Available on line at : www.eijppr.com

International Journal of Pharmaceutical and Phytopharmacological Research

(ICV-5.09)

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2012, 2(2): 71-74

(Research Article)

Acute and Subacute Toxicity Evaluation of Ethanolic Root Extract of *Saccharum spontaneum* Linn. (Poaceae) in Experimental Rats

M. Sathya[1] and R. Kokilavani[2]

[1]M.Sathya, Research scholar, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore-29, India
[2]Dr.R.Kokilavani, Associate Professor and Head, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore-29, India

Received on: 18/09/2012 ***Accepted on:*** 10/10/2012

ABSTRACT

The present investigation was intended to evaluate the toxicity of the ethanolic root extract of a traditionally used plant Saccharum spontaneum Linn (S.spontaneum). 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. There was no change in the body weight and no mortality was found in the experimental animals during the study period. The record of biochemical parameters like calcium, oxalate, phosphorus, magnesium protein, urea, uric acid, creatinine, sodium, potassium and chloride in treatment groups of rats were non significant in comparison with control group of rats. The parameters remained within the normal range. 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.

Key Words: *Saccharum spontaneum*, Roots, Acute toxicity, Sub- acute toxicity, Calcium oxalate.

INTRODUCTION

Saccharum spontaneum L. known as Kasa (Family: Poaceae) is a traditional herb, it has excellence medicinal value; has been advocated in the treatment gynaecological troubles, respiratory disease. Roots are used as galactagogue and diuretic and in ayurveda system roots are also used as astringent, emollient, refrigerant, diuretic, purgative, tonic, and aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness (Khalid and Siddiqui, 2011)[1]. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation strongly, renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility (Suresh kumar *et al*., 2009)[2].

Toxicology is defined as any harmful effect of a chemical or drug on a target organism. Acute and sub acute toxicity has been defined by various experts. Toxicity can be acute, sub chronic, or chronic. Acute toxicity involves harmful effects in an organism through a single or short- term exposure. Sub acute toxicity is the ability of a toxic substance to cause effects for more than one year but less than the life time of the exposed organism. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effect over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism (Fauci and Anthony, 2008)[3].

The purpose of the study was to look at the toxicity profile of the *Saccharum spontaneum*. A 28 days study is considered as sub acute toxicity study, which is well accepted for eliciting any toxicity on long term feeding. It gives valuable information on the cumulative toxicity of a substance on the target organ or prolonged exposure. A wide verity of adverse effects can be detected from sub acute toxicity studies. The results from such studies can provide information, which will aid in selecting dose level.

MATERIALS AND METHODS

Chemicals Used

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

Collection of the Plant Material

Saccharum spontaneum Linn. was collected from Koorappalayam, Erode district, Tamil Nadu, India during the month of September to November, 2011. The plant was identified and authenticated by taxonomist Dr.K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore,

Tamilnadu, India. Voucher specimen was deposited in herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

Preparation of the Ethanolic Root Extract

Roots of the plants were washed, shade dried, powdered and stored in tight containers under refrigeration. 100g of *S.spontaneum* 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 37ºC 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 the purpose of sub acute toxicity 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 $28^oC \pm 2^o$ 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 beding material and changed twice a week.

Acute Toxicity Studies

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

Experimental setup

To find out the effective dosage of *S.spontaneum*, sub actute 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(Al-Mamary *et al*.,2002)[4].

On 29^{th} 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 was excised immediately and thoroughly washed in ice cold saline and weights were recorded.

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.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages, urine was collected for 24 hr. and freed from faecal contamination. Rats were provided with water but no feed. Urine was collected in 50 ml beaker maintained at 0^oC in an ice bath. The collected urine samples were centrifuged at 3000rpm for 10 min. and any sediment present was discarded. It was used for further analysis.

Biochemical parameters assayed for sub acute toxicity studies

Biochemical parameters such as calcium, oxalate, phosphate, magnesium, protein, urea, uric acid, creatinine, sodium, potassium, chloride and glucose in serum and urine were assayed.

Statistical analysis

The results of the biochemical estimations were reported as mean ± 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

The oral administration of ethanolic root extract of *S.spontaneum* caused no noticeable change in the general behavior of the rats. All rats showed significant increase in body weight compared to their initial values. However there was no significant difference between the different treatment groups and the control, indicating that it did not have any adverse effects on the body weight, which is used to assess the response to the therapy of the drug. Both the control and treated groups appeared relatively healthy during the period of study. There were no deaths reported in any of the groups (table-1).

