ACUTE AND SUB-CHRONIC TOXICITY EFFECT OF *JASMINUM SAMBAC* LINN. OLEACEAE FLOWER IN SWISS ALBINO MICE

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Summary

J.sambac is traditionally used in many countries for the treatment of various diseases and disorders. The aim of the present study was to evaluate the safety of the methanolic extract of *J.sambac* through acute and subchronic toxicity study in mice. For acute toxicity study 500-2000 mg/kg MEJS were administered orally and obvious toxic symptoms and mortality was studied upto 14days. In subchronic study, effect of multiple weekly dosing of 400 mg/kg (one-fifth of the maximum tolerated dose) of MEJS was investigated in mice for six weeks and the evaluation was done by the studies of hematological parameters, biochemical estimations of hepatorenal parameters, antioxidant status, histological observations of the tissue. The extract was found to be well tolerated upto 2g/kg in acute toxicity study. In subchronic toxicity study it showed no significant alteration on any of the parameters, which was evident by the histological studies. Hence the results suggest that methanol extract of *J. sambac* flower is quite safe and can be used in the treatment of the chronic diseases like diabetes without any toxicity.

Keywords: *J. sambac*, acute, subchronic toxicity, hematological parameters, biochemical estimations

Introduction

Medicinal plants play a very significant role in health care needs of rural populations in African and other third world countries especially in treatment of diseases[1]. Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti cancer, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity[2,3]. Most of the diseases which have no medicine in allopathic system can be cured successfully using traditional medicines[4].

Among all *Jasminum sambac*. Linn (Oleaceae) is commonly known as Jasmine. It is a well known glabrous twining shrub widely grown in gardens throughout India. The flower is acrid, bitter with a sharp taste. It is useful in treating diseases of the mouth and teeth, especially for toothache[5]. The *J. sambac* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of *J. sambac* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding[6]. It is widely used in the Ayurveda, as an antiulcerative, anti cancer antileprotic, skin diseases and wound healing. The dried leaves, soaked in water and made into poultice are used in ulcers.

The flowers are said to arrest the secretion of milk in puerperal states in case of threatened abscess. It is also reported to possess angiotensin converting enzyme inhibitory activity[7]. *J.sambac* contains major phytoconstituents as glycosides, saponins, flavonoids and terpenoids. *J. sambac* contains maximum amount of terpenoids out of different Jasminum species[8]. The present study is aimed to evaluate the acute and sub-chronic toxicity effect of *Jasminum sambac* (L.) oleaceae flower in swiss albino mice.

Materials and Methods

Plant material

The flower part of *Jasminum sambac* was collected in December, 2009, Coimbatore district, Tamilnadu, India and identified by the Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore, India. A voucher specimen (No.BSI/SRC/5/23/09-10/Tech-972) was retained in laboratory for further reference.

Preparation of Plant Extract

The *J.sambac* flower were dried and powdered in a mechanical grinder. About 50g of the powdered material were extracted with 250ml of methanol in a water shaker for 72h. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50^{o}C. A dried powered material was obtained and stored at 0-4^{o}C. The yield of the extract material was about 15.14%.

Experimental design

Animals

Healthy Swiss albino mice (20 ± 2 g) were used for the study. The animals were kept in polypropylene cages with sawdust bedding and maintained under standard laboratory conditions. Standard pellet diet (Hindustan Lever, Bangalore) and water were given ad libitum. The mice were acclimatized to laboratory condition for one week before commencement of experiment. This study was approved by the Institutional Animal Ethics Committee (IAEC) constituted for the purpose of CPCSEA, Government of India.

Acute toxicity study

Healthy Swiss albino mice (20 ± 2 g) of either sex, starved overnight, were divided into five groups (n=4). Group I (untreated) served as control, while group II-V animals were orally fed with MEJS in increasing dose levels of 0.5, 1.0, 1.5, 2.0g/kg b.wt for 14days. The animals were observed continuously for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and lethality. One-fifth of the maximum safe dose of the extract tested for acute toxicity was selected for the subchronic toxicity study (Saha et.al 2011). The extract was administered by oral gavage.

