

**EVALUATION OF ACUTE AND CHRONIC ORAL TOXICITY STUDY OF LC
(LIQUORICE AND CATECHU) - A POLYHERBAL DRUG ON EXPERIMENTAL RATS**

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ABSTRACT

The currently available locally acting formulations to treat recurrent aphthous stomatitis (RAS) are either less efficacious or they are not comfortable for the use in the patients. A single administration of LC was given orally at the highest dose level of 2000 mg/kg body weight in the acute toxicity study. Signs of toxicity were observed every hour for the first 6 h and every day for 7 days. In the repeat oral toxicity study, LC was administered to rats at doses of 250, 500, 750 and 1000mg/kg body weight for 28 days. Mortalities, clinical signs, body weight changes, biochemical and haematological parameters were monitored during the study period. There were no mortalities or clinical signs observed in rats in the acute toxicity study. The observable increase in the level of alkaline phosphatase (ALP) in the group administered 1000 mg/kg body weight of LC may be as a result of congestion or obstruction of biliary tract, which may occur within the liver. ALP activity on the other hand is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure. The increased level of lactate dehydrogenase (LDH) observed in the present investigation apparently indicated the toxic effect of LC in rat. There were no significant changes in total protein in rats treated with LC, which suggested that there was no sign of impaired renal function. The near-normal levels of total cholesterol observed in groups treated with LC may be attributed to the presence of hypolipidemic agents in the herbal drug. Similarly, the drug had no adverse effect on the concentration of creatinine and urea. This is suggestive of no kidney damage specifically by renal filtration mechanism. Increase in platelets observed in rats treated with 1000 mg/kg body weight may be attributed to enhanced production and secretion of thrombopoietin the primary regulator of platelet production by LC indicating that it has haemostatic property. NOAEL from this preclinical study was found to be 500mg/kg.

KEYWORDS: LC (Liquorice and Catechu), RAS (Recurrent Aphthous Stomatitis), acute toxicity.

INTRODUCTION

Canker sores (aphthous stomatitis) are small oval sores, red in colour, that affect the mucous membranes inside the mouth that are usually developed on the inner cheeks, gums or lips and occasionally the tongue.^[1, 2] There is intense or moderate pain and it heals in about 10 - 14 days for the more common type and more than 2 weeks for the severe type.^[3] The goals of treating aphthous stomatitis disease are to relieve pain and heal the ulcer. The studies of UNESCO states that use of plants for treating various diseases predates human history and forms origin of much of modern medicines since they have potential for producing new drug of great benefit to mankind.^[4] According to the survey, recently conducted by WHO, about 80% of the world population relies on plant medicine for their health care needs.^[5] India is one of the countries where different traditional systems of medicines are practiced. These systems depend upon

plant resources to great extent for the raw materials. To the best of my knowledge there are no reports available poly herbal formulation using Liquorice and Catechu extract (LC). Phytochemical investigations on liquorice and catechu have identified a number of secondary metabolites such as flavonoids, tannins. The ulcer can become infected and the area may not readily heal because of poor blood circulation. Proper treatment can help ulcers heal and prevent new ones from developing. Therefore, appropriate interventions for wound care for reducing amputation rates are essential. Herbal extracts are among the medications which can be used easily on wounds. There are several evidences about the putative clinical usefulness of Liquorice and Catechu. But there is no evidence on combination of both Liquorice and Catechu in the treatment of Aphthous Stomatitis.

Prior to the initiation of human clinical trials of novel drugs, the safety of their application is to be proved. Generally this is accomplished by the implementation of general preclinical toxicity experiments to uncover potential poisonous effects of any drug in animals. The aim of the present study was to evaluate the animal safety of LC as a new phytotherapeutic candidate for Aphthous Stomatitis.

MATERIALS AND METHODS

Drugs

LC herbal extract was prepared and delivered by Shreyas Pharmaceuticals Vapi.

Experimental Animals

Healthy Wistar albino rats of both sexes weighing between 200-250 g were obtained from Pretox Research Centre Surat. A total of ten animals of equal numbers of male and female rats were used. The animals were housed in a cross ventilated room and kept under standard environmental condition of 12/12 h light/dark cycle.^[6] They were housed in polypropylene cages (5 animals per cage) and were fed with standard rat pellet and water *ad libitum*. They were allowed to acclimatization for 7 days to the laboratory conditions before the experiment the experiment was performed in accordance with the guidelines established by the Organization for Economic Cooperation and Development (OECD) guidelines.^[7] This study was approved by the Institutional Ethics Committee of the Pretox Research Centre Surat.

