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Toxicity Assessment of *Lindernia ciliata* (Colsm.) Pennell in Albino Rats: An Acute Oral Toxicity Study

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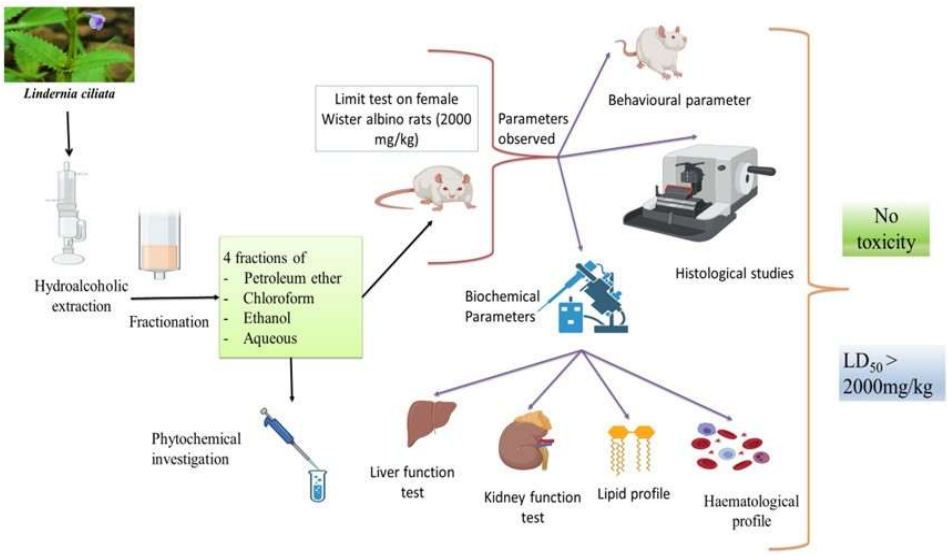
Graphical abstract



ABSTRACT

Background: *Lindernia ciliata (Colsm.)* Pennell (Family: Linderniaceae) is mostly a weed found in Asia's tropics and subtropics. It is used traditionally by the common people of Assam for different ailments.

Aim: We evaluated the acute oral toxicity of fractions of hydroalcoholic extracts of *L. ciliata* in female rats.

Method: We used the percolation method to prepare hydroalcoholic extract using 80% ethanol and fractionated with petroleum-ether, chloroform, ethanol and water. The toxicity assay was performed as per OECD guidelines. During the study (2 weeks), we monitored the body weight and animal behaviour after test dose administration (2000 mg/kg body weight). After the experimental period, we collected the blood samples and evaluated the haematological profile, lipid profile, liver function test and kidney function test. We performed histopathological studies of vital organs.

Results: The results suggested no significant changes in haematological profile, lipid profile, liver function test, kidney function test or histology of vital organs in test groups as compared to the control.

Conclusion: Therefore, we concluded, hydroalcoholic extract fractions of L. *ciliata* (up to 2000mg/kg) produced no acute toxicity after oral administration.

Keywords: *Lindernia ciliata;* Oral acute toxicity studies; OECD; Limit test; LD_{50};

INTRODUCTION

Lindernia ciliata (Colsm.) Pennell (Family: Linderniaceae) is mostly a weed found in Asia's tropics and subtropics, as well as North and South America's rice fields **[1]**. It is a stoloniferous, low-growing, annual, mat-forming plant with a height of 0.1 to 0.3 m **[2]**. *L. ciliata* has traditionally been used to treat gonorrhoea, urinary disturbances, anorexia, skin disorders, headaches, fevers, bronchitis, liver complaints, asthma, jaundice, constipation, cough, and spleen illness in Indians **[3, 4]**. It is used to treat diabetes among Assamese people **[5]**.

On a global scale, the use of medications has switched dramatically from synthetic to natural or semisynthetic medicines since herbal medicines have fewer adverse effects. As a result, the overuse of plant-derived treatments has increased **[6]**. Many plant-based treatments, on the other hand, are known to have substantial negative effects **[7, 8]**. Furthermore, as the father of toxicology, Paracelsus, put it, "All substances are poisons; none is not a poison." The correct dose is what distinguishes a medication from a poison **[9]**. As a result, we devised the current investigation to fractionate the hydroalcoholic extract and analyses its oral acute toxicity in

light of the use of *L. ciliata* in various traditional treatments.