Tables 2, 3, 4 and 5 shows the effect of the plant extract on calcium, oxalate, phosphorus, magnesium protein, urea, uric acid, creatinine, sodium, potassium and chloride in serum and urine of control and experimental rats respectively. This result showed that the plant extract at different levels tested did not produce considerable change in the levels of the different parameters tested.

In the present study, the insignificant difference in urea and creatinine levels between the treated groups and the control group probably indicate that the extract did not interfere with the renal capacity to excrete the metabolite. Indeed, creatinine is known as a good indicator of renal function. Any rise in creatinine levels is only observed if there is marked damage to functional nephrons Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Mukinda and Eagles, 2010)[5].

At the end of 28 days, the animals of both control and experimental groups were sacrificed and liver was isolated and a histopathological experiment was done. Histological observations (Fig.1) correlate the other results showing the normal cellular architectures in the treated group of animals, without any necrosis or fatty infiltration, which can substantiate the safety profile of the extract clearly.

Table 1: Changes in body weight of rats during the treatment with different doses of *Saccharum spontaneum* Linn

Dose mg/kg b.w	Body weight (g)				
	0th day	**7th day**	**14th day**	**21st day**	**28th day**
Control (Group I)	170.94 ± 0.18	173.97 ± 0.02	172.55 ± 0.02	175.59 ± 0.01	174.34 ± 0.02
100 (Group II)	171.12 ± 0.02 a^{ns}	172.89 ± 0.01 a^{ns}	172.45 ± 0.11 a^{ns}	174.54 ± 0.02 a^{ns}	175.74 ± 0.40 a^{ns}
200 (Group III)	170.94 ± 0.01 b^{ns}	172.96 ± 0.02 b^{ns}	171.62 ± 0.13 b^{ns}	174.96 ± 0.03 b^{ns}	174.58 ± 0.08 b^{ns}
300 (Group IV)	170.81± 3.44 c^{ns}	174.96 ± 0.02 c^{ns}	171.61 ± 0.02 c^{ns}	175.66 ± 0.02 c^{ns}	175.69 ± 0.03 c^{ns}
400 (Group V)	170.87± 0.15 d^{ns}	174.56 ± 0.15 d^{ns}	172.74 ± 0.20 d^{ns}	176.93 ± 0.49 d^{ns}	174.64 ± 0.01 d^{ns}
500 (Group VI)	170.94± 0.01 e^{ns}	172.93 ± 0.01 e^{ns}	173.86 ± 0.02 e^{ns}	175.85 ± 0.05 e^{ns}	175.91 ± 0.01 e^{ns}

Values are expressed as Mean ± SD of six animals

Statistical comparisons

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: Effect of alcoholic root extract of *S.spotaneum* on serum biochemical parameters in control and experimental rats

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**
Calcium (mg/ dl)	8.74 ± 0.01	8.72 ± 0.12 a^{ns}	8.79 ± 0.03 b^{ns}	8.75 ± 0.02 c^{ns}	8.86 ± 0.02 d^{ns}	8.83 ± 0.01 e^{ns}
Oxalate (mg/ dl)	1.77 ± 0.01	1.76 ± 0.02 a^{ns}	1.81 ± 0.02 b^{ns}	1.79 ± 0.03 c^{ns}	1.89 ± 0.02 d^{ns}	1.87 ± 0.02 e^{ns}
Phosphorus (mg/ dl)	5.03 ± 0.18	5.06 ± 0.01 a^{ns}	5.07 ± 0.15 b^{ns}	5.04 ± 0.03 c^{ns}	5.06 ± 0.22 d^{ns}	5.10 ± 0.21 e^{ns}
Magnesium(mg/ dl)	2.94 ± 0.01	2.90 ± 0.03 a^{ns}	2.95 ± 0.03 b^{ns}	2.99 ± 0.03 c^{ns}	2.91 ± 0.03 d^{ns}	3.01 ± 0.04 e^{ns}
Protein(g/ dl)	6.72 ± 0.03	6.81 ± 0.03 a^{ns}	6.79 ± 0.03 b^{ns}	6.78 ± 0.17 c^{ns}	6.84 ± 0.01 d^{ns}	6.86 ± 0.05 e^{ns}

Values are expressed as Mean ± SD of six animals

The comparison between groups and the statistical significance are as in table1.