Acute toxicity test

Healthy Wistar albino rats of both sexes weighing between 200-250 g maintained under standard laboratory conditions were used for the acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD guideline, 2002). A total of ten animals of equal numbers of male and female rats were used and each received a single oral-dose of 2000 mg kg body weight of LC. Animals were kept overnight fasting prior to drug administration by oral gavage.^[8] After administration of drug sample, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 7 days. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes were noted.

Repeat dose toxicity study

The study was performed as per the Organisation for Economic Co-operation and Development (OECD) test guideline 407. The rats were divided into 5 groups of 6 each. Animals in groups 2, 3, 4 and 5 were orally

administered 250, 500, 750 and 1000mg/kg body weight LC respectively. Once daily for 28 days. Animals in Group 1 served as control group and received distilled water through the same route. All the rats were observed for any physiological, behavioural changes and mortality. Food and water consumption was checked was checked daily. After 28 days of treatment the rats were fasted for 8 hours and anaesthetized in chloroform vapour and blood sample collected by cardiac puncture for biochemical analyses. The blood samples were collected for haematological parameters like haemoglobin(Hb), White Blood cells (WBC), Packed Cell Volume (PCV), Platelets, Alanine Amino Transaminase (ALT), Aspartate amino transaminase (AST), alkaline phosphatase (ALP), Lactate Dehydrogenase (LDH) and bilirubin, (Fasting Blood Sugar) FBS, Urea, Creatinine, Cholesterol.^[9]

Gross necropsy

At the end of the experiments, the animals were euthanized by over-dosage of chloroform vapour with pre-medication of droperidol in order to conduct histopathological examination of the internal organs and tissues. All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the body.

Statistical Analyses

The data are presented as mean \pm SEM. Results were analyzed statistically using one-way Analysis of one way variance (ANOVA) was employed for between and within group comparison while student's t-test was used for paired comparison. 95% level of significance ($p \leq 0.05$) was used for the statistical analysis.

Necropsy

Body orifices and organs of all animals were carefully observed after dissection for morphological and pathological changes. Wet weight of liver, kidney, spleen, brain, heart, adrenals and gonads (testes/ovaries) were recorded for all animals.

RESULTS

Herbal medicines are consumed by humans either as food or medicine. Traditional medicine has maintained greater popularity all over developing world and the use is rapidly on the increase.

Acute toxicity study

There were no LC treatment- related mortalities recorded in animals treated with a single dose of 2000mg/kg dose. Therefore, the approximate lethal dose (LD_{50}) of LC in the experimental rats was higher than 2000 mg/kg. There were no clinical signs in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, lacrimation urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000 mg kg dose of LC.

Repeat dose toxicity study

Generally, a steady increase in the body weight was observed in all the treated rats compared.

With control up to the 3rd week. There was a significant decrease ($p \leq 0.05$) in the activities of Alanine amino transferase (ALT), fasting blood sugar (FBS) level in the treated rats when compared with the control. However, Aspartate amino transferase (AST), alkaline phosphatases (ALP) and lactate dehydrogenase (LDH) showed a significant ($p \leq 0.05$) increase in rats treated with LC when compared with the control. There were however, no significant ($p \leq 0.05$) changes in total bilirubin, conjugated bilirubin, malondialdehyde (MDA), total protein, cholesterol, triglycerides, creatinine and urea of the treated groups compared with control.

The packed cell volume (PCV) in treated groups showed a marginal decrease when compared with control. The platelets of rats administered 1000 mg/kg body weight were significantly ($p \leq 0.05$) higher when compared with the control group. However, there was a significant decrease in WBC count when compared to control group.

Gross pathological examination of all animals did not reveal any abnormality attributable to the treatment in the species. No significant histopathological changes were noted in different organs that were examined.

DISCUSSION

Traditional medicine has maintained greater popularity all over developing world and the use is rapidly on the increase.^[10] Despite this, the safety of herbal medicine use has recently been questioned due to reports of illness and fatalities. In the present study, single dose of oral administration of LC to Wistar albino rats at 2000 mg/kg body weight had no effect on mortality and clinical signs such as changes in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion). Therefore, no acute toxicity was found in rats treated with LC and the approximate medium acute toxicity lethal value (LD50) were determined to be higher than 2000 mg/kg and as such could be generally regarded as safe. Any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe Serum marker