MATERIALS AND METHODS:

Collection of Plants:

We collected the whole plant of *L. ciliata* from the fields of Baghmara, District-Bajali, PIN-781328, Assam, India between January-March 2019. A taxonomist from the Botanical Survey of India (BSI), Eastern regional centre, Shillong, identified and authenticated the plant (Authentication letter No. BSI/ERC/Tech/2019/101 dated 02/05/2019).

Preparation of crude extract and dose preparation:

We shade-dried the *L. ciliata*, crushed it to a coarse powder (1700g), and macerated it for 7 days in 80% ethanol to get the extract. The prepared hydroalcoholic extract was then suspended in 50 mL of water and partitioned three times (60 mL each) with different solvents in the following order: petroleum ether (LC-Pf, 22.7 mg), chloroform (LC-Cf, 178.8 mg), ethanol (LC-Ef, 206.7 mg), and the remaining aqueous solution, which will be referred to as the aqueous fraction (LC-Af, 24.93 g). The individual fractions were lyophilized to make a dry powder, which was then suspended in 0.3 % CMC for dosing into animals.

Phytochemical investigation

Preliminary Phytochemical investigation

Using the procedures given in Khandelwal (2008), we investigated the existence of several secondary metabolites in various fractions **[10]**.

Total Phenolic Content and Total Flavonoid Content

To estimate the total phenolic content (TP) and total flavonoid content (TF), a solution of dimethyl sulfoxide (DMSO) containing a particular amount of extract of *Lindernia ciliata* (1 mg/ml) was produced.

The Folin-Ciocatteu method was used to calculate the TP **[11, 12]**. In a nutshell, Folin-Ciocalteu reagent was used to oxidise an adequate dilution of the fraction of *L. ciliata*. The reaction was neutralised with sodium carbonate, resulting in a blue colour that was detected at 700 nm using a UV spectrophotometer after 30 minutes. Using a standard calibration curve and a variety of gallic acid dilutions, the result of TP is expressed as mg of gallic acid equivalents per 100 g of the dry weight of the extract.

Biglari *et al*. (2008) described an aluminium chloride colourimetric test which was used for measuring TF **[13]**. About 1 ml of the sample and 4 ml of water were put together in a 10 ml volumetric flask. After that, 0.3 ml of 5% $NaNO_2$ was added, followed by 0.3 ml of 10% $AlCl_3$ after 5 minutes. Around 2 ml of 1M NaOH was added after one minute, increasing the total volume to 10 ml. Using a standard calibration curve made up of various catechin dilutions, the absorbance was measured at 510 nm. Milligrams of catechin equivalents per 100 grammes of dry extract weight are used to express the TF result. The outcomes were verified twice.

Animals and approval from the animal ethical committee:

We obtained healthy, nulliparous, female, non-pregnant Wistar albino rats weighing 150-200g from the "College of Pharmaceutical Sciences, Dayananda Sagar University" animal house facility. The tests were carried out after receiving previous approval from Dayananda Sagar University's Institutional Animal Ethical Committee (IAEC) (Approval number: DSU/Ph.D/IAEC/39/2019-20).

Acute toxicity assay

We performed an acute toxicity assessment for all fractions of *L. ciliata* hydroalcoholic extract using the Limit test indicated in OECD standards 425 according to Hazarika *et al*., [12]. In a nutshell, the animals were divided into five groups (n=5). We used the vehicle (0.3% CMC) as the control group and the other four groups as the test group. A single animal was administered orally with 2000 mg/kg of fractions of *L. ciliata* hydroalcoholic extract and examined for 24 hours in the test group. If no signs of toxicity or mortality were noticed, further four animals were given 2000 mg/kg of *L. ciliata* hydroalcoholic extract. The animals were individually identified and observed for the first 30 minutes, four hours, and at a regular interval of time. Up until the 14th day, the animal's weight and behaviour were documented.

The animals were euthanized on the 15th day, and blood samples were taken through heart puncture for haematological studies, lipid profiles, liver function tests, and renal function tests. The brain, liver, kidney, and heart were all removed and sent for histological analysis.

