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Acute and Sub chronic Toxicological evaluation of ethanolic leaf extract of *Sida acuta* Burm.F in Wistar albino rats by analyzing biochemical, heamatological and histochemical parameters

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ABSTRACT

Sida acuta burm.f belongs to *Malvaceae,* the mallow family and enjoys tropical and pantropical distribution. The plant is usually known as wireweed in the countryside, and it is highly medicinally valued traditionally and ethnobotanically promised. The present study is concerned with acute and subchronic toxicity evaluation of ethanolic extract of *Sida acuta* Burm.f leaves in Wistar albino rats. Acute toxicity evaluation was conducted for 14 days. Acute doses of 100, 250, 500, 1000 and 2000 mg/kg BW were administrated to test groups of animals under consideration on the first day of experimental evaluation with three animals in each of total six groups along with control. For the remaining 13 days, animals were observed for noted behavioural changes and body weight were recorded respectively for 7^{th} and 14^{th} day of experimental analysis. At the end of the trial period, all the animals were euthanised, and various biochemical parameters and histopathological examination were carried out to assess the toxicity of extract. The present study revealed that the ethanolic extract of *Sida acuta* Burm.f leaves is non-toxic up to 2000mg/kg body weight. Subchronic toxicity evaluation was conducted for 28 days with several doses 100, 200, 300, 400 and 500mg/kg BW. Control rats without any treatment were maintained during the entire period of experimental analysis. The results of subchronic toxicity parameters indicate no significant changes to the biochemical parameters (glucose, urea, uric acid, creatinine, AST, ALT and Cholesterol) haematological and histopathological observation in comparison to the control groups. Based on subchronic toxicity parameters data, effective doses (200 and 400mg/kg BW) is determined for further cancer (colon) study in Wistar albino rats.

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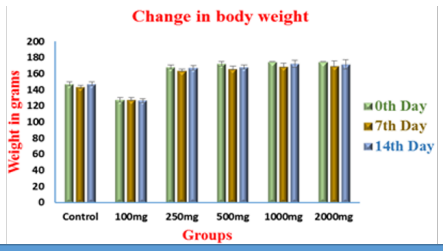
INTRODUCTION

Herbal medicines and medicinal herbs are receiving considerable importance worldwide. According to the report of the WHO (World Health Organization), about 80% of people in developing countries depend on herbal medicine for their preliminary health care due to its easy availability, low cost and convincing results. In developed countries also, the public interest in herbal prescription has dramatically increased (WHO, 2002). The bioactive principles of medicinal plants constitute the essential ingredients for drug development and therapeu-

tics. Researchers have shown that medicinal plants have antimicrobial, analgesic, anti-candidiasis activities, hypotensive activity, antidiabetic, antiproliferative, anti-inflammatory and antioxidant (Brogi *et al.*, 2019; Zahoui *et al.*, 2017; Gnahoué *et al.*, 2015).

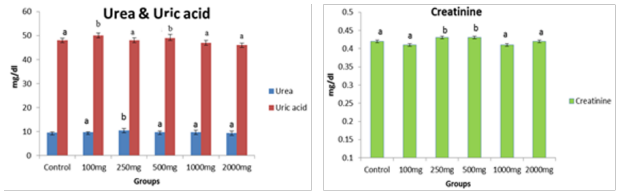
However, although medicinal plants have several therapeutic virtues, they have to be adequately screened for toxicological parameters in a biological system as some of them are at risk of toxicity. Several researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants and vegetables (Peyrin-Biroulet *et al.*, 2004; Poornima *et al.*, 2012). Seen, the dangers of toxicity, studies on the safety and effectiveness of medicinal plants have become one of the main concerns to guarantee the use (Stone, 2008; Yadava *et al.*, 2011).

Sida acuta, is an erect perennial shrub found throughout the warmer provinces of India and Nepal. In traditional and indigenous system of medicine, it is used to treat gonorrhoea, elephantiasis and ulcers and is claimed to have aphrodisiac properties. The juice of the root is applied to wounds. The whole plant is used to treat snakebite, and it lessened the hemorrhagic effect of *Bothrops atrox* venom (Muneeswari *et al.*, 2016; Sreedevi *et al.*, 2009) . Alkaloid cryptolepine isolated from *Sida acuta* was proved to be responsible for the antiplasmodial activity of the present species (Otero *et al.*, 2000; Karou *et al.*, 2003). The aerial part of the plant is the most frequently used part. In Central America, the plant is used to treat renal inflammation, asthma, colds, fever, ulcers, worms and headache, (Kirtikar and Basu, 1975). The present study aims to investigate the toxicological evaluation of the ethanolic extract of *Sida acuta* burm.f leaves using Wistar albino rats.