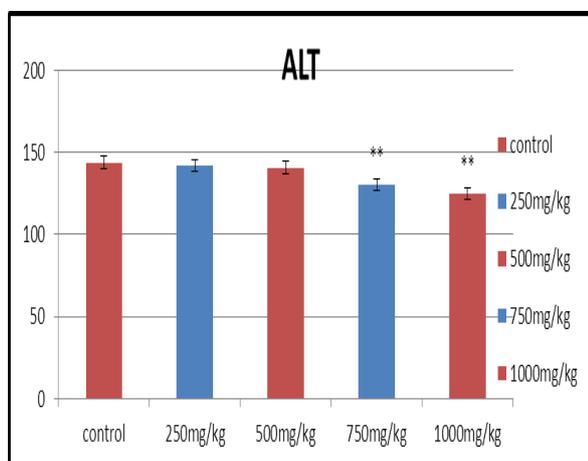
enzymes are biochemical parameters associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health.^[11] Alanine amino transferase (ALT) and Aspartate amino transferase (AST) are largely used in the assessment of liver damage by drugs or any other hepatotoxin. The liver and heart release ALT and AST and an elevation in their plasma concentrations are indicators of liver and heart damage. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury.^[12] The significant increase observed in the level of AST is suggestive that the herbal formula may possess hepatotoxic effect and equally could have caused some toxic effects on high dose. The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage.^[13] The observable increase in the level of alkaline phosphatase (ALP) in the group administered 1000 mg/kg body weight of LC may be as a result of congestion or obstruction of biliary tract, which may occur within the liver. ALP activity on the other hand is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure.^[14] However, the increased level of lactate dehydrogenase (LDH) observed in the present investigation apparently indicated the toxic effect of LC in rat. There were no significant changes in total protein in rats treated with LC, which suggested that there was no sign of impaired renal function. Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver diseases and many other conditions.^[15] The near-normal levels of total cholesterol observed in groups treated with LC may be attributed to the presence of hypolipidemic agents in the herbal drug. Similarly, the drug had no adverse effect on the concentration of creatinine and urea. This is suggestive of no kidney damage specifically by renal filtration mechanism or probably indicates that LC did not interfere with the renal capacity to excrete these metabolites. Therefore, it was evident that the drug at doses employed did not cause renal impairment or kidney damage. Increase in platelets observed in rats treated with 1000 mg/kg body weight may be attributed to enhance production and secretion of thrombopoietin^[15] the primary regulator of platelet production by LC indicating that it has haemostatic proper. The study also revealed that the herbal formulation may reduce cardiovascular risk and may have hypoglycaemic effect.

TABLES AND FIGURES

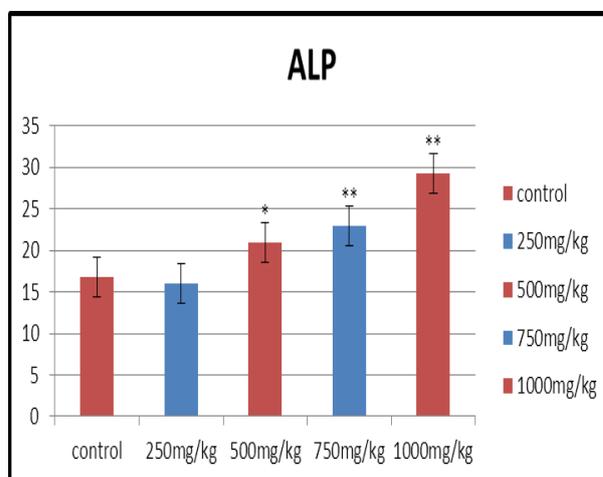
Serum biochemical parameters in rats treated orally with LC-LOZ for 28 days

Parameter	Control	250mg/kg	500mg/kg	750mg/kg	1000mg/kg
ALT	143.7± 0.3333	142.0± 0.01	140.7± 0.3333	130.3± 0.3333	124.7± 0.8819
T.B. (µmol/L)	8.417± 0.3333	6.300 ± 0.05774	7.840± 0.02	8.233± 0.03333	6.200± 0.01
AST	234.0± 0.5774	234.3± 0.3333	234.8± 0.6227	236.0± 0.5774	282.0± 2.000

