305±0.0083	5.33±0.0083	4.5±0.28
Globulin	2.153±0.0096	2.198±0.0165	3.185±0.0083	2.595±0.0083	2.35±0.146
A/G ratio	2.715±0.0083	1.97±0.01*	1.35±0.016	2.065±0.0083	0.75±0.052

UF = Female rabbits treated with drug; UM = Male rabbits treated with drug

All values are mean ± SEM; n = 6; * = Significant ($p<0.05$), ** = Highly significant ($p<0.01$).

3.3 Effects of *U. urens* on cardiac enzyme parameters of treated male (UM) and female (UF) rabbits.

In female test group (UF), LDH and CPK enzymes level were found elevated ($p<0.01$); whereas CK-MB enzymes level was observed to be significantly lowered ($p<0.01$) in comparison with its respective control group. In male test group (UM), all the cardiac enzymes levels were found elevated ($p<0.01$) as compared to its respective male control group (Table 3).

Table 3: Chronic toxicity test: Effect of *U. urens* extract on cardiac enzymes parameters of rabbits.

Biochemical Parameters	Control C (female)	Test Animal (UF)	Control C (male)	Test Animal (UM)	Reference Range
LDH	163.5±0.836	192.5±0.836**	270.5±0.83	351.16±42.58**	331.67±40.34
CPK	729.5±0.83	2370.5±0.836**	421.5±0.83	586.5±1.37**	90.33±25.03
CK-MB	852.5±0.83	569.5±0.836**	194.5±0.83	673.33±0.96**	16.67±2.46

UF = Female rabbits treated with drug; UM = Male rabbits treated with drug

All values are mean ± SEM; n = 6; * = Significant ($p<0.05$), ** = Highly significant ($p<0.01$).

3.4 Effects of *U. urens* on lipid function tests of treated male (UM) and female (UF) rabbits.

All the lipid function test parameters (cholesterol, triglycerides, HDL, LDL and VLDL) were found elevated ($p<0.01$) in female test group (UF) as compared to its respective control group. In male test group (UM), $p<0.01$ significant lowering effect was observed in cholesterol, triglycerides and LDL levels whereas, HDL level was observed to be slightly raised ($p<0.05$) and VLDL was found lowered ($p<0.05$) in male test group (UM) treated with *U. urens*as compared to its control group (Table 4).

Table 4: Chronic toxicity test: Effect of *U. urens* extract on lipid profile parameters of rabbits.

Biochemical Parameters	Control C (female)	Test Animal (UF)	Control C (male)	Test Animal (UM)	Reference Range
Cholesterol	30.5±0.83	77±1.058**	58.5±0.83	19.42±0.845**	109.16±22.24
Triglycerides	0.5±0.83	87.83±1.036**	131.5±0.83	51.83±1.036**	111.67±13.68
HDL	12.5±0.83	19.5±0.836*	6.5±0.83	9.5±0.836*	19.67±3.18
LDL	16.5±0.83	40.5±0.836**	38.5±0.83	7.67±0.96**	103.33±15.14
VLDL	7.5±0.83	17.5±0.836*	26.5±0.83	11±1.058*	30±5.83

UF = Female rabbits treated with drug; UM = Male rabbits treated with drug

All values are mean ± SEM; n = 6; * = Significant ($p<0.05$), ** = Highly significant ($p<0.01$).

3.5 Effect of *U. urens* on liver enzymes of treated male (UM) and female (UF) rabbits.

In female test group treated with *U. urens* (UF), SGOT, SGPT and alkaline phosphatase were lowered ($p<0.05$), while Gamma GT level was observed to be raised ($p<0.01$) as compared with its control group. In male test group (UM), SGOT and alkaline phosphatase levels were significantly lowered ($p<0.01$). SGPT level was lowered ($p<0.05$). Direct bilirubin and gamma GT levels were found raised ($p<0.05$) (Table 5).

Table 5: Chronic toxicity test: Effect of *U. urens* extract on liver enzymes parameters of rabbits.

Biochemical Parameters	Control C (female)	Test Animal (UF)	Control C (male)	Test Animal (UM)	Reference Range
SGOT	26.5±0.83	17.5±0.836*	42.5±0.83	18.83±1.03**	21.83±3.11
Total Bilirubin	0.275±0.0083	0.24±0.021	0.265±0.0083	0.286±0.0096	1.75±0.083
Direct Bilirubin	0.021±0.005	0.0165±0.0027	0.041±0.0065	0.115±0.0083*	0.029±0.0008
SGPT	41.5±0.83	39.5±1.224*	68.5±0.83	56.16±1.036*	27.5±4.18
Alkaline Phosphatase	37.5±0.83	33.5±0.836*	228.5±0.83	40.67±0.966**	91.67±17.30
Gamma GT	6.5±0.83	12.83±1.036**	9.5±0.83	13.08±0.639*	29.16±6.39

UF = Female rabbits treated with drug; UM = Male rabbits treated with drug

All values are mean ± SEM; n = 6; * = Significant ($p<0.05$), ** = Highly significant ($p<0.01$).