Haematological analysis

The blood sample from the animals in both groups was taken employing heart puncture and maintained in an EDTA tube to examine the effect of fractions of *L. ciliata* hydroalcoholic extract on haematological profile. The blood samples were then run through a haematological analyser (HumaLyser 4000) to determine the CBC parameters, which included total red blood cell (RBC) count, haemoglobin (Hb) level, packed cell volume (PVC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total white blood cell (WBC) count, and differential WBC count, which included neutrophil (M).

Biochemical analysis

To separate the blood serum from the blood acquired by cardiac puncture, the blood was centrifuged. Using Randox kits in a biochemical analyser, blood serum was then utilised to assess indicators of liver function, renal function, and lipid profile.

Histopathological study:

The animals were euthanized when the experiment was completed, and vital organs such as the brain, heart, liver, and kidney were separated. The organs were fixed in 10% formalin and paraffin wax processed. Organ sections were cut into 5mm sections and stained with hematoxylin and eosin after being

treated in paraffin wax. The slides were then examined under light microscopy, with the images being saved for future research.

Statistical analysis

All experimental results were presented as mean ± SEM. Dunnet's multiple comparison test was used to determine the statistical significance of the differences between the groups and $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

L. ciliata has been used by Indians for centuries to treat a variety of ailments. Pallerla *et al*. (2019) conducted a toxicity investigation on the fraction of *L. ciliata* hydroalcoholic extract, but no thorough report of its toxicity was published[1]. It is critical to understand the correct drug dosage to achieve the best therapeutic results **[14]**. According to OECD standards 425, the current study was designed to assess the acute oral toxicity of different fractions of *L. ciliata* hydroalcoholic extract in female albino rats. The results of the phytochemical investigation are presented in **Table 1 and Figure 1**.

The results of behavioural studies suggested that all parameters were normal when compared to control. When comparing the bodyweight of the group animals treated with fractions of *L. ciliata* to the vehicle group, the bodyweight of the group animals treated with fractions of *L. ciliata* showed no significant difference. The toxicity level of a medicine can be determined using clinical signs and symptoms as a model **[15]**. Up to 14 days following oral administration of 2000mg/kg of body weight of LC, no signs of death or toxic behaviour were found in the current investigation. Furthermore, there was no difference in the animals' food intake or body weight when compared to the control group, implying that food ingredients were processed normally and metabolised properly for proper growth **[16-18]**.

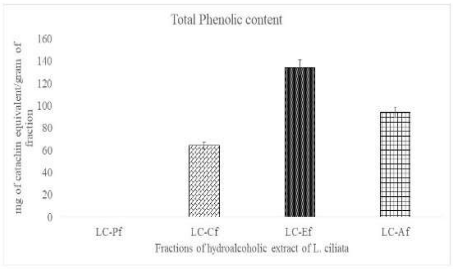
Figure 2(a)- (d) shows the findings of the haematological investigations. When compared to the control, the result indicated that the level of Hb had increased significantly ($p<0.05$). All other indicators, such as Total RBC, HCT, MCH, WBC count (both differential and total), and platelet count (as shown in **Figure 3**) were unchanged.

Table 1: Phytochemical constituents identified in all fractions of *L. ciliata*

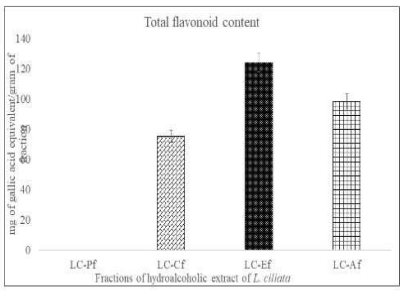
Class o f phytoconstituents	LC-Pf	LC-Cf	LC-Ef	LC-Af
Alkaloids	-	+	+	-
Flavonoids	-	+	+	+
Steroids/triterpenoids	+	+	+	-
Carbohydrates	-	-	+	+
Saponins	+	+	+	+
Phenolic compounds	-	+	+	+
Tannins	-	-	+	-