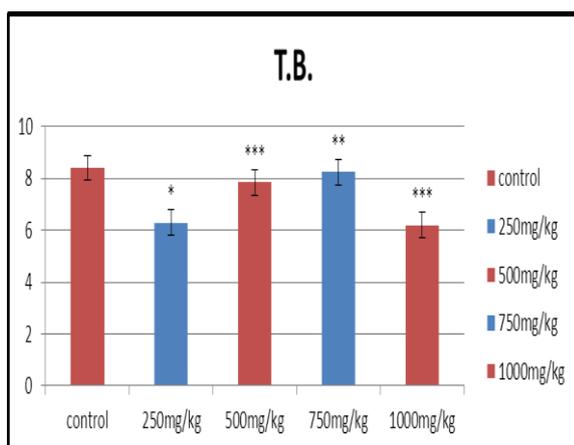
ALP	19.33± 0.6064	16.83± 0.6667	25.93± 0.1333	32.93± 0.3283	34.30± 0.05774
LDH	404.3± 1.202	451.0± 0.5774	478.0± 1.155	501.0± 0.5774	521.0± 0.5774
MDA(μmol/L)x10-6	2.323± 0.02848	2.270± 0.02082	2.173± 0.01202	1.663± 0.03180	1.067± 0.03333
C.B. (μmol/L)	2.623± 0.01453	2.620± 0.01	2.610± 0.01	2.507± 0.003333	2.423± 0.01453
Total protein(g/dl)	40.03± 0.9062	39.58± 1.225	39.38± 1.174	37.46± 1.861	37.30± 1.897
Cholesterol(μmol/L)	2.317± 0.07265	2.310± 0.06658	2.300± 0.07234	2.257± 0.08686	2.230± 0.09074
Creatinine(μMol/L)	67.70± 0.5774	68.03± 0.3333	68.10± 0.3100	68.26± 0.2633	68.20± 0.01
Urea(μMol/L)	2.190± 0.005773	2.180± 0.005773	2.173± 0.003333	2.170± 0.005773	2.167± 0.003333
FBS(μMol/L)	9.610± 0.005773	9.587± 0.006667	9.487± 0.02728	8.733± 0.1202	7.433± 0.2186



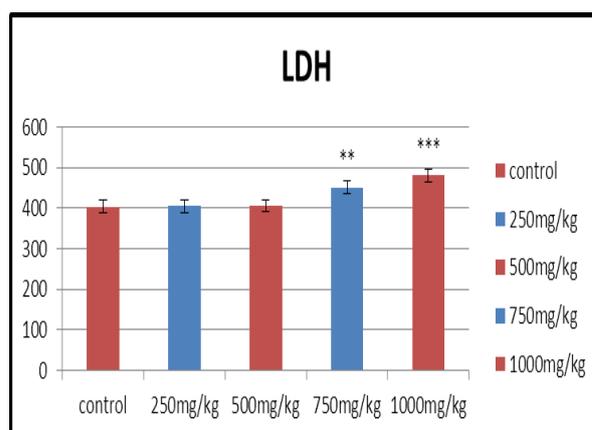
Values are expressed as mean ± S.E.M using one-way ANOVA; $p > 0.05$ vs. control group, *significantly different from control, $p < 0.05$.



