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ORAL ACUTE TOXICITY STUDY OF PURIFIED *STERCULIA FOETIDA* EXUDATE GUM

Pravin R. Bhushette[1], Satish V. Rojekar[2] and Uday S. Annapure[1*]

[1]Department of Food Engineering and Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai – 400 019, India.

[2]Department of Pharmaceutical Sciences & Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai – 400 019, India.

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***Corresponding Author**
Uday S. Annapure
Department of Food Engineering and Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai – 400 019, India.

ABSTRACT

The study aims to investigate the biologically active Sterculia foetida exudate gum's safety in terms of adverse reaction and mortality. The Sterculia foetida exudate gum was subjected to the toxicity studies; a sterculia foetida exudate gum was evaluated in Sprague-Dawley female rats by feeding the animals with serial doses of the gums between 50 to 2000 mg/kg bodyweight orally. Acute toxicity study in rats administered with 2000 mg/kg exudate gums, the toxicity in the animals was carried out by assessing the effects on body weight, relative organ weight, biochemical parameters and histopathological study for heart, lung, liver, kidneys, spleen, following oral administration of a solution of the Sterculia foetida exudate gum. The LD50 value of Sterculia foetida exudate gum was calculated and it was found to be more than 5000 mg/kg. Serum biochemical findings show no significant differences in comparison to control. No considerable bodyweight or relative organ weight changes occurred throughout the study; histopathology of selected organs showed no remarkable pathology. The above results show that the *Sterculia foetida* exudate gum did not produce any toxic effect in rats. Therefore it is safe for food and pharmaceutical applications as excipients.

KEYWORDS: Exudate Gum; Toxicity**;** Haematological study; Histopathological study; Food Additive.

INTRODUCTION

India has biodiversity with different climatic and physiographic features and is blessed with all types of vegetation. Out of nearly 425 families of flowering plants in the world, 328 families with 21,000 species occur in India.[1] Among these species, many yield gum, which is used in local areas from many years. Due to the dearth of literature, several plant gum exudates are still unexplored and are categorized as minor plant gum exudates.[2]

The *Sterculia foetida* plant belongs to the *Malvaceae* family, subfamily *Sterculioideae. It* is found in tropical areas[3] like India, Taiwan, Indochina, Philippines, United States (Hawaii), Indonesia, Ghana, Australia, Mozambique, and Togo. It is colloquially named as Indian almond, *Java olive*, *Arbre puant (*French*), jangli badam (*India*), kepoh (*Indonesian*), kalumpang (*Philippines*), anacagüita (*Spanish*).*[4,5] *Sterculia foetida* is one of the gums yielding plants[2], and its gum resembles gum tragacanth.[3] The exudate gum of *Sterculia foetida is* used for bookbinding[3], drug delivery system[6], film.[7] However, the toxicity studies of *Sterculia foetida* exudate gum were not reported.

The objective of this study is to of *Sterculia foetida* exudate gum, which can help in its further industrial applications.

MATERIAL AND METHODS

Collection and purification of exudate

SFG exudates were collected from the Mumbai area of Maharashtra, India. Selected *SFG* plants were subjected to stress by taping and injecting ethephon in it, as reported by Bhushette and Annapure.[8] Collected exudates were sundried and pulverised. Collected crude *Sterculia foetida* gum was partially soluble in water; to improve solubility and optimisation of purification method, it was treated at different conditions for like varying water temperature, pH of solution and volume of precipitating solution. The optimised methods, in brief, 5 gm of powdered gum was added in 100 ml water (30 ± 2°C) and pH adjusted to 12±0.1 by using 0.1N NaOH and stirred for 12 h at 500 rpm. After that, the solution was centrifuged at 5000 RPM to separate insoluble material. The supernatant was neutralised by using 0.1 N HCl and precipitated by adding 3 volumes of 90% ethanol. The precipitate was separated by centrifugation (5000 RPM for 20 min) and dried in an air oven at 45°C for 36 hours. The dried exudate was pulverised and stored at ambient temperature in an airtight container until further use.

ACUTE ORAL TOXICITY STUDY OF ISOLATED GUM IN SPRAGUE-DAWLEY RATS

Method of study and protocol information

The Sprague-Dawley rats were used for the acute oral toxicity of SFG as per The Organisation for Economic Co-operation and Development (OECD) 420 guidelines. The Institute Animal Ethics Committee approved the acute toxicity study protocol (Protocol No. ICT/IAEC/2019/P3) of the Institute of Chemical Technology, Mumbai, India.

Animal species

Six weeks old Sprague-Dawley female rats were obtained from Bombay Veterinary College, Parel, Mumbai, India.

Housing and feeding conditions

Animals were housed, having room temperature at 22 ± 2ºC and 50-60% relative humidity. A required supply of water and diets of the rat were provided to rats. The day and night cycle was maintained regularly.

Doses preparation and administration of dose

5-gram powder of SFG was dissolved in 20 ml of distilled water. The experiment was carried out as per the experiment design given in Fig. 1. Initially, a 300 mg/kg single dose was given to one animal with oral gavage. The animal was observed for 24 hours for toxic reactions. After 24 hours' animal was found to be safe, no toxic effect was observed, and no mortality was found. Hence another animal was dosed at a higher dose with 2000 mg/kg and watched for 24 hours. After 24 hours, the animal was safe, and no toxic effect was observed. It means that the animal was tolerated 2000 mg/kg, so we have given the same dose to another 4 animals, and they were observed for the next 24 hours for the gums mentioned above.[9–12]