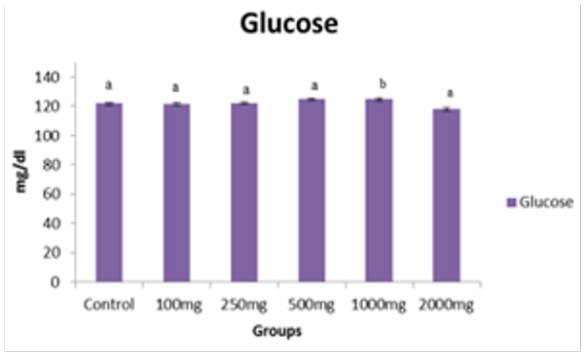
Values are expressed as Mean ± SD (n=3) (p<0.05) no significant changes in body weight of experimental groups compared to control

Figure 1: Effect of *Sida acuta* activity on changes in body weight of control and experimental groups



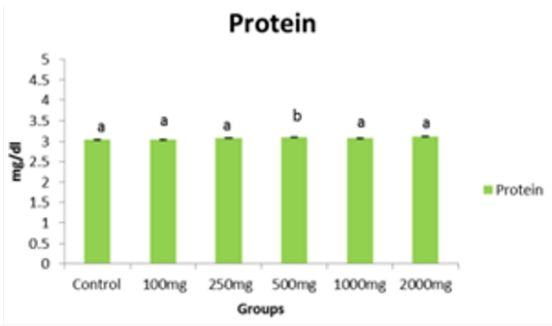
Values are expressed as mean ± SD n=3, (p<0.05) using One way ANOVA followed by DMRT:a-b

Figure 2: Effect of ethanolic extract of *Sida acuta* on serum urea, uric acid and creatinine in experimental rats



Values are expressed as mean ± SD n=3, (p>0.05) using One way ANOVA followed by DMRT:a

Figure 3: Effect of ethanolic extract of *Sida acuta* on serum glucose in experimental rats



Values are expressed as mean ± SD n=3, (p<0.05) using One way ANOVA followed by DMRT:a,b

Figure 4: Effect of ethanolic extract of *Sida acuta* on serum protein in experimental rats

MATERIALS AND METHODS

Plant collection

The whole plant *S.acuta* was collected from Tuticorin (DT) and authenticated in Botanical Survey of India, TNAU Campus, Coimbatore (Voucher number: BSI/SRC/5/23/2016/ Tech./348).

Preparation of crude extract

50g of powdered leaf material was weighed and extracted with 250 ml of ethanol for 72 hrs with