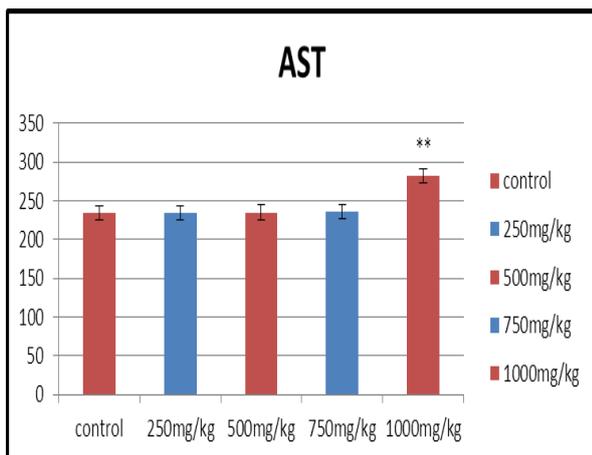
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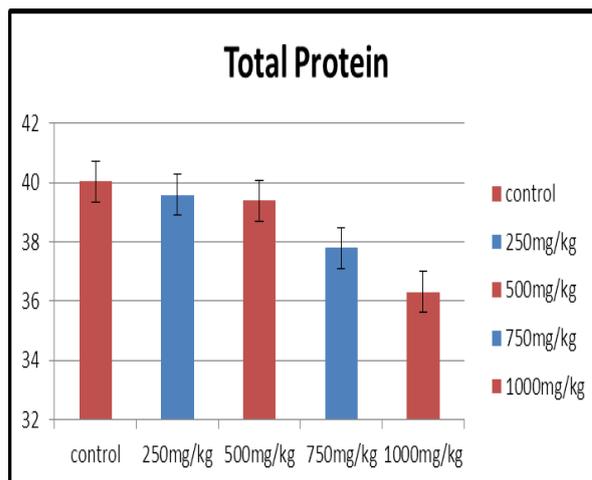
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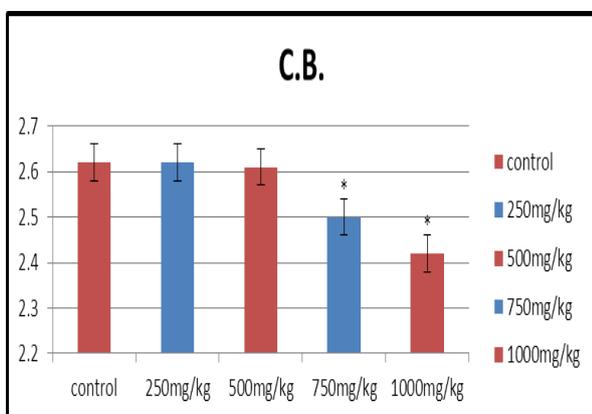
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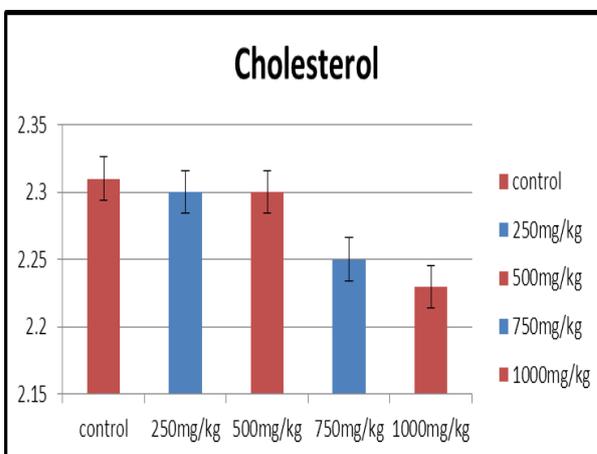
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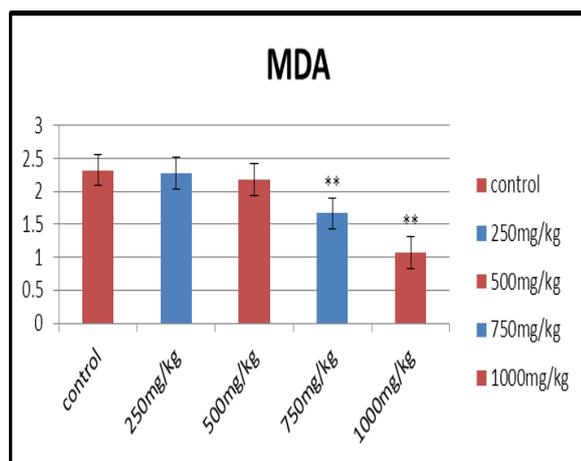
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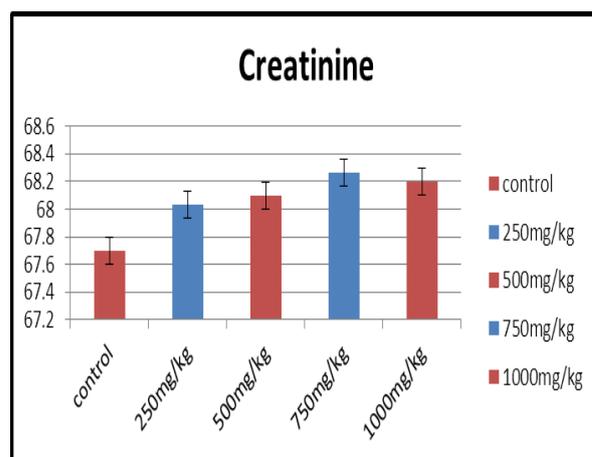
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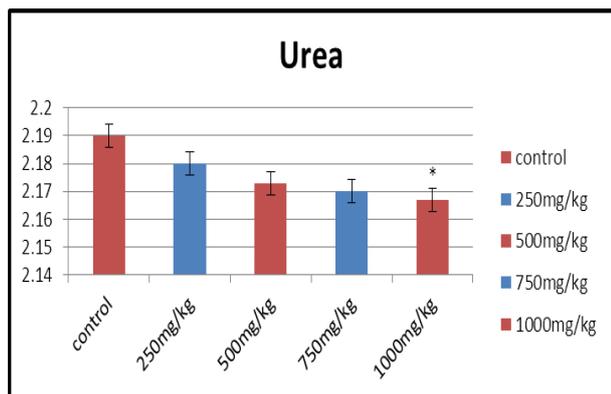
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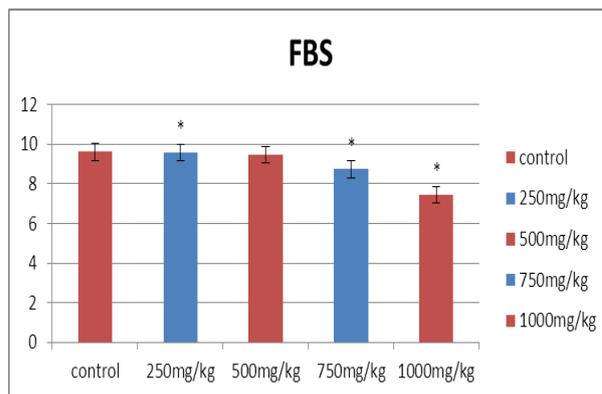
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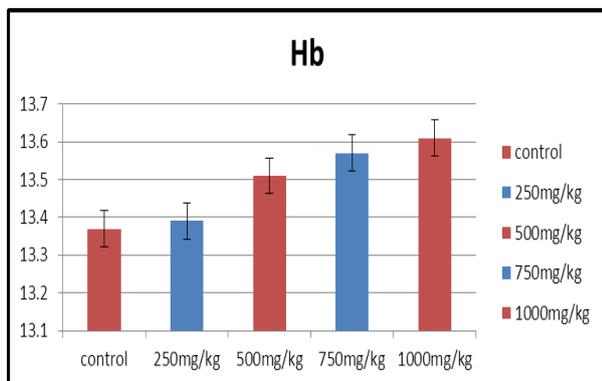
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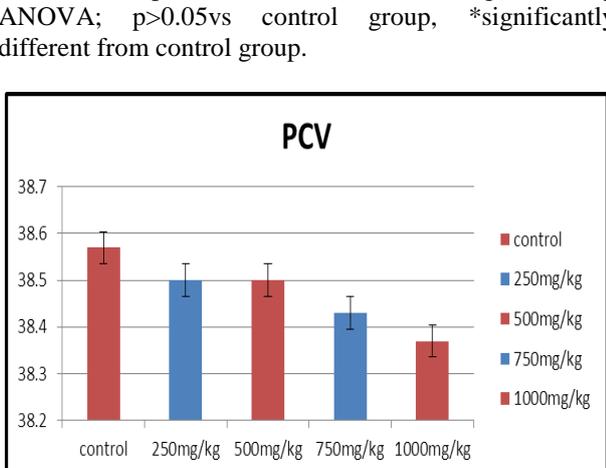
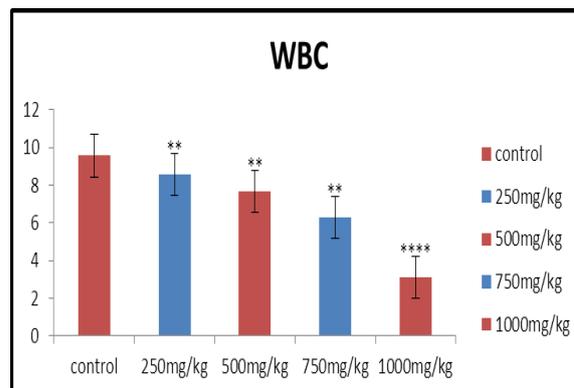
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Haematological parameters in rats treated orally with LC-LOZ for 28 days