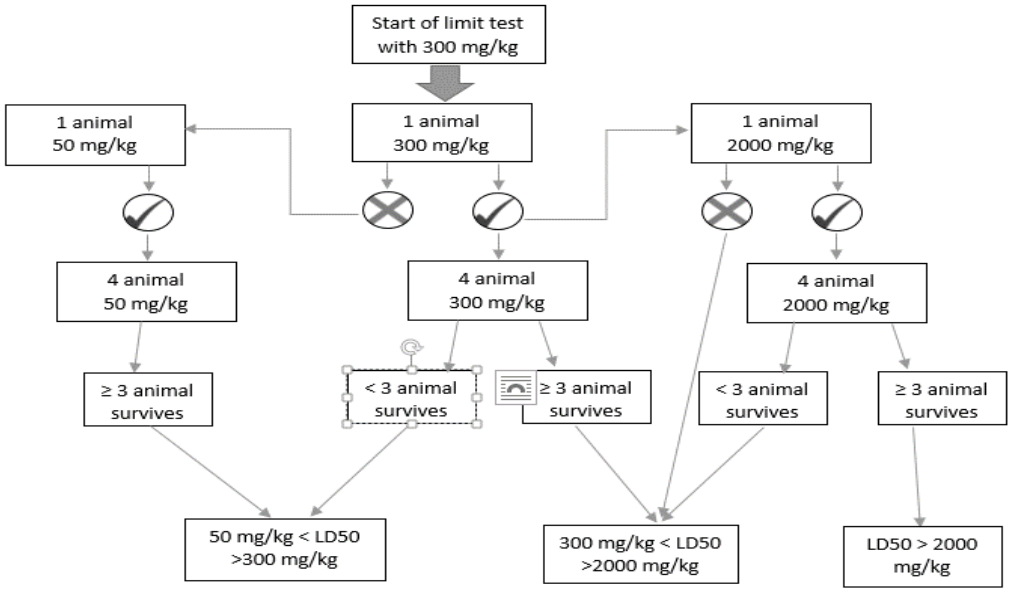


Figure. 1: Experimental Design.

OBSERVATIONS

Toxic signs and pre-terminal deaths

After dose administration, the rats were thoroughly observed every 30 min for the first 6 hours, then they observed daily for 14 days. However, the observation was noted for both the gums for the changes in the rats' skin, eyes, body weight, and behavioral pattern.

Bodyweight

The weight of each animal was taken before starting the study and after that weekly basis. To find out the changes in the weight of rats of both gums.

HEMATOLOGY

Euthanasia method

CO_2 asphyxiation, followed by cervical dislocation methods, have been used to kill experimental animals before dissection. "Euthanasia" is a term meaning "good death." The objective of euthanasia is to offer a rapid, painless, stress-free death. Carbon dioxide (CO_2) overdose causes instant unconsciousness, followed by death.[13]

Primary Method: CO_2 asphyxiation method

The 2 rats of the weight $\leq$ 200 gm were transferred in each rat cage (10" x 19" x 9", rat weight <500 gm allowed 2 rats per cage). For the Compressed CO_2 gas cylinder, the flow of CO_2 was maintained by the pressure reducing regulator and flow meter (5.6 L/min) for the rat

Cage size of (10" x 19" x 9"). The complete breathing cessation was observed for each animal and waited for 2 min before turning off the CO_2. After completing euthanasia in animals, the tank's main knob was turn off, and lids were returned to their racks.[14]

Secondary method: cervical dislocation

After completing the CO_2 asphyxiation procedure, the cervical discoloration was performed by a trained person on unconscious rats due to the small weight ≤ of 200 gm. The rats' death was confirmed by complete cessation of breathing, and further, it was used for the dissection.[14,15]

Clinical biochemistry

The blood samples were collected on the 1st day of dose administration and then subsequently on the 7th and 14th day of the study. However, collected blood samples were used for hematology and clinical biochemistry for both gums.

Gross necropsy

All the major organs were removed and thoroughly clean with phosphate buffer saline. Moreover, it was thoroughly examined for both gums' gross morphology and visual toxicology.[9]

Organs collection

After completing the study on the animal's 15th-day survival were observed for the SFG and ANG, further animals were sacrificed and dissected. All major organs will be collected and thoroughly washed by using phosphate buffer saline for additional evaluations.[9]

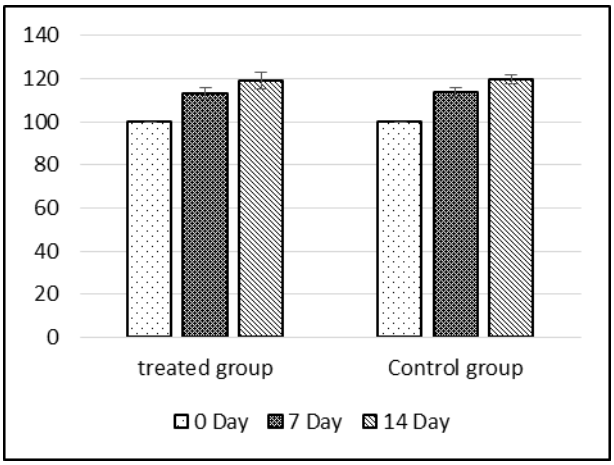
HISTOPATHOLOGY

Organ and tissue were fixed in the 10% v/v buffered neutral formalin solution overnight at 4 °C. After the organ/tissue mounting or fixing, all the organs entirely and thoroughly washed 3 times using 70% ethanol. After washing, the organs/tissues were processed and embedded in paraffin. A 5 µm thick paraffin sections were taken after washing and stained with Hematoxylin-Eosin. Then, the sectioned (at 5 µm) organs/tissues were placed on the clean glass slides and observed using microscopes for both gums.[9]