Subchronic toxicity study

Sixteen healthy mice were randomly divided into two groups of eight mice in each. Group I served as normal control and Group II animals received MEJS (400mg/kg, p.o., ie., one-fifth of the maximum tolerated dose) for six weeks. During the experimental period, the animals were weighed every three days and food and water intake were monitored daily. At the end of the experiment, after 24h of the last dose and 18h fasting, animals were sacrificed and blood was collected from jugular vein and taken into heparinized tube for hematological studies and non-hepainized centrifuge tube for biochemical estimations. Liver tissue was collected from the animals for the evaluation of *in vivo* antioxidant status and part of the liver tissue was taken for the histological studies.

Hematological studies

RBC, WBC and differential counts using Neubauer hemocytometer and estimation of hemoglobin using Sahli's Hemoglobinometer were carried out by standard procedures from the blood obtained from jugular vein[9,10].

Biochemical estimation

The effect of MEJS treatment on the biochemical parameters of the experimental mice were evaluated by the estimation of serum biochemical enzymes such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities by the method of ALT[11], acid phosphatase (ACP) and alkaline phosphatase (ALP) activities by[12], Phospholipids[13], Free fattyacids[14], total bilirubin[15], total protein[16], urea[17], uric acid[18], creatinine[19], glucose, total cholesterol, triglyceride, HDL and LDL cholesterol[20,21]. All the analysis was performed by standard methods using commercially available kit from Span Diagnostics Ltd.

***In vivo* antioxidant assay**

The antioxidant assay was performed with the liver tissues of the experimental animals and evaluation of the antioxidant status was carried out by measuring the level of lipid peroxidation[22] and the amount of enzymatic (Catalase: CAT) and nonenzymatic antioxidant system (reduced glutathione: GSH) by the methods of[23,24] respectively.

Histological studies

After sacrificing the mice, parts of liver tissues were collected for the histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, and embedded in paraffin, cut into 4-5µm thick sections and stained with hematoxylin-eosin dye for photomicroscopic observation

Statistical Analysis

The values were represented as the mean of six values ± S.D. The results were statistically analyzed using the statistical package (SPSS). One-way analysis of variance was employed for comparison among the six groups followed by LSD. Statistical significance was set at $P < 0.05$.

Results

In acute toxicity study, MEJS did not show any mortality or toxic effect upto the dose of 2g/kg during the observational period of 14days. It did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, and gastrointestinal effects in mice. These results showed that in single dose, there are no adverse effects of MEJS, indicating that the medium lethal dose (LD_{50}) is higher than 2000 mg/kg in mice. Accordingly one-fifth of the maximum tolerated dose ie, 400 mg/kg was considered as the high dose of MEJS and used for the subchronic toxicity study in the present investigation.

In sub-chronic toxicity study, MEJS administration did not show any significant effect on water and food intake and body weight of the treated animals (data not shown).

Effect of MEJS on hematological parameters has been presented in Table.1. RBC, WBC and differential counts remained unaltered in MEJS treated animals, while hemoglobin content was slightly decreased in group II mice, however it was within the normal range.

Table 1: Effect of methanolic extract of *Jasminum sambac* flower on hematological parameters in serum of mice

Groups	Hb (g%)	RBC(million/ cu.mm)	WBC (T/cu.mm)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	ESR (mm/1sthr)
Control	13.25±0.70	10.11±0.18	7.02± 0.05	19.20±2.19	31.7± 1.26	4.7 ± 0.96	12.5±4.4	1.8 ±0.9	13.25±1.26
MEJS (400mg/kg)	13.82±1.27	10.23±0.2	7.0± 0.09	19.7±2.05	31.8± 1.26	4.5±0.96	12.4±4.1	1.9±0.9	13.8±1.29

Values are mean±SD, (n=8), * $p < 0.05$ for MEJS treated group vs normal group

The normal levels of hepatic biomarker enzymes (SGPT, SGOT, ACP and ALP), total bilirubin and protein in serum and the unaltered values of renal biochemical parameters (urea, uric acid and creatinine), as shown in Table.2, indicate that subchronic treatment with MEJS does not posses any significant adverse effect on hepato-renal functioning of the animals.