3.6 Effects of *U. urens* on urine analysis of treated male (UM) and female (UF) rabbits

The urine parameters of the male test group (UM) and female test group (UF) were similar to that of their respective control groups except for the presence of blood in the urine of the male test group (UM) (Table 6).

Table 6: Chronic toxicity test: Effect of *U. urens* extract on urine parameters of rabbits.

Urine Parameters	Control Animal C (female)	Test Animal (UF)	Control Animal C (Male)	Test Animal (UM)	Reference Range
Urine Physical					
Volume	30.08±0.11	9.98±0.31	25.01±0.136	14.95±0.31	179.17±61.81
Colour	Yellow	Yellow	Yellow	Yellow	Pale yellow-red brown
Appearance	Turbid	Turbid	Turbid	Turbid	Clear
Sp. Gravity	1.0045±0.00037	1.0046±0.00046	1.0045±0.00037	1.0046±0.00046	1.019±0.007
pH	9±0.063	8.95±0.083	9±0.063	9±0.11	8.53±0.195
Urine Chemical					

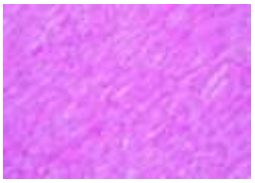
Protein	Nil	Nil	+1 (30 mg/dL)	Nil	Negative
Glucose	Nil	Nil	Nil	Nil	Negative
Ketone Bodies	Negative	Negative	Negative	Negative	Negative
Urobilinogen	Normal	Normal	Normal	Normal	Negative - weak positive
Blood	Negative	Negative	Negative	Positive +	Negative
Bilirubin	Nil	Nil	Nil	Nil	Negative
Urine Microscopy					
RBC	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF
WBC	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF
Epithelial Cell	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF

UF = Female rabbits treated with drug; UM = Male rabbits treated with drug

3.7 Effects of *U. urens* on histo-pathological parameters of male treated rabbits (UM)

No significant pathology was observed in heart, liver and stomach tissues; whereas, acute tubular necrosis was found in kidney tissues of the male group treated with *U. urens* extract (UM) (Figures 1-8).

Figure 1: Histo-pathological examination of heart of control male rabbit

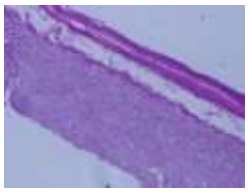


Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

Figure 2: Histo-pathological examination of heart of treated male rabbit (UM)

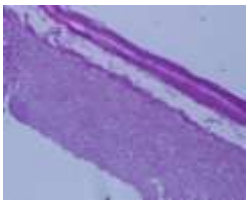
Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

Figure 3: Histo-pathological examination of stomach of control male rabbit



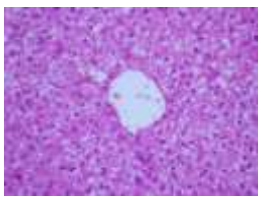
Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandularstructures. Underlying sub-mucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

Figure 4: Histo-pathological examination of stomach of treated male rabbit (UM)



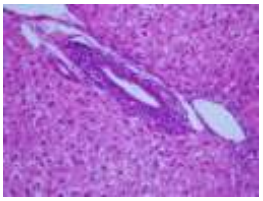
Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying sub-mucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

Figure 5: Histo-pathological examination of liver of control male rabbit



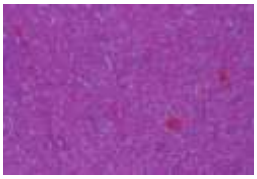
Sections show liver tissue with overall preserved lobular architecture. Portal tracts aremildly dilated with lymphocytic infiltrate and minimal fibrosis. Centri-lobularhepatocytic degeneration also noted. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen.

Figure 6: Histo-pathological examination of liver of treated male rabbit (UM)



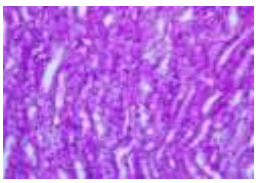
Sections show liver tissue with overall preserved lobular architecture. Portal tracts are within normal limits, containing portal triad and scanty fibrous tissue. No significant portal or lobular inflammation seen. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen.

Figure 7: Histo-pathological examination of kidney of control male rabbit



Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows focal lymphocytic infiltrate. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

Figure 8: Histo-pathological examination of kidney of treated male rabbit (UM)



Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Severe degree of acute tubular necrosis is seen. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

IV. Discussion

In this study we evaluated the safety profile of *U. urens* extract when administered in low dose for three months.The effectiveness and safety of *U. urens* may be attributed to its rich chemical constituents. The presence of flavonoids and volatile oils content in *U. urens* are responsible for its anti-oxidant, anti-microbial and anti-inflammatory activities. The recent evidence based research supports the utilization of *U. urens* as anti-diabetic, hypocholesterlaemic, for the treatment of benign prostratic hyperplasia, urinary tract infections, neuralgia, arthritis and other related conditions[18-24].