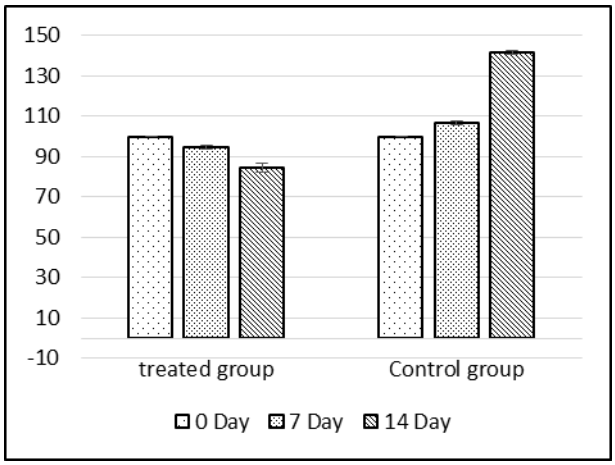
RESULTS

Effect on Growth: Treatment with 2000 mg/kg of *Sterculia foetida* gradually increased the bodyweight of rats, with the effect reaching statistical significance ($p < 0.01$-0.05)

after two weeks when compared to the control group (Figure 2a). However, this increased in weight was not statistically significant compared with the pre-treatment bodyweight of the animals. Besides, the degree of weight increased was not influenced by the *Sterculia foetida* treatment level. Furthermore, the animals showed a marked weight gain one week following the termination of *Sterculia foetida.* It was observed that the animals fed with *Sterculia foetida* exudate gum were healthy. No unusual changes in behavior or locomotor activity, no ataxia, and no signs of intoxication were observed during the 14 days. No differences were found in growth between the control group and the animals fed with different levels of the *Sterculia foetida* exudate gum Figure 2a. No change in fur coating, eyes, and respiratory functions. There was no significant difference in the food and water consumption between the treatment and control groups.



a)



b)

Figure 2 a) %Weight increase, b) blood glucose level in rats.

Effect on Biochemical and Haematological Measurements

Haematological parameters, Haemoglobin, Red blood cells, WBC, Neutrophils, Lymphocytes, Eosinophils, Monocytes, Packed cell volume, Platelet count. Total Eosinophils count (TEC), Total leucocyte count (TLC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) in both control and experimental rats were not significantly different (P >.05) for *Sterculia foetida* exudate Gum (Table 1). All values were found to be within the normal range for rats, and there were no differences between the groups.

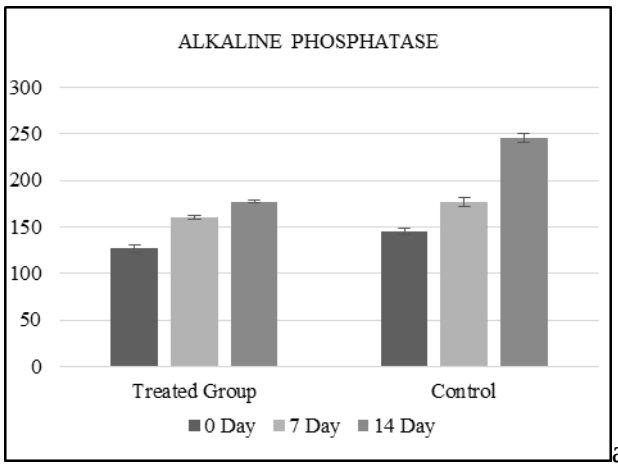
Table 1: Haematological Parameters Before and After Treatment.

Groups/ Parameters		Hb (mg/dl)	TEC (106/µl)	PCV (%)	MCV (fl)	MCH (Pg)	MCHC (gm/dl)	TLC (103/µl)	NEUTRO (%)	LYMPHO (%)	MONO (%)
REFERRENCE RANGE		11-19.2	8.48 – 15.15	36-54	48-70	27-33	40	06 to 18	10 to 30	65-85	0-5
Treated groups	0 day	12.4 ± 0.1	5.66 ± 0.2	38.5 ± 1.0	69 ± 1.6	19.4 ±0.5	31.0 ± 1.1	11.9 ± 0.9	10.6 ± 0.9	84 ± 1.0	1.5 ± 0.3
	7 day	12.3 ± 0.2	6.4 ± 0.3	41.1 ± 1.2	64 ± 1.6	19.2 ± 0.6	29.9 ± 1.1	7.7 ± 0.9	14 ± 0.9	76 ± 2.0	12 ± 1.0
	14 day	11.7 ± 0.2	6.1 ± 0.3	38.5 ± 1.3	63 ± 1.5	19.1 ± 0.7	30.4 ± 2.5	13.8 ± 0.9	10 ± 0.1	87 ± 1.7	3 ± 0.3
Control groups	0 day	12.3 ± 0.1	5.4 ± 0.1	36.2 ± 0.1	62 ± 1.5	20.5 ± 0.3	30.12 ± 1.1	12.7 ± 0.8	9.8 ± 0.3	80 ± 1.2	1.5 ± 0.3
	7 Day	12.3 ± 0.1	5.8 ± 0.2	37.1 ± 0.1	63 ±0.6	21.3 ± 0.1	33.5 ± 0.7	11.3 ± 0.9	10.4 ± 0.6	81.5 ± 0.6	8.1 ±0.1
	14 day	12.3 ± 0.1	6.6 ± 0.2	39.8 ± 0.8	60 ± 0.6	18.7 ± 0.2	31.1 ± 0.8	8.6 ± 1.0	15 ± 0.4	76 ± 0.6	9 ±0.1

Values = Mean ± SD

Clinical biochemistry

The level of serum glucose was increased in the control group over the 14 days, and the blood glucose level was significantly reduced in the treated group over the 14 days (Figure 2 b). The clinical biochemistry of control and *Sterculia foetida* gum-dosed rats were shown in Figure 3 a-j. In which found that cholesterol, alkaline phosphatase, total bilirubin, urea, BUN, total protein, and creatinine were not significantly different between the control and the experimental groups of rats (P>0.05) when fed with *Sterculia foetida* exudate gum. On the other hand, serum glutamate pyruvate (SGPT), Alkaline phosphatase, did not see any significant changes compared with *Sterculia foetida* exudate gum fed rats in comparison with controls. However, glutamate oxaloacetate transaminase (SGOT) was significantly changed with control groups. It was observed that the SGOT level was increased in the initial period, and recovery will be observed over time. However, analysis of the other blood metabolite levels (blood urea nitrogen, creatinine, and total bilirubin) did not see any significant changes in both experimental animals fed with *Sterculia foetida* exudate gum in comparison with controls. However, in the lipid profile like total cholesterol and triglycerides, it was observed that there are no significant changes in triglycerides and cholesterol in control animals and animals fed with the *Sterculia foetida* exudate gum. There is no effect on the lipid profile (Figure 3 a-j).