+ represents present and – represents absent

(a) % yield of different fractions

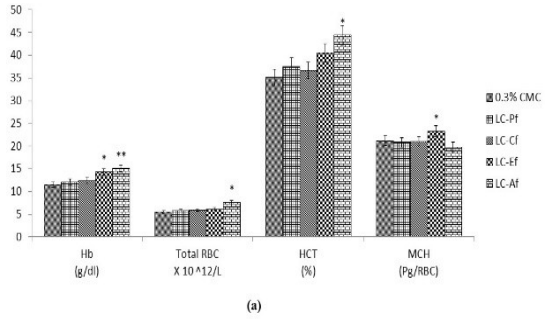


(b) Total Phenolic content different fractions

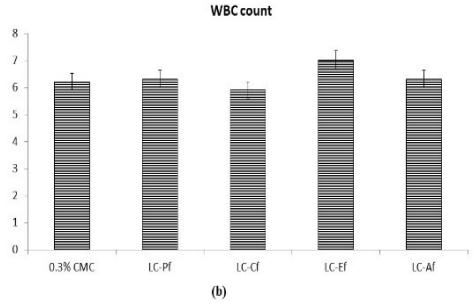


(c) Total flavonoid content of different fractions

Figure 1: Results of % Yield, total phenolic content and total flavonoid content of different fractions of hydroalcoholic extract of *L. ciliata*



(a)



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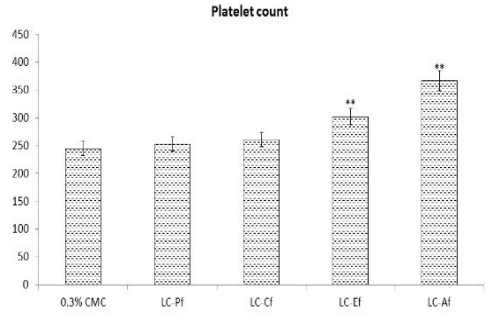
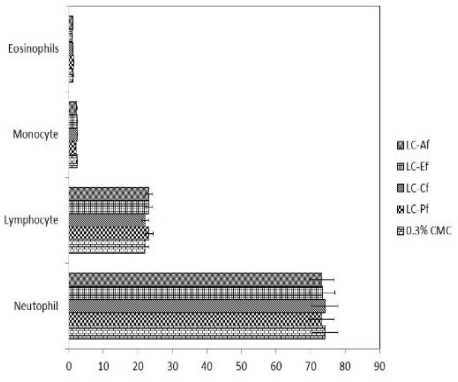


(c)



(d)

Figure 2: Effect of a single dose of *L. ciliata* extract (2000mg/kg) on Haematological profile. All values are expressed as Mean ± SEM; CMC: Carboxymethyl cellulose; LC-Pf: Petroleum ether fraction of hydroalcoholic Extract of *L. ciliata;* LC-Cf: Chloroform fraction of hydroalcoholic Extract of *L. ciliata;* LC-Pf: Ethanol fraction of hydroalcoholic Extract of *L. ciliata;* LC-Af: Aqueous ether fraction of hydroalcoholic Extract of *L. ciliata;*

The lipid profile result is shown in **Figure 3**, and it shows that there is no significant difference in cholesterol, triglycerides, HDL, LDL, VLDL, or Cholesterol to HDL ratio between the groups treated with different fractions of hydroalcoholic extract and the control group.

Figure 4 (a) and (b) shows the results for the indicators of liver function. When compared to the control, the biomarkers of liver function were shown to be statistically insignificant. The indicators of renal function test yielded similar results, as shown in **Figure 5 (a)-(b)**.

We evaluated the influence of *L. ciliata* on haematological profile, lipid profile, kidney and liver function to discover more definite data on the safety profile of *L. ciliata*. We calculated indicators including SGOT, SGPT, and alkaline phosphatase to assess the liver's functional condition **[6, 19-22]**. The results of our present investigations show that there was no significant difference in liver function biomarkers when compared to the control group.

Multiple hyperlipidemia symptoms may appear as a result of a toxicant's toxic effects **[23]**. In our current experiments, we discovered that the lipid profile does not alter following the administration of the limit dose of 2000 mg/kg of *L. ciliata* extracts, indicating that it does not affect the lipid profile.

