



Acute and sub acute toxicological assessment of the ethanolic root extract of *Saccharum spontaneum* Linn. (Poaceae) in male wistar albino rats

M. Sathya and R. Kokilavani

Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore-29

ABSTRACT

The present investigation was intended to evaluate the toxicity of the ethanolic root extract of a traditionally used plant *Saccharum spontaneum* Linn. The acute toxicity studies was done on male wistar albino rats which showed no clinical signs, no mortality of the rats even under higher dosages levels (50, 150, 300, 500, 1000, 2000mg/kg b.wt) indicating the high margin of safety of the plant extract. The sub acute toxicity study was done to find out the effective dosage of the plant in rats. The varying doses(100,200,300,400, and 500mg/kg b.wt) of the plant extract were administered orally to different groups of male wistar strain of albino rats on daily basis and sacrificed after 28 days of administration. The administration of plant extract produced no significant change in organ weight and hamaetological parameters like hemoglobin, RBC, Hb, WBC, MCV, MCH, MCHC, PCV, platelet, neutrophil and lymphocyte. The record of biochemical parameters like ACP, ALP, AST, ALT, LDH, and NAG in treatment groups of rats were non significant in comparison with control group of rats. The parameters remained within the normal range. Physical, hematological parameters as well as biochemical were unaltered throughout the study. The results of study have suggested there was no obvious toxicity observed with the treatment of *S.spontaneum* It was found to be safe alternative for various severe infections.

Keywords: *Saccharum spontaneum*, Roots, Acute toxicity, sub- acute toxicity, Hamaetological parameters

INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern medicines in a number of studies. In developing countries, all over the world, 80% of population continues to use traditional medicine for primary medical problems [1].Herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based on long-term clinical experience. Despite the usage of plants in folk medicine over ages, only lately has pharmacology and toxicity of these plants begun to receive attention from scientists. With the upsurge in the use of herbal remedies in the last two decades, there is need for a thorough scientific evaluation of these medicinal plants [2].

Research carried out in last few decades has validated several such claims of use of traditional medicinal plants. Human beings has recognized the need for better control of the present use and the future development of chemicals which should chemically tested and retested before researching of chemicals primary and cumulative toxicity and its mutagenic, teratogenic and carcinogenic potential which can be obtained from animal studies

Experimental screening method is an imperative method, in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products [3]. The purpose of toxicity testing is to provide adequate database to make decision concerning the toxicologic properties of chemicals and commercial products and to decide whether a drug or chemical will be safe or not. Hence to validate their claimed pharmacological properties and investigate their possible toxicity, preclinical toxicity studies were carried out on the ethanolic root extract of *Saccharum spontaneum* in rat models.

EXPERIMENTAL SECTION

Collection of the plant material

Saccharum spontaneum Linn. was collected from Koorappalayam, Erode district, Tamil Nadu, India during the month of September to November, 2008. The plant was identified and authenticated by taxonomist Dr.K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Preparation of the ethanolic root extract for *in vivo* studies

Roots of the plants were washed, shade dried, powdered and stored in tight containers under refrigeration. 100g of *S.spontaneum* root powder was taken in a conical flask. To this 500ml of 99% ethanol was added. The content of the flask was kept in the shaker for 48 hr. and the suspension was filtered and residue was resuspended in an equal volume of 99% ethanol for 48hr. and filtered again. The two filtrates were pooled and the solvents were dried in an oven at 37°C and a crude residue was obtained. The yield was 21.8 g, and the residue was suspended in water and administered orally to the experimental rats.

Selection of animals for *In vivo* studies

For the purpose of sub acute toxicity and urolithiatic studies, adult male wistar albino rats weighing about 150 to 200 g were collected from animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The ethical committee permission license number is 659/02/a/CPCSEA. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hr. light and dark cycle at 28°C ± 2° C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC). Paddy husk was used as bedding material and changed twice a week.

Acute toxicity studies of the ethanolic plant extract

Thirty six male wistar albino rats weighing 150-200g were used for the acute toxicity study. They were randomly distributed into one control group and five treated groups, containing six animals per group and were on standard normal diet provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory condition before the experiment. The treated group received orally varying doses (50, 150, 300, 500, 1000, 2000mg/kg b.wt) at a rate of 1.0ml /rat/day to different sets of animals for 14days. Animals treated with 5% acacia served as control. They were continuously observed for 4hr. to detect any changes in autonomic or behavioral responses. viz alterness, spontaneous activity, irritability, corneal reflex, urination and salivation. Any mortality during the experimentation period of 14 days was also recorded. The percentage in mortality in each group was noted.