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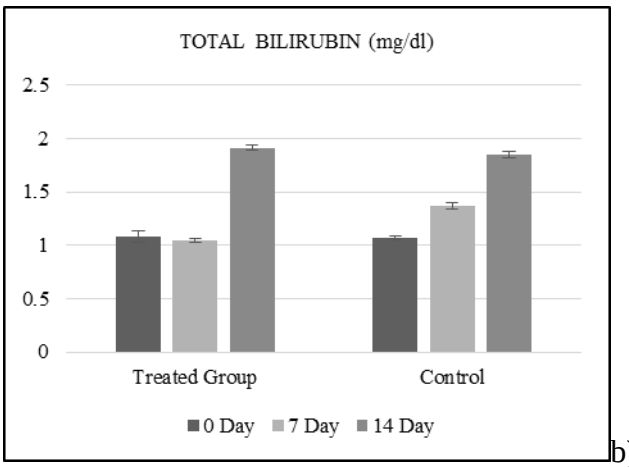

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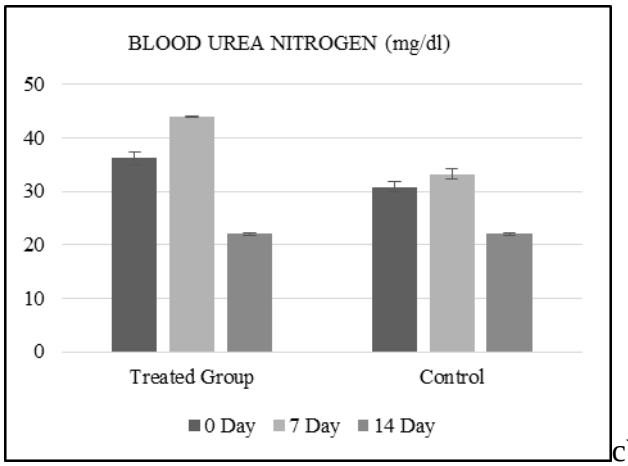
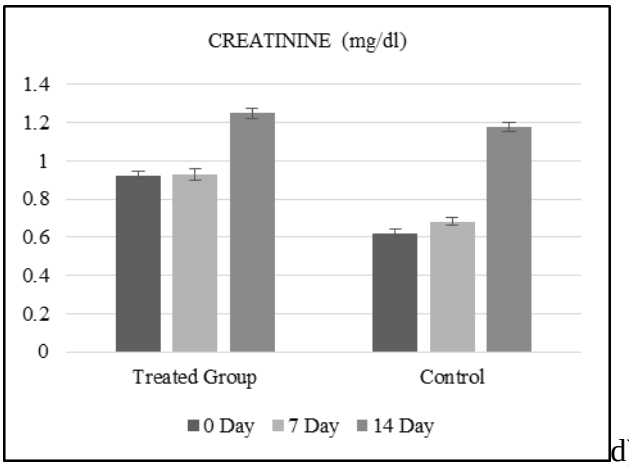
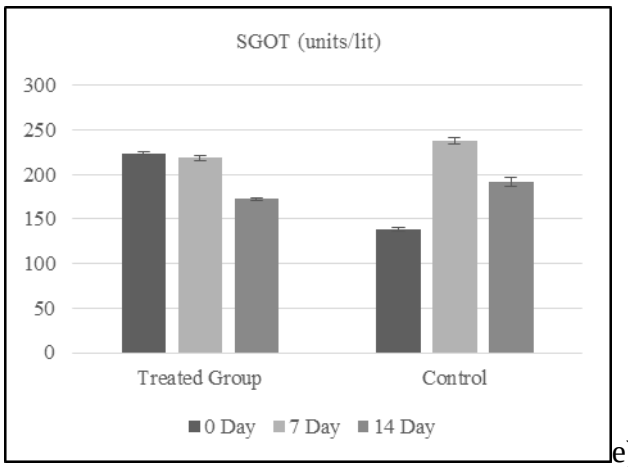

BLOOD UREA NITROGEN (mg/dl)
50
40
30
20
10
0
Treated Group
Control
0 Day
7 Day
14 Day

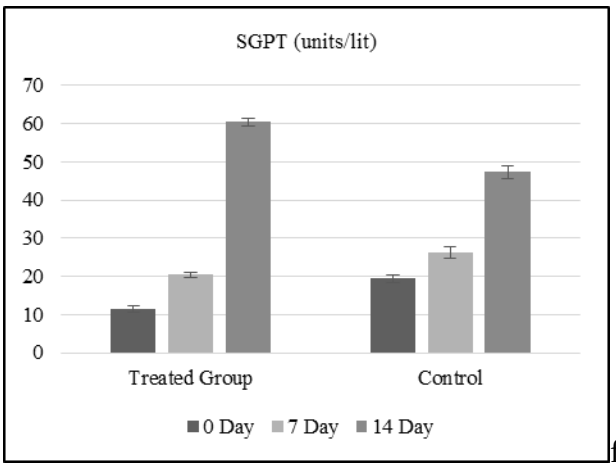
c)