Many studies show that when someone has renal impairment, their serum creatinine and urea levels rise **[24]**. As a result, serum creatinine and serum urea can be used as biomarkers to assess renal function. The serum creatinine and urea levels did not alter in the current investigations. In haematological measures, similar results were seen.

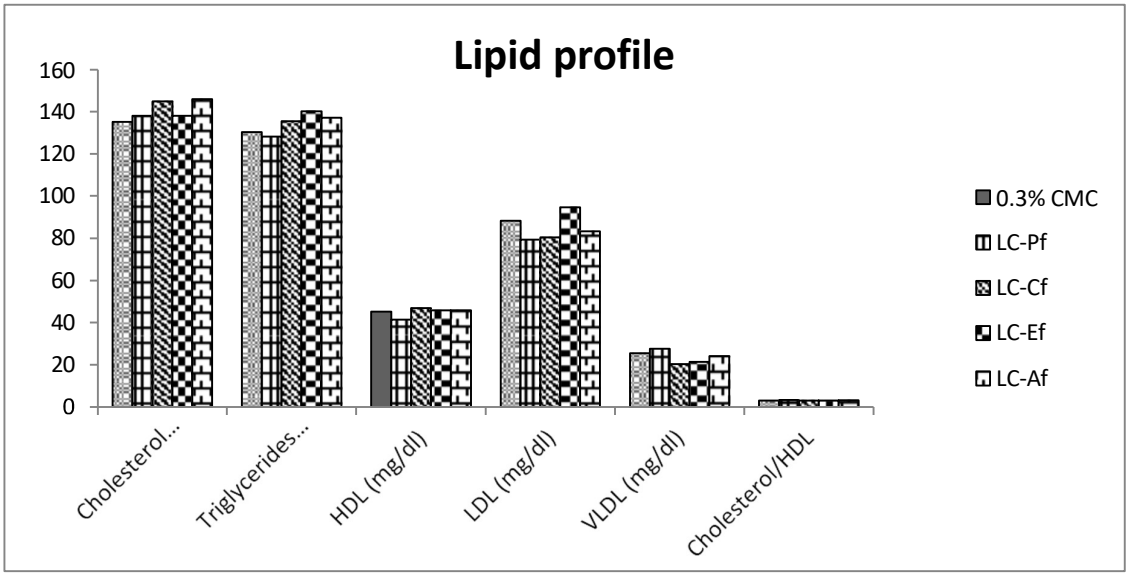


Figure 3: Effect of single dose of L. ciliata extract (2000mg/kg) on blood lipid profile. All values are expressed as Mean ± SEM; CMC: Carboxymethyl cellulose; LC-Pf: Petroleum ether fraction of hydroalcoholic Extract of L. ciliata; LC-Cf: Chloroform fraction of hydroalcoholic Extract of L. ciliata; LC-Pf: Ethanol fraction of hydroalcoholic Extract of L. ciliata; LC-Af: Aqueous ether fraction of hydroalcoholic Extract of L. ciliata;