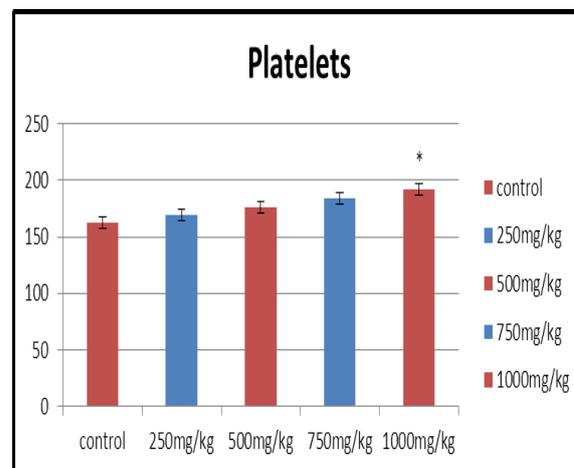
Parameter	Control	250mg/kg	500mg/kg	750mg/kg	1000mg/kg
Hb(mg/dl)	13.37 \pm 0.5776	13.39 \pm 0.5803	13.51 \pm 0.6558	13.57 \pm 0.6592	13.61 \pm 0.6654
PCV(%)	38.57 \pm 0.5044	38.50 \pm 0.4726	38.53 \pm 0.4410	38.43 \pm 0.4055	38.37 \pm 0.3180
WBC(x106/ μ L)	9.567 \pm 0.03333	8.567 \pm 0.03333	6.677 \pm 0.09062	5.300 \pm 0.1000	3.107 \pm 0.05812
Platelets(x103/ μ L)	163.0 \pm 2.517	169.7 \pm 0.3333	175.7 \pm 1.453	184.3 \pm 2.603	192.3 \pm 1.453