Sub acute toxicity studies of the root extract *Saccharum spontaneum* L.

a. Experimental setup

To find out the effective dosage of *S.spontaneum*, sub acute toxicity studies were carried out by the method Biswas (1998). The residue was suspended in water administered orally at varying doses (100,200,300,400, and 500mg/kg b.w.) at a rate of 1.0ml /rat/day to different sets of animals for 28 days as follows,

Group I : Control rats

Group II : Plant extract treated rats (100mg/kg b.wt)

Group III: Plant extract treated rats (200 mg/kg b.wt)

Group IV: Plant extract treated rats (300mg/kg b.wt)

Group V : Plant extract treated rats (400 mg/kg b.wt)

Group VI: Plant extract treated rats (500 mg/kg b.wt)

Mortality and clinical signs

During the four-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to 4 hr. after dosing[4].

On 29th day, the animals were anaesthetized with light chloroform anesthesia, blood was collected by Sino – orbital puncture and centrifuged for 30 min. at 2000rpm to separate serum for biochemical analysis. The liver and kidney were excised immediately and thoroughly washed in ice cold saline and weights were recorded.

Relative organ weight

On 29th day, all the animals were by anaesthetized under light chloroform anesthesia. Heart, liver, lungs, spleen, and kidneys were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows,

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Bodyweight of rat on sacrifice day (g)}} \times 100$$

Hematological assay

On the 29th day the blood samples were collected from external jugular vein under mild chloroform anaesthesia for the estimation of hematological parameters like hemoglobin concentration Hb, RBC, WBC, MCV, MCH, MCHC, PCV, platelets and differential counts were performed. Blood samples were collected in 10% EDTA/ saline of pH 7.2.

Collection of serum sample

After the experimental regimen, the animals were sacrificed by cervical decapitation under chloroform anesthesia. Blood sample of each animal was collected separately and centrifuged for 10 min. at 2500 rpm. The serum supernatant was collected and then diluted in the ratio of 1:10 with saline. Aliquots of the diluted serum were then used for the determination of serum constituents and serum enzymic activities.

Biochemical parameters assayed for sub acute toxicity studies

Biochemical parameters such as SGOT (Serum glutamate oxalo acetate transaminase), SGPT (Serum glutamate pyruvate transaminase), ACP (Acid phosphatase), ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase), NAG (N- Acetyl glucosamine), & XAO (Xanthine oxidase) in serum.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Statistical analysis

The results of the biochemical estimations were reported as mean \pm SD of six animals in each group. Total variations, present in a set of data were estimated by one way Analysis Of Variance (ANOVA) followed by the analysis of level of significance between different groups based on ANOVA using SPSS statistical package (Version 15.0). Difference among means were analysed by least significant difference (LSD) at 5% level ($p < 0.05$).

RESULTS AND DISCUSSION**Preliminary animal studies****Acute toxicity studies of ethanolic root extract**

In the acute toxicity study, the rats were treated with different concentration of *S.spontaneum* root extract in the range of 50, 150, 300, 500, 1000, 2000mg/kg b.wt which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. During acute toxicity evaluation, none of the animals died at the dose administered indicating that the LD₅₀ of *S.spontaneum* is 2000mg/kg b.wt. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

Sub acute toxicity studies of the ethanolic root extract of the *Saccharum spontaneum* Linn.

The oral administration of ethanolic root extract of *S.spontaneum* caused no noticeable change in the general behavior of the rats. An increase in kidney weight indicates nephrotoxicity The *Saccharum spontaneum* did not

induce any adverse effect on kidney and the other organs such as liver, heart, brain and spleen, since absolute and relative weight of the organs were not significantly different from control value (Table 1). All the values remained within normal limits throughout the experimental period. An increase in kidney weight indicates nephrotoxicity [5]. The *Saccharum spontaneum* did not induce any adverse effect on kidney and the other organs such as liver, heart, brain and spleen, since absolute and relative weight of the organs were not significantly different from control value (table 1). All the values remained within normal limits throughout the experimental period.

Our results agrees well with that of Koshy et al. (2011). who reported that *Elytraria acaulis* did not induce any adverse effect on kidney and the other organs such as Liver Heart, Lung, Spleen and Brain, since absolute and relative weight of the organs were not significantly different from control value.