Table 1: Hematological parameters of sub chronic toxicity study

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Total WBC Count Cells/Cu.mm	8,201 ± 0.4[a]	8,251 ± 0.5[a]	8,204 ± 0.52[a]	8,200 ± 0.57[a]	8,201 ± 0.13[a]	8,201 ± 0.3[a]
Total RBC Count Million/Cu.mm	4 ± 0.25[a]	3.9 ± 0.15[a]	3.9 ± 0.16[a]	4 ± 0.72 [a]	4 ± 0.08[a]	4 ± 0.07[a]
Hemoglobin g/dL	13 ± 0.40[a]	13 ± 0.1[a]	13 ± 0.40[a]	13 ± 0.35[a]	13± 0.10[a]	14± 0.36[a]
HCT/PCV %	38 ± 0.60[a]	39 ± 0.36[a]	40 ± 0.85[a]	40 ± 0.76[a]	38 ± 0.2[a]	39 ± 0.26[a]
Platelet Count Lakhs/Cu.mm	4 ± 0.22[a]	3.9± 0.25[a]	3.8± 0.29[a]	3.8 ± 0.35[a]	4 ± 0.31[a]	3.9 ± 0.16[a]
MCV Cu.um	83 ± 1[a]	82 ± 1[a]	82 ± 1[a]	81 ± 1[a]	83 ± 1[a]	82 ± 1[a]
MCH pg	25 ± 1[a]	24 ± 0.20[a]	25± 0.25[a]	24 ± 0.55[a]	25 ± 0.70[a]	24 ± 0.1[a]
MCHC g/dL	47 ± 1[a]	46 ± 0.5[a]	48 ± 0.64[a]	46± 0.6[a]	45 ± 0.68[a]	45 ± 0.36[a]
RDW %	15 ± 0.90[a]	15 ± 0.60[a]	16 ± 0.40[a]	15 ± 0.68[a]	15 ± 0.85[a]	15± 0.26[a]
MPV Cu.um	7 ± 0.60[a]	7 ± 0.45[a]	7 ± 0.25	7 ± 0.3[a]	8 ± 1[a]	7 ± 0.1[a]
PCT %	0.326 ± 0.05[a]	0.4 ± 0.17	0.3 ± 0.15	0.3 ± 0.06	0.4 ± 0.14	0.3 ± 0.06
PDW %	8 ± 0.152[a]	9 ± 0.60[a]	9 ± 0.68[a]	8± 0.1[a]	9 ± 0.20[a]	8± 0.1[a]
Polymorphs %	40 ± 1[a]	40 ± 1[a]	40 ± 0.2[a]	41 ± 0.76[a]	41 ± 0.68[a]	40 ± 0.76[a]
Lymphocytes %	88 ± 1[a]	87 ± 1[a]	87 ± 0.3[a]	86 ± 0.35[a]	89 ± 0.5[a]	87± 1[a]
Monocytes %	5 ± 0.5[a]	5 ± 0.76[a]	5 ± 0.5[a]	5 ± 0.25[a]	6 ± 0.76[a]	5 ± 1[a]
Lymphocyte Count Cells/Cu.mm	3,601 ± 1[a]	3,603 ± 3.51[a]	3,601 ± 0.7[a]	3,600 ± 0[a]	3,601 ± 1[a]	3,601 ± 1[a]
Monocyte Count Cells/Cu.mm	1201 ± 0.76[a]	1,202 ± 1.52[a]	1301 ± 0.2[a]	1,201 ± 1[a]	1,201 ± 1[a]	1201 ± 0.76[a]
Granulocyte Count Cells/Cu.mm	1,401 ± 0.01[a]	1,401± 0.02[a]	1,403 ± 0.01[a]	1,40 1± 0[a]	1,400 ± 0[a]	1,401 ± 0.01[a]

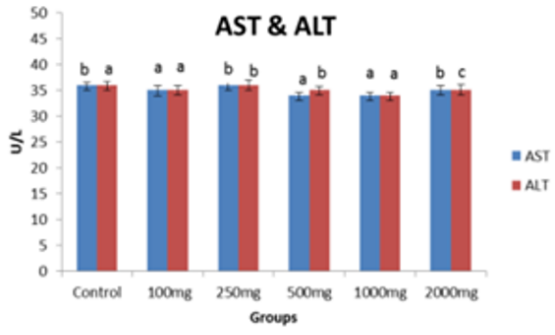
occasional shaking. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in air-tight bottles for further studies.

Experimental Animals

Healthy Wistar albino rats were procured for the toxicity study. The study was accepted by IAEC (Institutional Animal Ethical Committee) constituted for CPCSEA, Government of India (KAHE/IAEC/Ph.D./163).

Experimental procedure

Acute toxicity assessment was performed for 14 days according to the guidelines as per the Organization for Economic Cooperation and Development (OECD) guidelines 425 (OECD, 2001).A total of 18 healthy Wistar albino male rats were divided similarly into six groups of 3 each. Different doses of 100, 250, 500, 1000 and 2000mg/kg BW of ethanolic extract of *S.acuta* were given to Wistar albino rats in the very first day of experimental analysis. Control group receives normal saline and water. They were all placed under observation after treatment for 24h, 48h and 72h for any behavioural, neurological changes and then mortality for the 14 days.



Values are expressed as mean ± SD n=3, ($p<0.05$) using One way ANOVA followed by DMRT[a,b]

Figure 5: Effect of ethanolic extract of *Sida acuta* on serum AST & ALT in experimental rats