Table 3: Effect of alcoholic root extract of *S.spotaneum* on serum biochemical parameters in control and experimental rats

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**
Urea(mg/ dl)	10.56 ± 0.02	10.55 ± 0.02 a^{ns}	10.57 ± 0.02 b^{ns}	10.58 ± 0.03 c^{ns}	10.61 ± 0.06 d^{ns}	10.59 ± 0.21 e^{ns}
Uric acid(mg/ dl)	4.73 ± 0.02	4.76 ± 0.12 a^{ns}	4.74 ± 0.02 b^{ns}	4.78 ± 0.26 c^{ns}	4.81 ± 0.01 d^{ns}	4.84 ± 0.19 e^{ns}
Creatinine (mg/ dl)	0.86 ± 0.02	0.85 ± 0.01 a^{ns}	0.88 ± 0.23 b^{ns}	0.87 ± 0.23 c^{ns}	0.91 ± 0.12 d^{ns}	0.94 ± 0.23 e^{ns}
Sodium(mEq/L)	130.73± 0.17	130.97 ± 0.16 a^{ns}	130.67 ± 0.13 b^{ns}	130.70 ± 0.12 c^{ns}	130.82 ± 0.39 d^{ns}	130.98 ± 0.21 e^{ns}
Potassium(mEq/L)	4.18 ± 0.03	4.26 ± 0.02 a^{ns}	4.20 ± 0.12 b^{ns}	4.25 ± 0.02 c^{ns}	4.29 ± 0.02 d^{ns}	4.27 ± 0.02 e^{ns}
Chloride(mEq/L)	100.25± 0.01	100.31 ± 0.02 a^{ns}	100.32 ± 0.23 b^{ns}	100.28 ± 0.21 c^{ns}	100.35 ± 0.25 d^{ns}	100.41 ± 0.05 e^{ns}

Values are expressed as mean ± SD of six animals

The comparison between groups and the statistical significance are as in table1.

Table 4: Effect of alcoholic root extract of *S.spotaneum* on urine biochemical parameters in control and experimental rats

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**
Calcium★	0.80 ± 0.05	0.79 ± 0.004 a^{ns}	0.82 ± 0.06 b^{ns}	0.83 ± 0.21 c^{ns}	0.86 ± 0.21 d^{ns}	0.89 ± 0.24 e^{ns}
Oxalate★	0.58 ± 0.01	0.55 ± 0.03 a^{ns}	0.57 ± 0.01 b^{ns}	0.60 ± 0.02 c^{ns}	0.63 ± 0.06 d^{ns}	0.67 ± 0.21 e^{ns}
Phosphorus★	6.73 ± 0.06	6.69 ± 0.21 a^{ns}	6.72 ± 0.23 b^{ns}	6.74 ± 0.24 c^{ns}	6.82 ± 0.02 d^{ns}	6.87 ± 0.21 e^{ns}
Magnesium★	5.25 ± 0.02	5.18 ± 0.24 a^{ns}	5.23 ± 0.018 b^{ns}	5.26 ± 0.28 c^{ns}	5.19 ± 0.35 d^{ns}	5.27 ± 0.08 e^{ns}
Protein Ψ	1.09 ± 0.01	1.15 ± 0.01 a^{ns}	1.12 ± 0.01 b^{ns}	1.11 ± 0.02 c^{ns}	1.14 ± 0.01 d^{ns}	0.98 ± 0.305 e^{ns}

Values are expressed as Mean ± SD of six animals

The comparison between groups and the statistical significance are as in table1.

Units :★ - mg/24 hour urine, **Ψ -** g/ 24 hour urine

Table 5: Effect of alcoholic root extract of *S.spotaneum* on urine biochemical parameters in control and experimental rats.

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
Urea★	25.27 ± 0.15	25.34 ± 0.12 a^{ns}	25.31 ± 0.15 b^{ns}	25.35 ± 0.12 c^{ns}	25.41 ± 0.12 d^{ns}	25.77 ± 0.11 e^{ns}
Uric acid★	45.36 ± 0.14	45.49 ± 0.18a^{ns}	45.42 ± 0.15 b^{ns}	45.37 ± 0.18c^{ns}	45.54 ± 0.28 d^{ns}	45.87 ± 0.10e^{ns}
Creatinine★	146.40 ± 0.15	146.60 ± 0.22 a^{ns}	146.43 ± 0.18 b^{ns}	146.44 ± 0.10 c^{ns}	146.79 ± 012 d^{ns}	146.86 ± 0.14 e^{ns}
Sodium★★	64.32 ± 0.17	64.34 ± 0.19 a^{ns}	64.35 ± 0.21b^{ns}	64.37 ± 0.16 c^{ns}	64.75 ± 0.19 d^{ns}	64.79 ± 0.14 e^{ns}
Potassium★★	80.24 ± 0.16	80.53 ± 0.17 a^{ns}	80.30 ± 0.12 b^{ns}	80.54 ± 0.16 c^{ns}	80.58 ± 0.23 c^{ns}	80.59 ± 0.12 e^{ns}
Chloride★★	170.51 ± 0.29	170.57 ± 0.22 a^{ns}	170.54 ± 0.23 b^{ns}	170.57 ± 0.19c^{ns}	170.64 ± 0.23 d^{ns}	170.67 ± 0.19 e^{ns}