Organ weight changes

Table 1. Effects of *S. spontaneum* root extract on change in organ weight of control and experimental rats (sub acute toxicity studies)

| Organ weight(g) | Dose (mg/kg b.w) / Groups | | | | | |
|-----------------|---------------------------|------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Control /Group I | 100/ Group II | 200/ Group III | 300/ Group IV | 400/ Group V | 500/ Group VI |
| Heart | 1.25 ± 0.02 | 1.24 ± 0.01 a ^{ns} | 1.26 ± 0.02 b ^{ns} | 1.25 ± 0.02 c ^{ns} | 1.23 ± 0.29 d ^{ns} | 1.28 ± 0.01 e ^{ns} |
| Kidney | 1.34 ± 0.02 | 1.36 ± 0.055 a ^{ns} | 1.35 ± 0.04 b ^{ns} | 1.36 ± 0.04 c ^{ns} | 1.39 ± 0.02 d ^{ns} | 1.41 ± 0.03 e ^{ns} |
| Liver | 6.73 ± 0.02 | 6.72 ± 0.08 a ^{ns} | 6.73 ± 0.07 b ^{ns} | 6.74 ± 0.06 c ^{ns} | 6.78 ± 0.08 d ^{ns} | 6.81 ± 0.08 e ^{ns} |
| Spleen | 1.14 ± 0.19 | 1.15 ± 0.15 a ^{ns} | 1.16 ± 0.02 b ^{ns} | 1.17 ± 0.02 c ^{ns} | 1.12 ± 0.01 d ^{ns} | 1.18 ± 0.16 e ^{ns} |
| Brain | 1.54 ± 0.02 | 1.60 ± 0.19 a ^{ns} | 1.54 ± 0.17 b ^{ns} | 1.55 ± 0.02 c ^{ns} | 1.59 ± 0.01 d ^{ns} | 1.61 ± 0.02 e ^{ns} |

Values are expressed as mean ± SD of six animals

a - Group II is compared with group I b - Group III is compared with group I

c - Group IV is compared with group I d - Group V is compared with group I

e - Group VI is compared with group I ns - non-significant at 5% level * - p < 0.05

Table 2. Haematological values of the rats treated with ethanolic *S. spontaneum* root extract (sub acute toxicity studies)

| Haematological parameters | Dose (mg/kg b.w) / Groups | | | | | |
|-----------------------------|---------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| | Control / Group I | 100/ Group II | 200/ Group III | 300/ Group IV | 400/ Group V | 500/ Group VI |
| RBC (x 10 ¹² /l) | 9.23 ± 0.01 | 9.24 ± 0.12 a ^{ns} | 9.25 ± 0.015 b ^{ns} | 9.26 ± 0.14 c ^{ns} | 9.27 ± 0.01 d ^{ns} | 9.29 ± 0.08 e ^{ns} |
| Hb(g/dl) | 13.53 ± 0.01 | 13.52 ± 0.01 a ^{ns} | 13.54 ± 0.10 b ^{ns} | 13.55 ± 0.19 c ^{ns} | 13.58 ± 0.18 d ^{ns} | 13.61 ± 0.25 e ^{ns} |
| WBC(10 ⁹ /l) | 5.82 ± 0.01 | 5.83 ± 0.01 a ^{ns} | 5.84 ± 0.02 b ^{ns} | 5.85 ± 0.17 c ^{ns} | 5.86 ± 0.12 d ^{ns} | 5.89 ± 0.07 e ^{ns} |
| MCV(μm ³) | 57.77 ± 0.01 | 57.75 ± 0.15 a ^{ns} | 57.78 ± 0.17 b ^{ns} | 57.77 ± 0.01 c ^{ns} | 57.81 ± 0.14 d ^{ns} | 57.84 ± 0.02 e ^{ns} |
| MCH(pg) | 19.63 ± 0.01 | 19.64 ± 0.15 a ^{ns} | 19.64 ± 0.16 b ^{ns} | 19.65 ± 0.13 c ^{ns} | 19.68 ± 0.13 d ^{ns} | 19.71 ± 0.12 e ^{ns} |

Values are expressed as mean ± SD of six animals

The comparison between groups and the statistical significance are as in table 1.