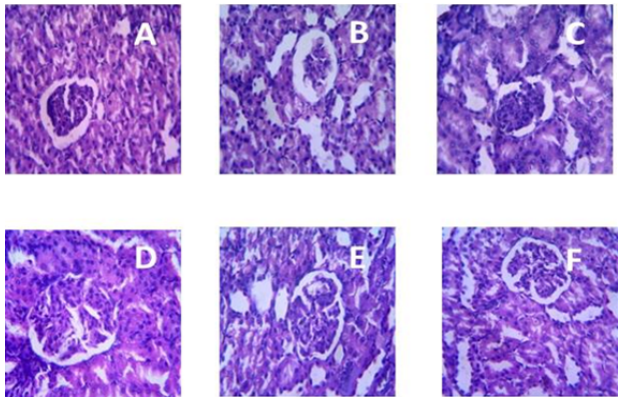
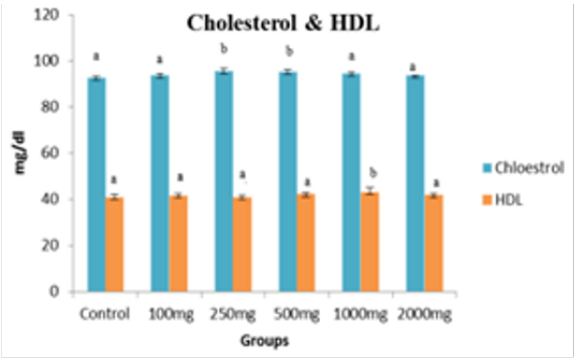


Figure 8: Histopathology of kidney. A –Control, B – 100mg/kg BW, C – 250mg/kg BW, D – 500mg/kg BW, E –1000mg/kg BW, F– 2000mg/kg BW(Magnification 40x)



Values are expressed as mean ± SD n=3, ($p<0.05$) using One way ANOVA followed by DMRT[a,b]

Figure 6: Effect of ethanolic extract of *sida acuta* on serum HDL and cholesterol in experimental rats

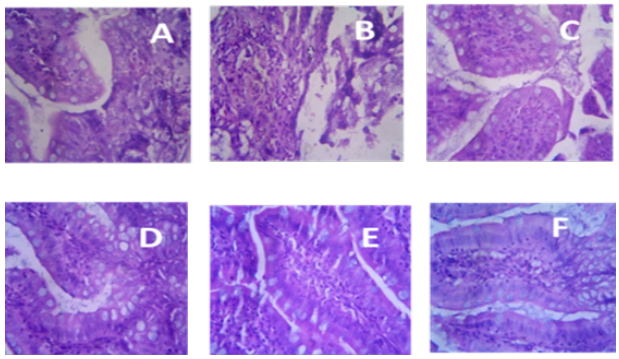


Figure 9: Histopathology of colon. A –Control, B – 100mg/kg BW, C – 250mg/kg BW, D – 500mg/kg BW, E –1000mg/kg BW, F– 2000mg/kg BW(Magnification 40x)

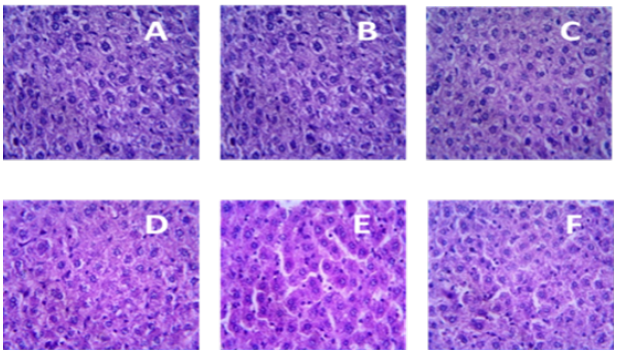
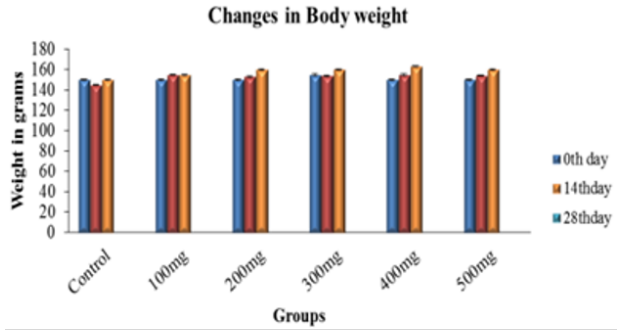
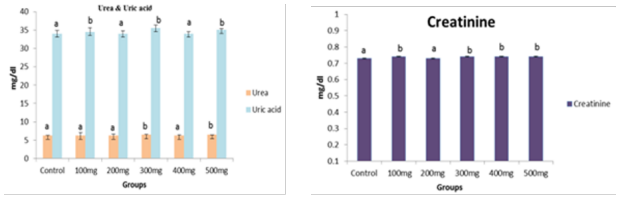


Figure 7: Histopathology of liver. A –Control, B – 100mg/kg BW, C – 250mg/kg BW, D – 500mg/kg BW, E –1000mg/kg BW, F– 2000mg/kg BW(Magnification 40x)