Values are expressed as mean ± SD of six animals
The comparison between groups and the statistical significance are as in table1.
Units :★- mg/24 hour urine, **★★-** mEq/ 24hour urine

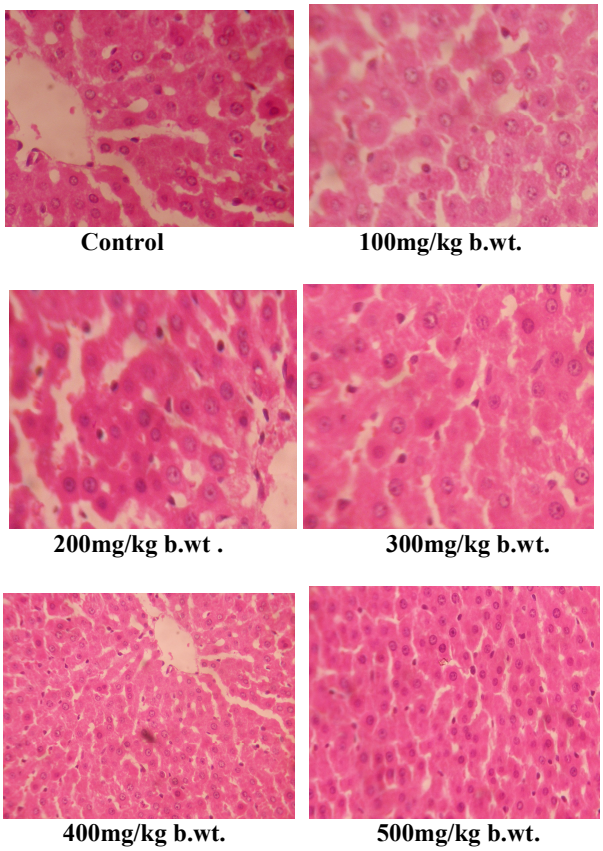

Fig.1: Histopathological investigation of liver- Sub acute toxicity studies

CONCLUSION

Since, there were no significant adverse effects on the biochemical parameters, it may be concluded that the ethanolic extract of *Saccharum spontaneum* did not induce any noteworthy 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 body weight and biochemical parameters indices during the acute and sub-acute periods of study.

REFERENCES

1) Mohammad Khalid, Hefazat H. Siddiqui Pharmacognostical Evaluation and Qualitative Analysis of *Saccharumspontaneum* (L.) Root, *International Journal of Pharmaceutical Sciences and Drug Research*.,2011, 3(4): 338-341.
2) Suresh kumar C.A., Varadharajan R., Muthumani P., Meera R., Devi P. and Kameswari B., Pharmacognostic and Preliminary Phytochemical Investigations on the stem of *Saccharum spontaneum journal of pharmaceutical science and research.*, 2009,1 (3), 2009, 129- 136.
3) Fauci, Anthony S., et al. Harrison's Principles of Internal Medicine. 17th ed. United States: McGraw-Hill Professional. 2008.
4) Al-Mamary, M., Al-Habori., M., Al-Aghbari, A.M. and Baker M.M., Investigation in to the toxicological effects of *Catha edulis* leaves: a short - term study in animals. *Phytotherapy research*.,2002,16:127 – 132.
5) Mukinda, J.T. and Eagles.P.F. Acute and sub-chronic oral toxicity profiles of the aqueous extract of Polygala fruticosa in female mice and rats., *J.ethanopharmacol*., 2010,128(1): 236-240.

Corresponding Author:
M. Sathya,
Research scholar,
Department of Biochemistry,Kongunadu Arts and Science College, Coimbatore-29,India
Email: *sathya_biom84@yahoo.co.in*