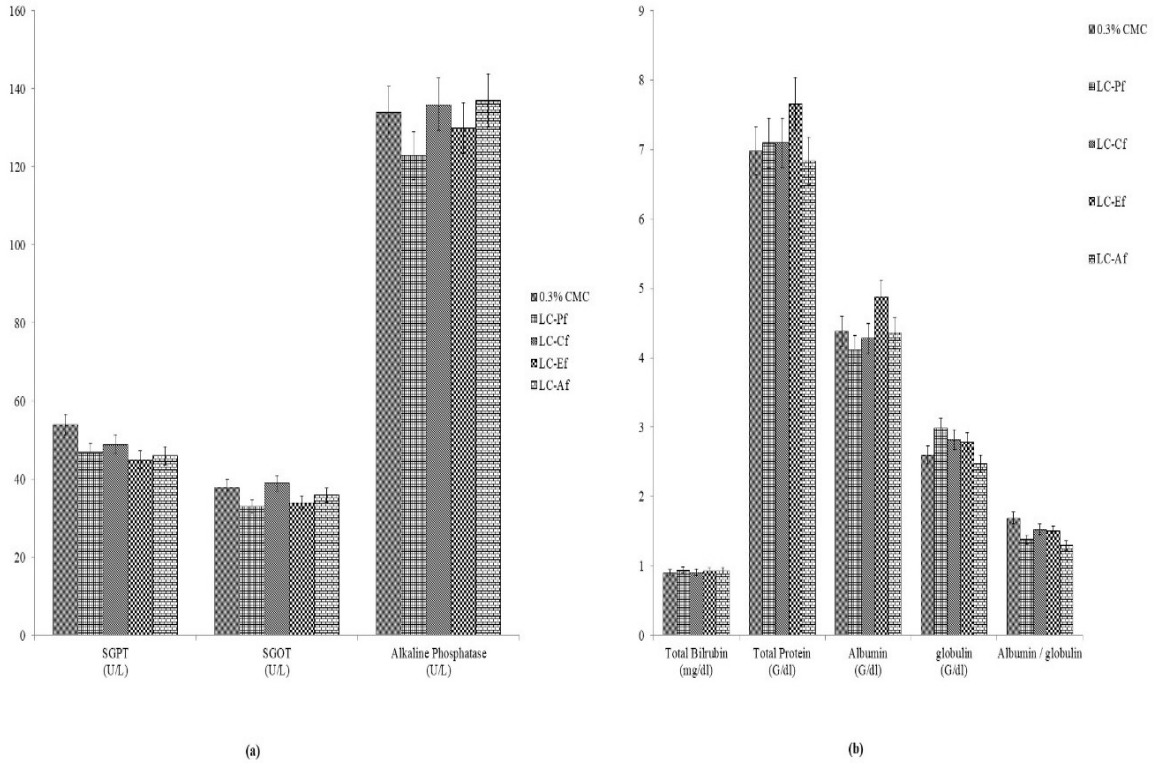


Figure 4: Effect of a single dose of *L. ciliata* extract (2000mg/kg) on the biomarkers of Liver function test a) SGPT, SGOT and Alkaline Phosphatase expressed as U/L (b) Total Bilirubin level, Total protein, Albumin and globulin level and albumin to globulin ratio. All values are expressed as Mean ± SEM; CMC: Carboxymethyl cellulose; LC-Pf: Petroleum ether fraction of hydroalcoholic Extract of *L. ciliata;* LC-Cf: Chloroform fraction of hydroalcoholic Extract of *L. ciliata;* LC-Pf: Ethanol fraction of hydroalcoholic Extract of *L. ciliata;* LC-Af: Aqueous ether fraction of hydroalcoholic Extract of *L. ciliata;*