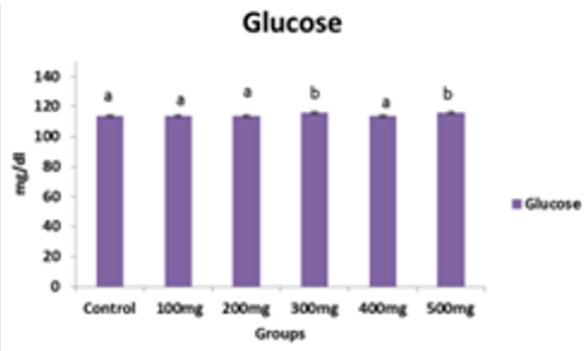
Values are expressed as Mean ± SD (n=3)

Figure 10: Body weight changes in sub-chronic toxicity study



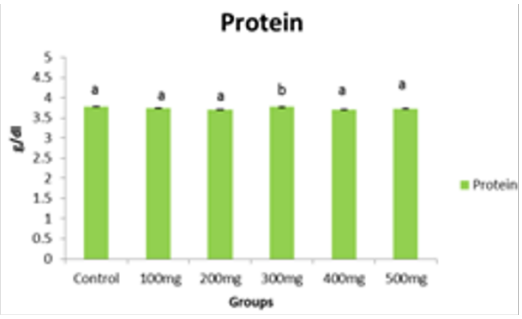
Values are expressed as Mean ± SD (n=3(p<0.05) using One way ANOVA followed by DMRT[a,b]

Figure 11: Effect of plant extract on Serum urea, uric acid and creatinine level of control and experimental rats



Values are expressed as Mean ± SD (n=3(p<0.05) using One way ANOVA followed by DMRT[a,b]

Figure 12: Effect of plant extract on Serum Glucose level of control and experimental rats

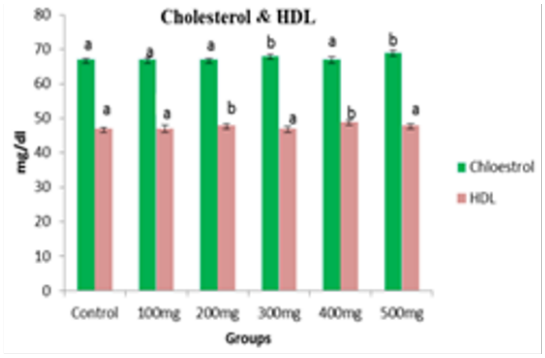


Values are expressed as Mean ± SD (n=3) (p<0.05) using One way ANOVA followed by DMRT[a,b]

Figure 13: Effect of plant extract on Serum protein level of control and experimental rats

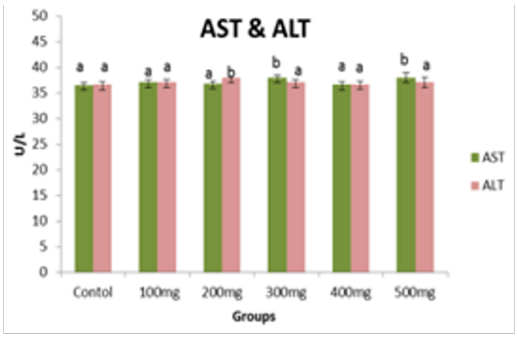
Later, the animals were euthanised on the 15th day. Biochemical parameters in serum and histological (liver, kidney and colon) examinations were determined by standard protocols.

For subchronic toxicity study ethanolic leaf extract of *s.acuta* was administrated to a total of 15 albino rats, randomly grouped into five groups of three in each (Group II, III, IV, V and VI) Group I served as control and was administered with 0.5 ml of normal saline once daily for 28 days.



Values are expressed as Mean ± SD (n=3) (p<0.05) using One way ANOVA followed by DMRT[a,b]

Figure 14: Effect of plant extract on serum cholesterol and HDL of control and experimental rats



Values are expressed as Mean ± SD (n=3) (p<0.05) using One way ANOVA followed by DMRT[a,b]

Figure 15: Effect of plant extract on Serum AST & ALT level of control and experimental rats