CREATININE (mg/dl)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Treated Group
Control
0 Day
7 Day
14 Day

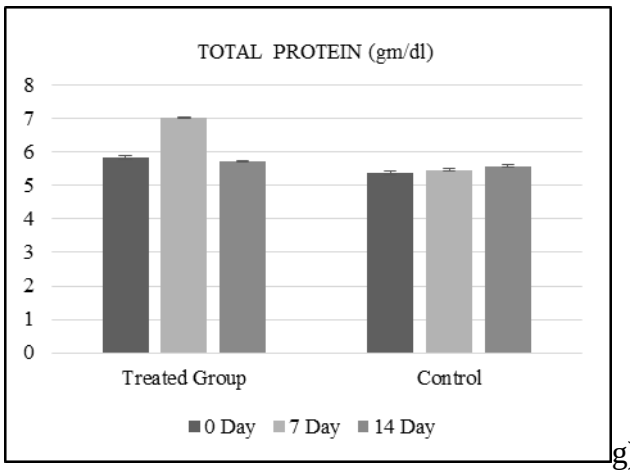
d)


SGOT (units/lit)
300
250
200
150
100
50
0
Treated Group
Control
0 Day
7 Day
14 Day

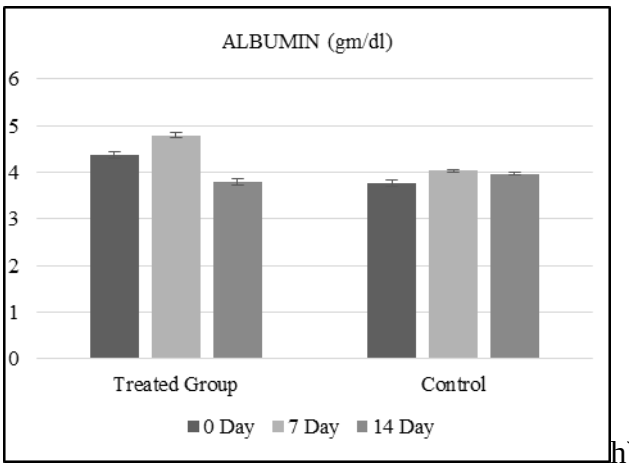
e)


SGPT (units/lit)
70
60
50
40
30
20
10
0
Treated Group
Control
0 Day
7 Day
14 Day

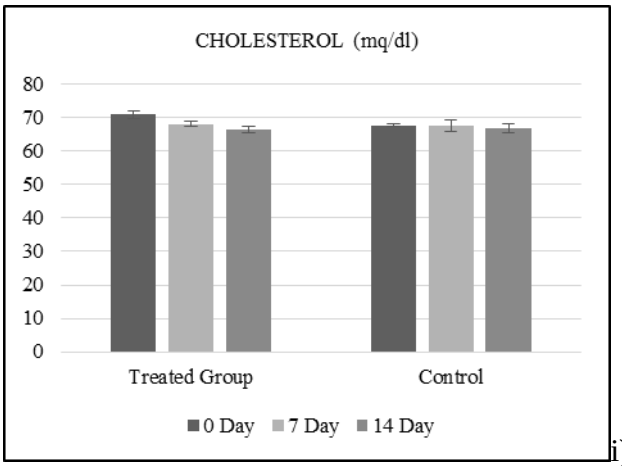
f)


TOTAL PROTEIN (gm/dl)
8
7
6
5
4
3
2
1
0
Treated Group
Control
0 Day
7 Day
14 Day

g)


ALBUMIN (gm/dl)
6
5
4
3
2
1
0
Treated Group
Control
0 Day
7 Day
14 Day

h)



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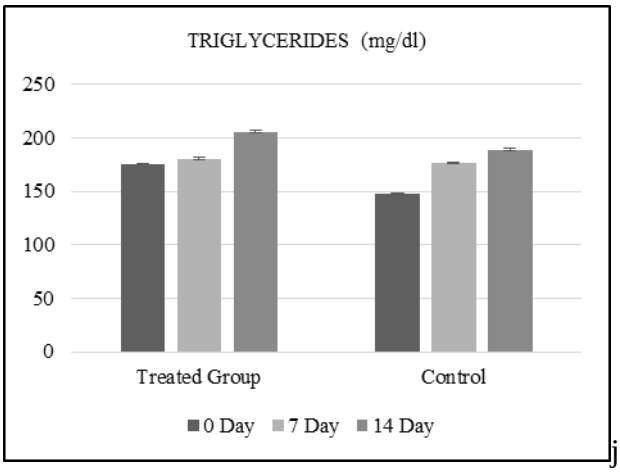


j)

Figure 3: Effect of *Sterculia foetida* gum feeding on haematological parameters of rats.

Acute toxicity study (LD_{50})

Acute toxicity has been defined variously by various experts. The organization for economic cooperation and development (OECD) panel of experts has defined acute toxicity as "the adverse effect occurring within a short time of (oral) administration of a single dose of a substance or multiple doses given within 24 hours. The purpose of acute toxicity studies is to determine the LD_{50} values, which help determine the safe dose range at which the drug can be used such that there is no harmful or lethal effect on the animal.

The gum exudate did not cause death or change in physical appearance and morphological characteristics in the treated animals throughout the 14-day observation period after single oral administration of 50, 300 and 2000 mg/kg doses of aqueous extract of *Sterculia foetida* in the acute toxicity study (Table 2). The estimated oral median lethal dose (LD_{50}) in mice is more than 5000 mg/kg body weight.

Table 2: Toxicity and mortality during acute toxicity study of aqueous extract of *Sterculia foetida* in rats.