It is important to note that *U. urens* extract showed increase and decrease in some blood parameters but within limits. Along with this differences in values of male and female rabbits' blood parameters were also observed. This action may be due to the physiological and biochemical functions of the sexes. In blood analysis, hemoglobin (10.308±0.063), hematocrit (35.89±0.063), MCV (67.63±0.048), MCH (19.425±0.0418) were slightly raised; whereas platelet count (504.08±0.11) was significantly elevated. Red blood cells (5.316±0.0096) count, white blood cells (5.315±0.0083) count and MCHC (28.775±0.068) levels were somewhat lowered in test group treated with *U. urens* when compared with its respective male control group. In female test group treated with *U. urens*, hemoglobin (9.218±0.022), RBC count (4.485±0.0083), hematocrit (31.79±0.063), MCV (70.8±0.063) and MCH (20.34±0.077) were found to be lowered while MCHC (28.85±0.0836), white blood cells (8.675±0.0418) count and platelet count (404.11±0.118) were elevated in comparison to its respective female control group.

Few lowering and elevation of the kidney function parameters were observed in both sexes. Urea (48±1.058), serum calcium (14.643±0.02), total protein (7.925±0.0083), albumin (5.33±0.0083) and A/G ratio (2.065±0.0083) were raised while creatinine (0.643±0.0083), phosphorus (4.845±0.0195), uric acid (0.105±0.01) and globulin levels (2.595±0.0083) were lowered in male test group treated with *U. urens* as compared to the male control group. Serum calcium (14.867±0.24), uric acid (0.08±0.0058) and globulin (2.198±0.0165) levels were found elevated whereas, urea (48.5±0.836), creatinine (0.525±0.0083), phosphorus (0.988±0.023), total protein (6.495±0.0083), albumin (4.306±0.0096) and A/G ratio (1.97±0.01) were observed lowered in female test group treated with *U. urens* in comparison to the female control group. According toESCOP(1997);ESCOP(2003); Blumental*etal*. (1998) and Bisset(1994), *U. urens* is recommended as a diuretic, for irrigation in inflammatory conditions of the lower urinary tract and treatment of kidney gravel [19, 25-27]. According to Bradley (1992), flavonoid and high potassium and other mineral content of *U. urens* may be attributed for its diuretic effect [28].

Some effects were also observed in cardiac enzymes evaluation of both sexes of rabbits. The levels of the cardiac enzymes; LDH (351.16±42.58), CPK (586.5±1.37) and CK-MB (673.33±0.96) were found towards elevated side in the male test group treated with *U. urens* as compared to its respective male control group. LDH (192.5±0.836) and CPK (2370.5±0.836) enzymes were observed elevated whereas CK-MB (569.5±0.836) levels were found reduced in female test group treated with *U. urens* as compared to the control female group. The previous researches revealed the effectiveness of *U. urens* in cardiovascular diseases. Tahri*etal*. (2000) in his research work reported the association of hypotensive effect of *U. urens* with its diuretic and natriuretic effects [29].

Our results of lipid profile were different than that reported for its closely related species, that is, *U. diocia* on rats. The reported results were observed in rats (lowering effects in LDL, HDL and cholesterol [3, 30]. Our results were observed in male and female rabbits; where in, female rabbits' blood all lipid profile

parameters were observed to be raised.In male rabbits, HDL level was found raised while cholesterol, triglycerides and LDL levels were observed to be on lower side. Precisely, according to the reported and observed results *U. urens*and *U. diocia*were overall found to improve blood lipid profile[3].

The effects of the drug on liver enzymes of male and female rabbits showed variation within some parameters in both sexes that may be due to physiological, biochemical function and the type of molecule exerting action on the site.Daher*etal*. (2006) evaluated the effect of *U. diocia* on liver enzymes of rats and found no liver damage during the study period [30].

No change in urine analysis parameters were observed after drug introduction in both male and female rabbits, except for the presence of blood in male treated group.

No significant histo-pathology was observed in histo-pathological slides of heart, liver and stomach tissues, while acute tubular necrosis was seen in kidney tissues of treated group (UM) with respect to its control group.

V. Conclusion

Our results of chronic toxicity studies confirms the effective utilization of *U. urens*in allergic conditions, reducing inflammation, alleviating pain, increasing urine flow, dilating blood vessels, lowering blood pressure and healing wounds. Safety profile of *U. urens* supports its use in medicine.

Conflict Of Interest

Authors have no conflict of interest.

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