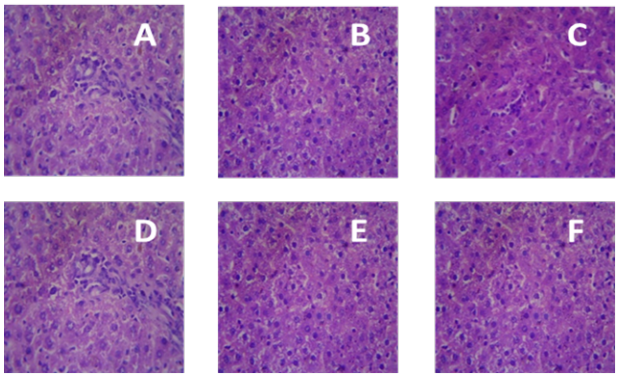


Figure 16: Histopathology of liver. A –Control, B – 100mg/kg BW, C – 200mg/kg BW, D – 300mg/kg BW, E –400mg/kg BW, F– 500mg/kg BW(Magnification 40x)

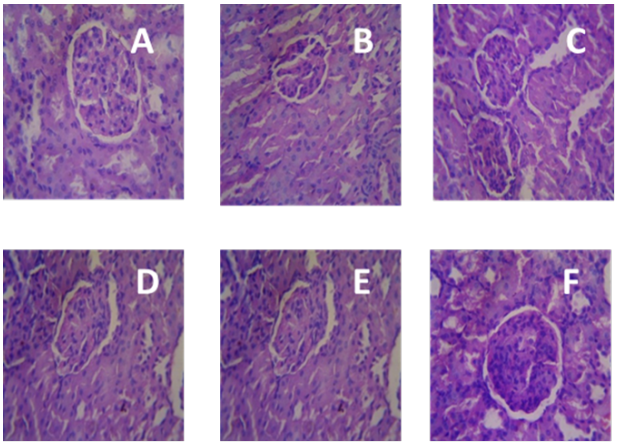


Figure 17: Histopathology of kidney. A –Control, B – 100mg/kg BW, C – 200mg/kg BW, D – 300mg/kg BW, E –400mg/kg BW, F– 500mg/kg BW(Magnification 40x)

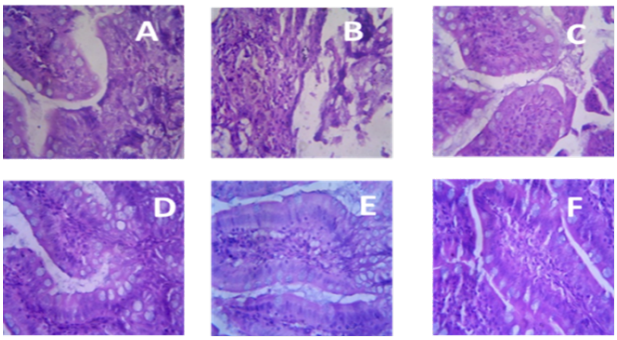


Figure 18: Histopathology of colon. A –Control, B – 100mg/kg BW, C – 200mg/kg BW, D – 300mg/kg BW, E –400mg/kg BW, F– 500mg/kg BW(Magnification 40x)

Rats in groups II, III, IV, V and VI were orally gavaged with 100, 200, 300, 400 and 500 mg/kg body weight of the ethanolic extract of *Sida acuta* respectively once daily for 28 days. The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period. Then the rats were euthanised on 29^{th} day, by keeping overnight fasting for 12 hours prior to euthanasia. Blood samples were collected by cardiac puncture into EDTA and non EDTA coated tubes for haematological and biochemical investigations, respectively. Liver, colon and kidneys were fixed in 10% formalin for histopathological examination.

Biochemical estimation

Blood samples were collected in non-anticoagulant tubes and centrifuged at 3000 rpm. The serum was separated and used for quantification. Commercially available kits from (Agappe Diagnostics) were used for the biochemical assays such as glucose, protein, urea, uric acid creatinine, cholesterol, HDL, AST and ALT. The haematology autoanalyser determined hematologic parameters like red and white blood cells, haemoglobin, MCV, MCH, MCHC, platelets, lymphocytes, monocytes, eosinophils, neutrophils and basophils.

Histopathology

After euthanasia, liver, kidney and colons were poised for the histology study. All the organs were secured in 10% formalin, fixed in paraffin, divided approximately four μm width and marked with H&E (hematoxylin and eosin) for examination under light microscopy.

Statistical analysis

Results expressed as Mean $\pm$ SD were evaluated by one way ANOVA, followed by DMRT comparison(5% level of significance) using SPSS.

RESULTS AND DISCUSSION

Acute Toxicity Study

Behavioural and body weight changes

The effect of ethanolic extract of *Sida acuta* Burm.f leaves on the bodyweight of rats is shown in Figure 1. The acute toxicity analysis did not show any signs and symptoms up to 2000 mg/kg BW. No morbidity or mortality was observed in the treated groups in all doses during the experimental period. The body weights were measured on 1^{st}, 7^{th} and 14^{th} day. As per observation, an initial gradual decrease in body weight in all groups were observed on 7^{th} day and increased to normal on 14^{th} day. So it can be assumed that the LD_{50} of the extract could be greater than 2000 mg/kg body weight.