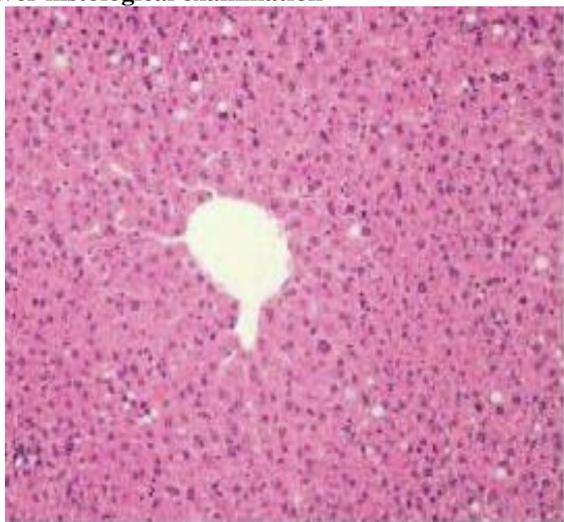
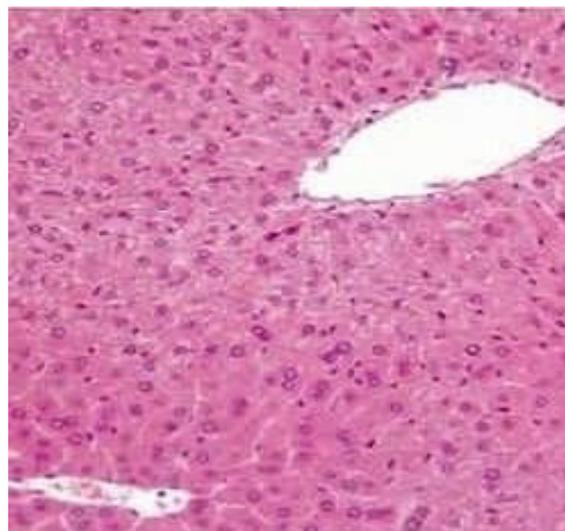
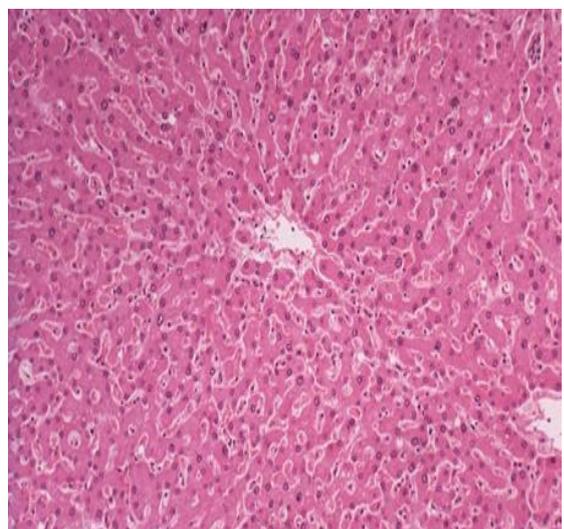
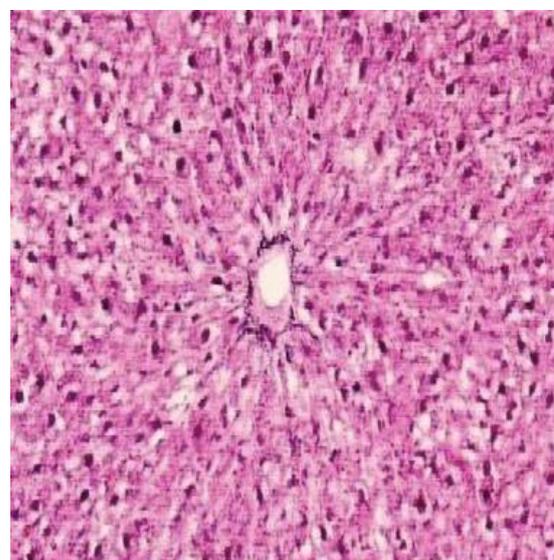
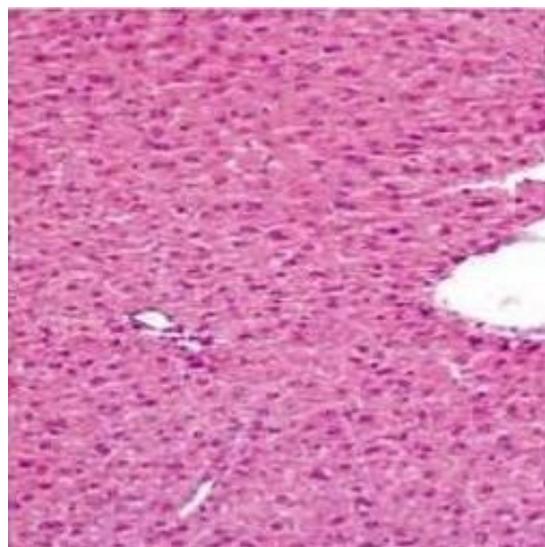


Values are expressed as mean \pm S.E.M using one –way ANOVA; $p > 0.05$ vs control group, *significantly different from control group.