Table 3. Effects of ethanolic extract on haematological parameters of the control and experimental rats (sub acute toxicity studies)

| Haematological parameters | Dose (mg/kg b.w) / Groups | | | | | |
|------------------------------|---------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control / Group I | 100/ Group II | 200/ Group III | 300/ Group IV | 400/ Group V | 500/ Group VI |
| MCHC(g/dl) | 32.63 ± 0.01 | 32.69 ± 0.01 a ^{ns} | 32.65 ± 0.17 b ^{ns} | 32.60 ± 0.12 c ^{ns} | 32.69 ± 0.14 d ^{ns} | 32.72 ± 0.11 e ^{ns} |
| PCV (%) | 42.44 ± 0.20 | 42.45 ± 0.10 a ^{ns} | 42.46 ± 0.12 b ^{ns} | 42.47 ± 0.18 c ^{ns} | 42.48 ± 0.21 d ^{ns} | 42.51 ± 0.01 e ^{ns} |
| Platelet(10 ⁹ /l) | 716.73 ± 0.18 | 716.79 ± 0.01 a ^{ns} | 716.75 ± 0.01 b ^{ns} | 716.74 ± 0.22 c ^{ns} | 716.76 ± 0.22 d ^{ns} | 716.62 ± 0.30 e ^{ns} |
| Neutro (%) | 53.54 ± 0.02 | 53.67 ± 0.03 a ^{ns} | 53.57 ± 0.29 b ^{ns} | 53.58 ± 0.03 c ^{ns} | 53.56 ± 0.17 d ^{ns} | 53.64 ± 0.54 e ^{ns} |
| Lympho (%) | 47.53 ± 0.02 | 47.54 ± 0.01 a ^{ns} | 47.55 ± 0.01 b ^{ns} | 47.54 ± 0.14 c ^{ns} | 47.59 ± 0.02 d ^{ns} | 47.61 ± 0.26 e ^{ns} |

Values are expressed as mean ± SD of six animals

The comparison between groups and the statistical significance are as in table 1.

Table 4. Effects of *S.spontaneum* root extract on serum biochemical markers in control and experimental rats (sub acute toxicity studies)

| Biochemical parameters | Dose (mg/kg b.w) / Groups | | | | | |
|------------------------|---------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control / Group I | 100/ Group II | 200/ Group III | 300/ Group IV | 400/ Group V | 500/ Group VI |
| ACP ^s | 69.30 ± 0.67 | 69.36 ± 0.32 a ^{ns} | 69.31 ± 0.16 b ^{ns} | 69.32 ± 0.04 c ^{ns} | 69.65 ± 0.03 d ^{ns} | 69.66 ± 0.03 e ^{ns} |
| ALP ^s | 77.53 ± 0.01 | 77.62 ± 0.01 a ^{ns} | 77.56 ± 0.02 b ^{ns} | 77.58 ± 0.03 c ^{ns} | 77.64 ± 0.03 d ^{ns} | 77.69 ± 0.04 e ^{ns} |
| AST [#] | 35.98 ± 0.69 | 36.43 ± 0.66 a ^{ns} | 36.02 ± 0.38 b ^{ns} | 36.09 ± 0.17 c ^{ns} | 36.60 ± 0.30 d ^{ns} | 36.71 ± 0.02 e ^{ns} |
| ALT [#] | 40.16 ± 0.01 | 40.13 ± 0.36 a ^{ns} | 40.14 ± 0.21 b ^{ns} | 40.16 ± 0.04 c ^{ns} | 40.21 ± 0.03 d ^{ns} | 40.25 ± 0.02 e ^{ns} |
| LDH [#] | 112.62 ± 0.19 | 112.73 ± 0.32 a ^{ns} | 112.67 ± 0.12 b ^{ns} | 112.63 ± 0.14 c ^{ns} | 112.58 ± 0.15 d ^{ns} | 112.83 ± 0.12 e ^{ns} |
| NAG | 15.52 ± 0.17 | 15.73 ± 0.19 a ^{ns} | 15.60 ± 0.17 b ^{ns} | 15.58 ± 0.18 c ^{ns} | 15.53 ± 0.12 d ^{ns} | 15.62 ± 0.17 e ^{ns} |

Values are expressed as mean ± SD of six animals

The comparison between groups and the statistical significance are as in table 1.