Effect of ethanolic extract of *Sida acuta* on serum biochemical parameters

In the present study, biochemical parameters urea, uric acid and creatinine in the treated groups 250mg/kg BW(Urea), 100 & 500mg/kg BW(Uric acid) and 250 & 500mg/kg BW(creatinine) shows statistically significant variation from control groups and the remaining groups are similar to that of control(Figure 2). Even though there is significant difference in groups (250mg/kg BW(Urea), 100 & 500mg/kg BW(Uric acid) and 250 & 500mg/kg BW(creatinine), the values are pertaining to the normal range of experimental animals. So it can be considered as safe as for as kidney markers as considered.

Figure 3 shows that there is a statistically significant change in blood glucose level in 500mg/kg BW treated rats, and the remaining treated groups are similar to that of the healthy control rats. As the statistically significant difference comes under the normal range and hence cannot be treated as toxicity.

In the experimental study, the level of protein in treated groups (500 mg/kg BW & 2000 mg/kg BW) shows a slight deviation from control rats (Figure 4). The slight elevation of protein level pertains within the normal range. So it can be suggested that there are no signs of toxicity.

Figure 5 shows that there are statistically significant changes in AST and ALT levels in treated groups compared with the control rats. Though there is a variation, the alterations are within normal range and hence cannot be counted as an implication of toxicity. In the present study, the level of cholesterol and HDL in the treated doses 250 & 500 mg/kg BW (cholesterol) and 1000 mg/kg BW (HDL) shows statistically significant variation when compared to the control rats and other remaining treated groups are showing typical values as that of control rats (Figure 6). The slight elevation of HDL and cholesterol within the normal range. So it may not be considered as a sign of toxicity.

Histopathological examination

Microscopic examination of liver, kidney and colon displayed in Figures 7, 8 and 9 showed no histopathological changes in both control and the treated groups. Thus the histopathological examination supports the protective nature of *Sida acuta* on the liver. Study of morphological and anatomical features of kidney tissues of the rats showed the standard architecture of the kidney with glomeruli and tubules signifying that the drug is devoid of nephrotoxic effect. All the control and treated animals showed the standard architecture of the colon.

Sub chronic toxicity study

Bodyweight changes

Neither death, no toxicity signs were observed on animals following 28 days of treatment with various doses of *Sida acuta* (100 -500mg/kg BW). From the $0^{th,}$ 14^{th} and 28^{th} day of treatment, a gradual increase in body weight gain was noted in all experimental animals, except control group (Figure 10).

Haematological Parameters

The effect of the ethanolic extract of *Sida acuta* on haematological indices was examined at the end of treatment (Table 1). Results showed no significant variance on haematological parameters of experimental groups compared to control. Indicates that the plant extract is devoid of toxic substance that can cause anaemia or other defects.

Effect of ethanolic extract of *Sida acuta* on serum biochemical parameters

Results of the biochemical parameters (urea, uric acid and creatinine) are shown in Figure 11. The level of uric acid in the following groups 100, 300 and 500 mg/kg BW exhibited a significant increase from control and 200 & 400 mg/kg BW found to be statistically similar to that of control indicating the efficacy of respective doses. The statistical variation mentioned above falls in the normal range and hence no evidence of toxicity.

In the present sub chronic toxicity study, statistical change in blood glucose level in treated doses 300 & 500 mg/kg BW from control rats and doses 200 &400mg/kg BW shows no statistically significant variation with control rats (Figure 12). The slight elevation level of experimental groups is pertaining within the normal range. So we can consider it as no evidence of toxicity.

In the present study, the level of protein is slightly increased in 300 mg/kg BW treated rats, and all the other groups show normal range as that of control (Figure 13). The slight elevation is also within the normal range we can consider it as devoid of toxicity as far as protein is considered.

In the level of cholesterol and HDL in the treated doses 200 & 400mg/kgbw (cholesterol) and 300 & 500mg/kgbw(HDL) shows statistically significant variation when compared to the control rats and other remaining plant treated groups are no statistically significant variation from control rats (Figure 14). The slight elevation of HDL and cholesterol are within the normal range. So it cannot be considered as a sign of toxicity.