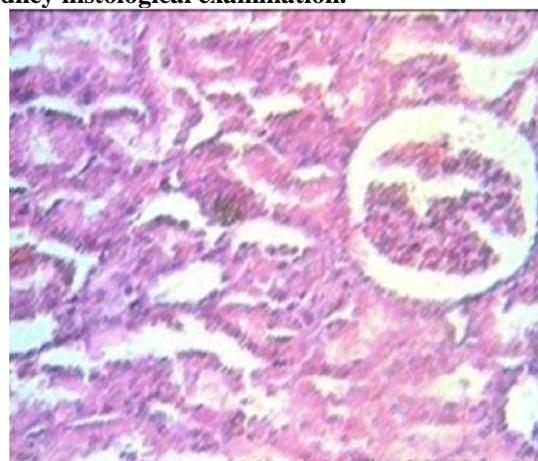


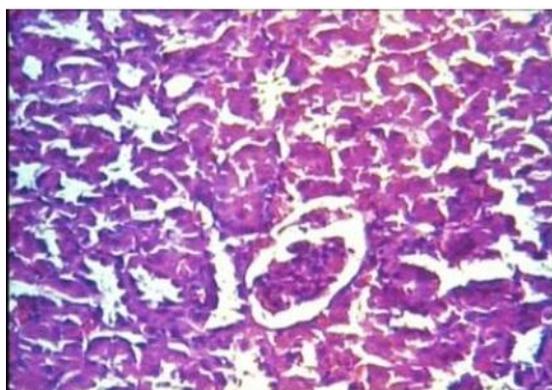
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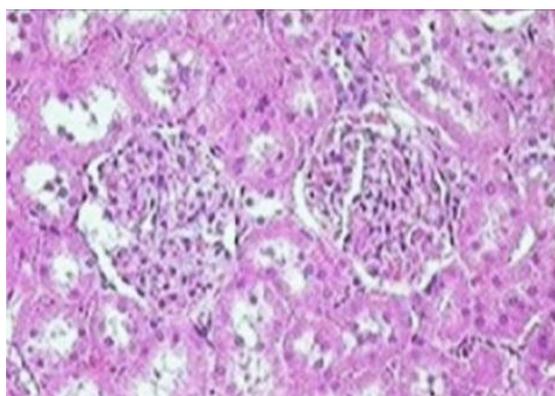
Histopathology**Liver histological examination****Control****750mg/kg****250mg/kg****1000mg/kg****500mg/kg**

Histological examination exhibited poor cellularity with extensive lipid depositions and enlarged hepatocytes compared to normal control animals.

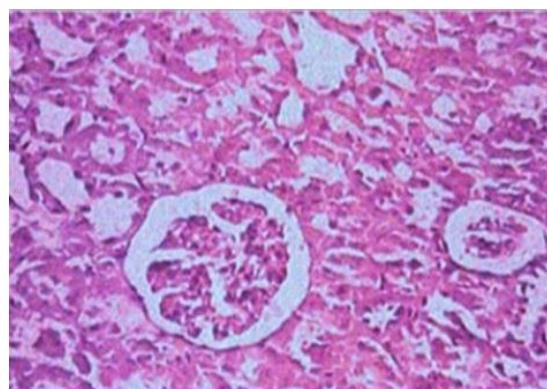
Kidney histological examination.**Control**



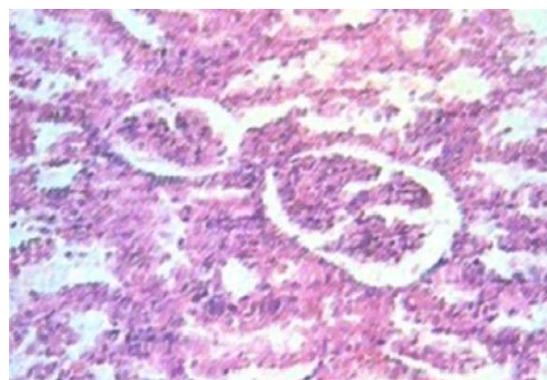
250mg/kg



500mg/kg



750mg/kg



1000mg/kg

There was no significant change in structure of tubules, no inflammation was found.

ACKNOWLEDGEMENT

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