Effect ethanolic root extract of *S.spontaneum* on hematological parameters of rats

Study of haematological status is one of the important ways for the diagnoses of root cause of diseases. Haematological disorders include a wide range of abnormal conditions indicating the profile of blood parameters, due to changes in metabolism. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals. The hematological analysis (Tables 2 and 3) showed no significant differences in any of the parameters examined in either the control or treated groups of animals. All the values remained within normal limits throughout the experimental period.

Our results are in accordance with that of Kripa *et al.* (2011). Who showed that hematological changes such as anemia are often accompaniments of bone marrow toxicity and analysis of blood parameters with respect to animal studies have a high relevance and predictive value for humans (Rhiouani, *et al.*, 2008). In addition, all of the changes were still within the normal limits.

Our findings are in accordance with the studies of Koshy *et al.* (2011). who reported that there is no significant changes in haematological parameters like Haemoglobin, RBC, WBC, ESR, Platelets, Clotting time and PCV in the extract treated animals compare with control which indicates that there is no lyses of blood cells and inhibition in blood cell thesis by the active constituent of *Elytraria acaulis* extract.

Effects of ethanolic root extract *S.spontaneum* Linn on biochemical parameters

The biochemical evaluation is important since there are several reports of liver and kidney toxicity related to the use of phytotherapeutic products [9,10,11]. In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites [12, 13]. Results of biochemical studies showed that there was no significant increase in the levels of the parameters at different doses ACP, ALP, AST, ALT LDH and NAG in the different groups of animals treated with (100,200,300,400,500mg/kg b.wt) of the extract compared with control. This implies that the extract at the doses tested had no effects on the liver and kidney tissues (Table 4).

Our findings are in accordance with the studies of Koshy *et al.* (2011). who reported that there are no significant changes in biochemical parameters like SGOT, SGPT and ALP reflects the structural and functional dysfunction of hepatocellular membrane or cell rupture, and thereby indicates liver damage. The normal value of the hepatic biochemical parameters reveals the safety profile of the extract on liver function even on its use.

CONCLUSION

Since, there were no significant adverse effects on the hematological and biochemical parameters it may be concluded that the ethanolic extract of *Saccharum spontaneum* did not induce any note worthy damage to the vital organs. In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the ethanolic extract of *Saccharum spontaneum* may be considered as relatively safe, as it did not cause either mortality or produce severe toxicological effects on selected body organs, biochemical indices and hematological markers of rats during the acute and sub-acute periods of study.

Acknowledgement

The authors are thankful to the college management for their guidance, valuable suggestion, encouragement and constant supporting during this investigation.

REFERENCES

- [1] Grover, J.K. and Yadav, S.P. *J Ethanopharmacol.*, **2004**, 93(1): 123-132.
- [2] Kripa, K.G. Chamundeeswari, D. and Thanka, *Int J Drug Res.*, **2011**, 3 (3): 339-347
- [3] Mythilipriya, R., Shanthi, P. and Sachdanandam, P. *J Health sci.*, **2007**, 53 (4): 351-358.
- [4] Al-Mamary, M., Al-Habori., M., Al-Aghbari, A.M. and Baker M.M. *Phytother. Res.*, **2002**, 16:127 – 132.
- [5] Kluwe, W.M., *Toxicol. Appl. Pharmacol.*, **1981**, 57(3): 414-424.
- [6] Kripa, K.G. Chamundeeswari, D. and Thanka, J, *Int. J. Drug Dev. & Res.*, **2011**, 3 (3): 339-347.
- [7] Rhiouani, H., EI-Hilaly, J., Israili, Z.H. and Lyoussi, B., *J. Ehanopharmacol.*, **2008**, 118(3):378-86.
- [8] Koshy IR.K. and Kapoor.R. and , Azmathulla, M. *Pharmacologyonline* **2011**, 3: 229-242
- [9] Obici, S., Otobone, F.J., Da Silva Sela, V.R, Ishida, K., Da Silva, J.C. and Nakamura,C.V., *J Ethanopharmacol.*, **2008**, 115(1) : 131-9.
- [10] Rhiouani H, EI-Hilaly J, Israili ZH, Lyoussi B., *J Ethanopharmacol.*, **2008**; 118(3):378-86.
- [11] Corns CM., *Ann clin Biochem.* **2003**, 40 (Pt5): 489-507
- [12] Greaves P. *Histopathology preclinical toxicity studies: Interpretatio and Revelance in Drug safety Evaluation.* 3rd ed. Newnork: Academic press; **2007**.
- [13] Schreiner GE, Maher JF., *Am J Med.* **1965**, 38: 409-49