Groups	Dose (mg/kg b.w)	No of rats with signs of toxicity/ Normal behaviour (ST/NB)	No of mortality/ Survival (D/S)
1 (Control)	0	0/3	0/3
2	50	0/3	0/3
3	300	0/3	0/3
4	2000	0/3	0/3

(b.w = body weight, ST = sign of toxicity, NB = normal behaviour, D = dead, S = survive)

Gross necropsy

The study Gum-treated rats did not show any death, behavioural changes, and toxic signs immediately after dosing, during 14 days, and at the end of the trial. On necropsy, no gross pathological abnormalities were observed in the vital organs, and hence the toxicity of the gum can be ruled out. On necroscopy, no gross pathological abnormalities were observed in the vital organs. Therefore, the acute toxicity study indicates that the gum is well tolerated [more than 2000 mg/kg] the therapeutic dose in tested Wistar rats.[9,10]

Histopathology

Observations of gross pathology immediately after dissection on rats of all groups were found to be uniformly healthy, lacking in any apparent pathological abnormalities. Histopathological examination of the liver, kidneys, spleen, heart, and lung in the control and the *Sterculia foetida* exudate gum fed groups showed no differences, indicating that feeding these *Sterculia foetida exudate* gum at these levels to the rats did not result in any adverse toxicological effect on these organs.[16,17]

Heart

The histopathological examination of H and E stained sections of healthy control rats' heart tissue did not reveal any significant pathological changes. No appreciable pathological changes were noticed in the heart in rats of this group. However, there were multifocal degenerative changes (Figure 4 a & 5 a) in myocardial muscles seen in places.

Liver

The histopathological examination of H and E stained sections of healthy control rats' liver tissue did not reveal any significant changes except minimal multifocal congestion. The microscopic examination of liver sections of rats revealed a distortion of hepatic architecture. Dissociation of hepatocytes and sinusoidal dilatation were observed. Vascular changes revealed central vein and sinusoidal congestion (Figure 4 b & 5 b) with focal areas of hemorrhages. Cellular swelling and hepatic degenerative changes (Plate-3) were also recorded in rats of this group under the microscopic field.

Lung

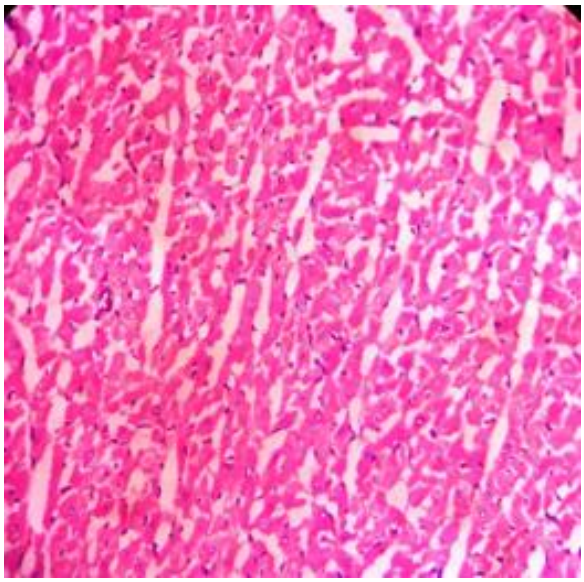
Lung tissue of healthy control rats did not reveal any significant changes except mild multifocal congestion and hemorrhages. Lung sections of treated rats revealed mild multifocal congestion and hemorrhages (Figure 4 c & 5 c).

Kidney

Kidney tissue of healthy control rats did not reveal any significant changes except mild multifocal hemorrhages. Microscopic examination of kidney sections from treated rats revealed multifocal vascular changes includes mild congestion, hemorrhages, focal areas of glomerular atrophy (Figure 4 d & 5 d), and multifocal necrotic changes were observed.

Spleen

Spleen tissue of healthy control rats did not reveal any significant pathological changes (Figure 4e & 5e).



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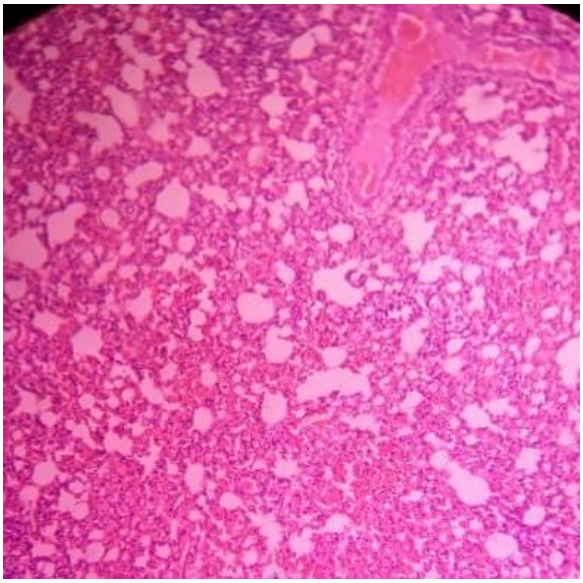

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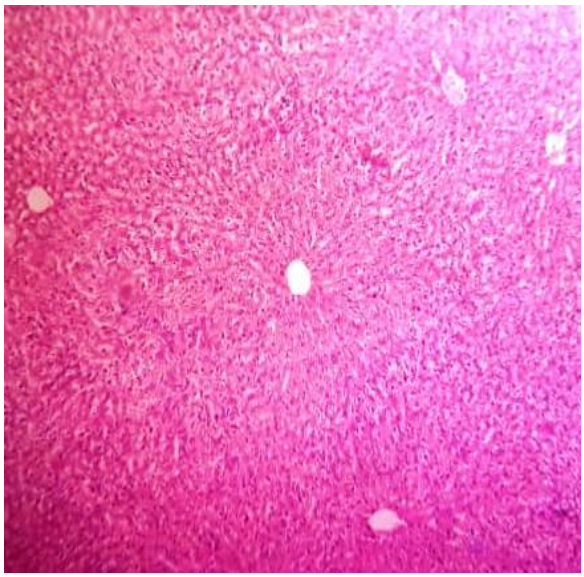