Figure 15 shows that there is a statistically significant change in AST (300 & 500mg/kg BW) and ALT (200 mg/kg BW) levels in treated groups compared with the control rats and the remaining plant extract groups show statistically no significant variation from control rats. But the alterations are within the control range and hence cannot be counted as an implication of toxicity.

Histopathology of liver, kidney and colon

Histopathological examination of the liver, kidney and colon sections showed normal histology of these organs at all treatment doses compared to the control group. No toxic effects on the histological appearances of the liver, kidney and colon of the treated rats (Figures 16, 17 and 18) were noticed.

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells (Das *et al.*, 2015; Vahalia and Sangle, 2011). Herbal constituent based drugs or herbal formulations, in general, have low toxicity index compared to chemical drugs. Toxicological evaluation is a preliminary step in pharmacol-

ogy to evaluate whether the respective plant has any altered modulations of biochemical and metabolic interactions. For acute toxicity study, animals did not exhibit any signs of toxicity for doses up to 2000 mg/kg BW in single-dose administration. No mortality and no changes in behavioural parameters were observed. Bodyweight parameters also fall in the normal range without any expression of toxicity.

Acute toxicity aimed to assess whether the respective extract is toxic in dangerous doses. From the study, it can be concluded that *S.acuta* ethanolic extract can be safely administrated orally up to 2000mg/kg BW. Sub chronic evaluation is significant because, in most of the diseases, there is a requirement for repeated administration of medications. Hence it is relevant to validate whether the plant sample has any toxicity in repeated dosage formulations in *in vivo* models. Sub chronic toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rats. This test illuminates whether the repeated dosage implementation provides any changes in biochemical and haematological parameters and any histological alterations in target vital organs. The results are convincing in having no observable toxicity in any of the parameters analysed.

In toxicological evaluation, biochemical parameters have significant roles as a marker because of their immediate response to toxicants and associated clinical signs and symptoms. The results on biochemical parameters have shown a statistically significant difference in relevant parameters such as glucose, AST, ALT, Urea, Uric acid, creatinine, cholesterol, HDL, and total protein in experimental groups compared to control rats. But observed variations of various parameters come under the normal range so it can be inferred that plant extract has got no toxic effect on serum biochemical parameters. Serum transaminases are relevant markers of hepatic toxicity because of their immediate response to hepatic infection or injury. In the present analysis, enzymatic markers don't show any alterations in the toxic range (Priyanga *et al.*, 2017).

An alteration in haematological parameters in treated rats is an indication of anaemia and erythropoiesis. In the present study, the haematological parameters are about the normal range is an indication of the safety nature of the plant extract. Bodyweight change is an essential index for assessment of toxicity as normal growth directly correlates with normal metabolism (Ghosh *et al.*, 2008).

In the present observation, body weight changes don't indicate any sign of toxicity. In the present study, all biochemical parameters remain relatively constant changes. Measurement of blood serum urea has been used for many years as an indicator of kidney marker (Burtis *et al.*, 2012; Féres *et al.*, 2006).

In the present study, the mean values of urea, uric acid and creatinine are in the same range as that of control groups. AST and ALT are found primarily in the liver and is the most sensitive marker for liver cell damage. In this study, the mean values of AST and ALT also fall in the normal range as that of control. Histological examination of treated and control rats showed that ethanolic extract *of S.acuta* does not cause any toxic and noted histopathological changes on kidney, liver and colon. Based on the results of the present study, it can be concluded that leaves of *S.acuta* ethanolic extract is being non-toxic for internal administration in animal models and could be well used for pharmacological and therapeutic purposes. Based on the sub chronic toxicity evaluation the lowest effective dose of 200mg/kg BW and a comparatively higher dose of 400mg/kg BW was determined for further in *vivo* anticancer study of *Sida acuta* burm,f ethanolic leaf extract in Wistar albino rats.

CONCLUSION

The acute toxicity study of the ethanolic extract of the *s.acuta* leaves did not produce adverse effects on behaviour changes and gross pathology of the rats up to 2000mg/kg BW. Hence lethal dose for *s.acuta* is above 2000mg/kg BW. Sub chronic toxicity study of the ethanolic extract of the *s.acuta* leaves did not have any adverse effect on body weight, biochemical and hematological parameters at tested doses. Kidney, liver and colon tissues exhibit no sign of toxicity. From sub chronic toxicity study, the doses 200 and 400mg/kg BW as lowest and highest effective dose respectively was standardised for further research in animal models using the individual plant extract.

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Conflict Of Interest

The authors declare no conflict of interest

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