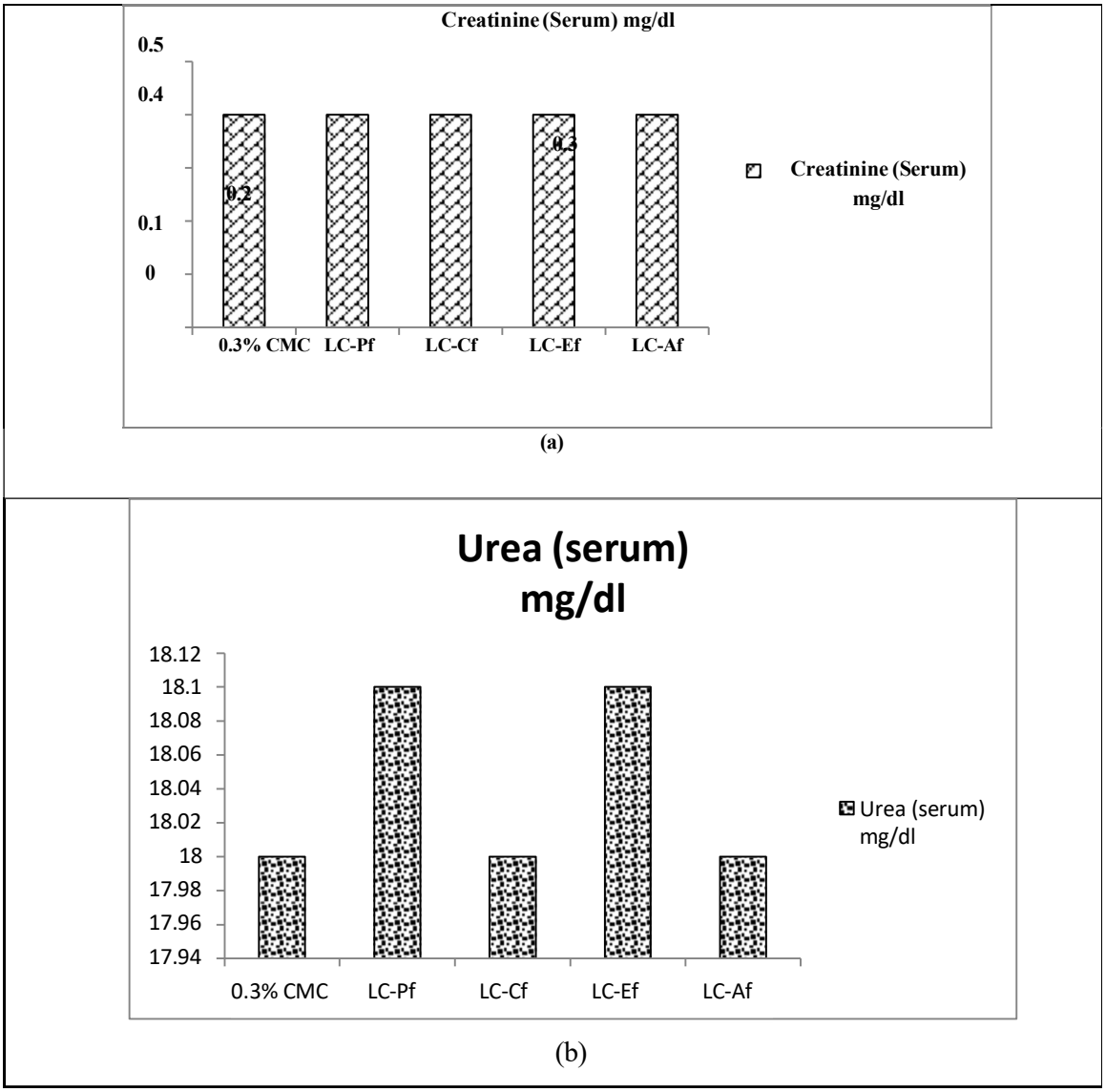


Figure 5: Effect of a single dose of *L. ciliata* extract (2000mg/kg) on Kidney function biomarkers (a) Serum creatinine level expressed in mg/dl (b) Serum urea level expressed in mg/dl. All values are expressed as Mean ± SEM; CMC: Carboxymethyl cellulose; LC-Pf: Petroleum ether fraction of hydroalcoholic Extract of *L. ciliata;* LC-Cf: Chloroform fraction of hydroalcoholic Extract of *L. ciliata;* LC-Pf: Ethanol fraction of hydroalcoholic Extract of *L. ciliata;* LC-Af: Aqueous ether fraction of hydroalcoholic Extract of *L. ciliata*

Any poisonous chemical has a significant impact on vital organs such as the brain, kidneys, liver, and heart **[25]**. Finally, the histological tests of the brain, kidney, liver, and heart have been assessed. The results show that the histology of important organs (brain, heart, liver, and kidney) in the treated group did not differ from that of the control group. The findings of the behavioral and biochemical experiments are perfectly consistent with the histopathological findings. The findings imply that the different fractions of LC's hydroalcoholic extract are safe up to a dose of 2000mg/kg body weight, and the median lethal dose (LD_{50}) is more than 2000mg/kg. Based on the findings, the fractions of *L. ciliata* hydroalcoholic extract can be classified as group 5 (LD_{50} > 2000 mg/kg) in the global harmonised categorization system (GHS) **[25]**.

CONCLUSION

The current investigation focused on the effects of a single 2000 mg/kg dose of the various fractions of *L. ciliata* hydroalcoholic extract. When compared to the control, all of the changes in parameters were statistically insignificant. As a result, we can conclude that the fractions of *L. ciliata* hydroalcoholic extract are quite safe (LD_{50}>2000mg/kg body weight) and can be categorised into GHS class 5. The extract's preliminary safety profile has been provided in our work; nevertheless, further research employing isolated phytoconstituents will be useful for its application in current treatments.

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