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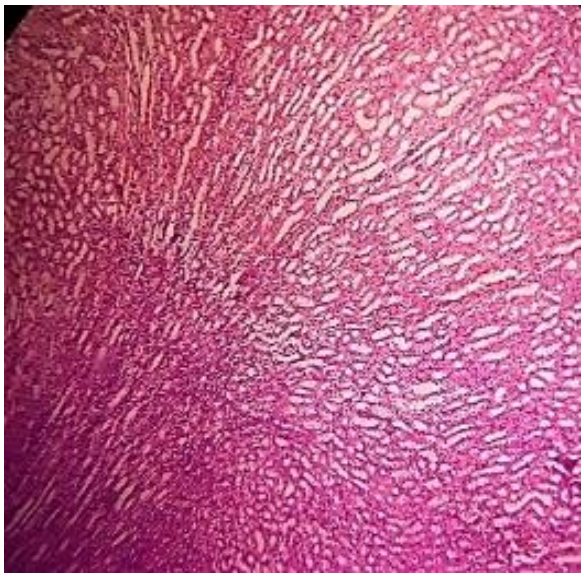
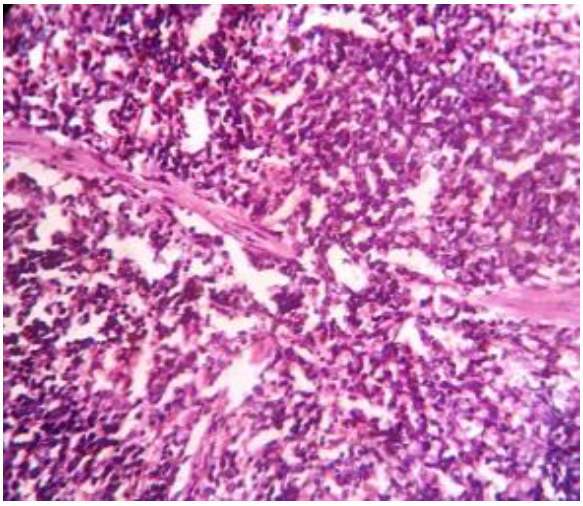
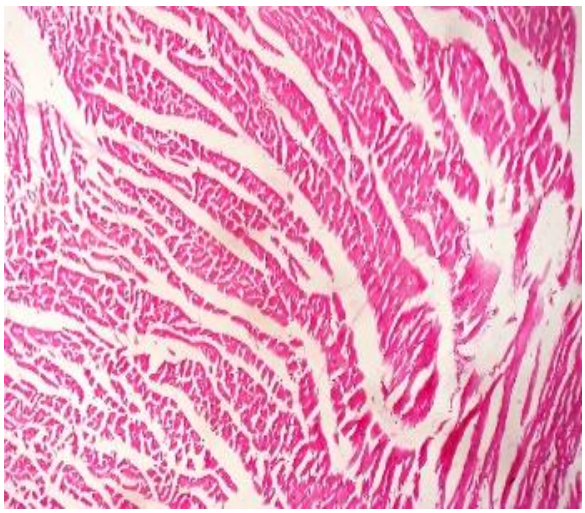


(d)



(e)

Figure. 4: Control group microphotograph of a) normal heart b) lungs c) liver d) kidney e) spleen



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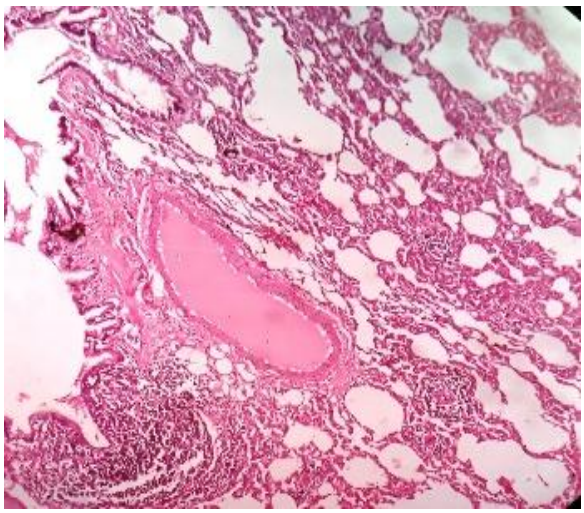

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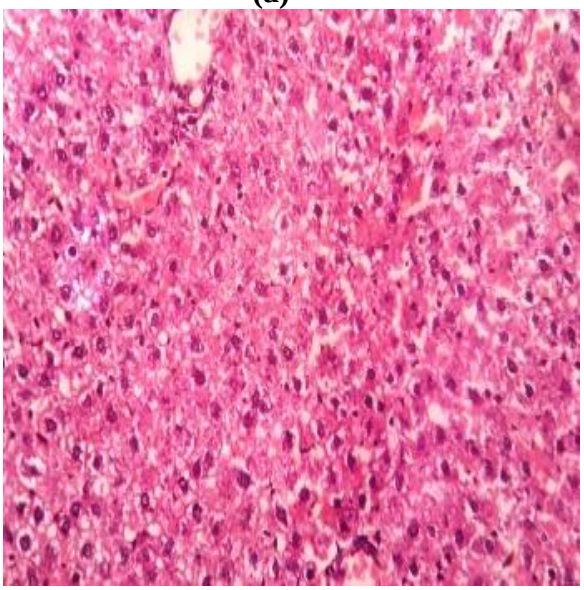

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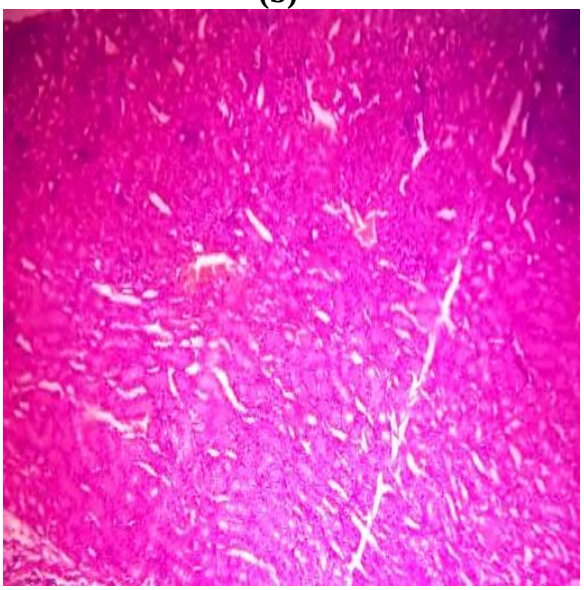

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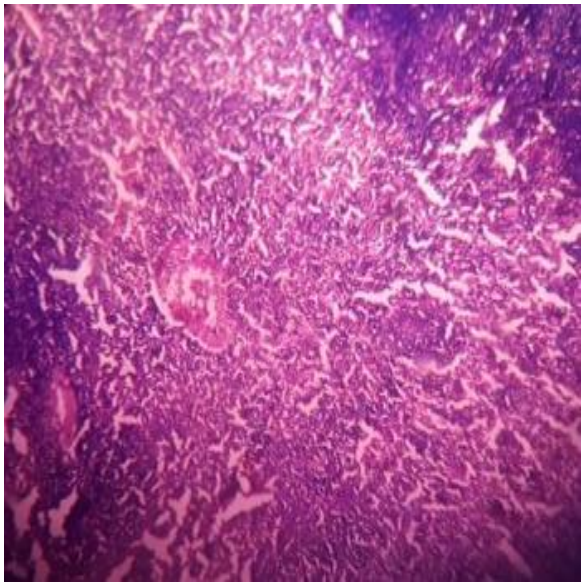

(e)

Figure. 5 *Sterculia foetida* gum Group microphotograph of a) normal heart b) lungs c) liver d) kidney e) spleen.

DISCUSSION

The intake of *Sterculia foetida* exudate gum 2000mg/kg bodyweight for 14 days was well tolerated. The ingestion of *Sterculia foetida* had no significant effect on glucose tolerance, serum cholesterol, cholesterol, triglycerides, and phospholipids, plasma biochemistry, haematological indices, urinalysis parameters.[18–20] Although these are indirect inferences, they agree with the report that *Sterculia foetida* was not metabolized by rats and appeared virtually quantitatively in the faeces. Thus *Sterculia foetida* seems to produce no toxic effects and to have a little metabolic effect on the host. This is in contrast to the effects established for some other plant gums and polysaccharides, i.e., Gum Arabic and whole raw carrot cause the average concentrations of breath hydrogen and methane to increase; guar galactomannan alters glucose tolerance significantly in both normal and diabetic subjects.[21,22]

The exudate gum was found to lower the blood glucose level, cholesterol, and triglycerides levels. Hence it is reported that leaf extract of *Sterculia foetida* showed significant anti-diabetic activity and antihyperlipidemic activity, which was comparable with that of the standard.[23]

Microscopic data, together with the data of macroscopic evaluation of the animals' organs, showed that both test and control groups were practically healthy. According to histological examination data, no toxic or allergic effects of *Sterculia foetida* exudate gum were detected

in the test group. No irritating local effects of the drug preparation were observed in the test group during the study period. Our experiments showed that *Sterculia foetida* exudate gum has safe. In histopathological studies, the liver of treated animals showed normal histological features at 2000 mg/kg. The kidney of treated rats showed normal glomeruli, and there is no necrosis of tubular epithelium in the kidney. Gross examination of liver and kidney on histology did not reveal any abnormalities. Also, other organs like the heart, lung, and spleen were not shown any toxic effect on control and gum treated organs. Thus, it was concluded that *Sterculia foetida* exudate gum did not produce any toxic effect in female Sprague Dawley rats.[16,17,24,25]

CONCLUSION

No mortality was recorded in experimental animals treated with the *Sterculia foetida* exudate gum orally at a dose of 2000 mg/kg. The toxicity study of the gum reflected the innocuous nature of hepatic, renal, cardiac, pulmonary, and speelinc and hemopoietic systems even at a high dose level of administration for14 days in rats. The above results show that the *Sterculia foetida* exudate gum did not produce any toxic effect in rats. Therefore it is safe for food and pharmaceutical applications as excipients. This gum has known promising anti-diabetic and anti-oxidant activity and would help treat diabetes.

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DECLARATIONS

Ethics approval and consent to participate

The Institute Animal Ethics Committee approved the acute toxicity study protocol (Protocol No. ICT/IAEC/2019/P03) of the Institute of Chemical Technology, Mumbai, India.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

ABBREVIATIONS

ALP - Alkaline phosphatase

ALT - Alanine Aminotransferase

AST - aspartate aminotransferase

Hb- hemoglobin

LD50 - Median lethal dose

LYMPHO - lymphocyte

MCH - Mean corpuscular haemoglobin

MCHC - Mean corpuscular haemoglobin concentration

MCV - Mean corpuscular volume

MONO - Mononucleosis

NEUTRO - neutrophils

NOAEL - No observable adverse effect level

OECD - Organisation for economic cooperation and development

PCV - Packed cell volume

SFG - *Sterculia foetida* exudate gum

SGOT - Serum glutamic-oxaloacetic transaminase

SGPT - Serum glutamate pyruvate

TEC - Total Eosinophils count

TLC - Total leucocyte count

WBC - white blood cells

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