Table 2: Effect of methanolic extract of *Jasminum sambac* flower on biochemical parameters in control and treated mice

Groups	SGOT (IU/dl)	SGPT (IU/dl)	ACP (IU/dl)	ALP (IU/dl)	Total Bilirubin (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal control	19.57±1.1	27.60±3.8	48.09±0.2	77.64±1.2	1.10±0.13	2.33±0.12	7.83±0.57	8.53±0.42	6.06 ±0.6	0.91 ±0.29
MEJS(400 mg/kg)	19.73±1.4	27.38±4.0	48.12±0.3	77.58±1.4	1.20±0.09	2.08±0.46	7.90±0.52	8.97±0.39	5.71±0.94	1.63±0.65

Values are mean±SD, (n=8), * $p < 0.05$ for MEJS treated group vs normal group

Table.3 explores the lipid profile and blood sugar level of normal and MEJS treated animals after the six week experimental period. The results revealed that the extract does not adversely alter the lipid profile and blood sugar level of the animals after subchronic supplementation.

Table 3: Effect of methanolic extract of *Jasminum sambac* flower on lipid profiles and glucose levels in control and treated mice

Groups	Phospholipids (mg/dl)	FFA (mg/dl)	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Glucose (mg/dl)
Normal control	19.57±1.10	27.60±3.8	48.09±0.21	77.64±1.18	1.10± 0.13	7.83±0.57	8.53±0.42
MEJS (400mg/kg)	19.73±1.36	27.38±4.0	48.12±0.24	77.58±1.43	1.20-± 0.09	7.90±0.52	8.97±0.39

Values are mean±SD, (n=8), * $p < 0.05$ for MEJS treated group vs normal group

No significant difference in case of endogenous antioxidant status among the normal control animal and extract treated mice were reported, however slight higher value for GSH was observed in group II mice after MEJS administration (Table.4).

Table 4: Effect of methanolic extract of *Jasminum sambac* flower on antioxidant status in control and treated mice

Groups	LPO	GSH	Catalase
Normal control	99.95±6.6	34.76±1.45	71.60±6.6
MEJS (400mg/kg)	110.10±3.08	36.98±2.79	80.02±5.60

Values are mean±SD, (n=8), * $p < 0.05$ for MEJS treated group vs normal group

Histological observation of the liver tissue of both normal control mice as well as extract treated mice (Fig.1.A and 1.B) showed normal cellular architecture with prominent central vein.

Fig.1.Histological observations of liver sections of normal mice and mice treated with methanolic extract of *Jasminum sambac*

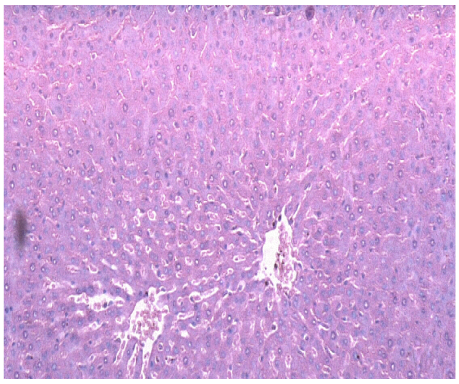

Fig.1.A.Photograph of liver section Normal mice

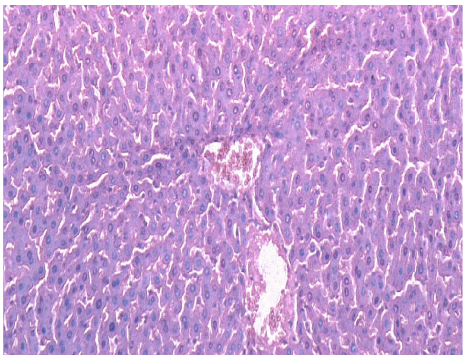

Fig.1.B.Photograph of liver section mice treated with 400mg/kg bw

Discussion

In recent years there is increasing trend for using alternative system of medicine. It is argued, that such drugs are not only effective but also very safe as compared to allopathic drugs for the similar indications. The claim that natural plant product are safe should be accepted only after the plant product passes through toxicity testing using modern scientific methods[25].

Acute toxicity studies showed the lack of mortality and toxicity upto oral treatment of 2000 mg extract/kg body weight which suggests that the methanol extract of *C.maxima* aerial parts is practically nontoxic at single dose. However in case of subsequent use in the treatment of the chronic diseases like diabetes or cancer, whether it will be safe that can be clear from its subchronic toxicity study.

During the experimental period, there was no treatment related effect on hemoglobin concentration and RBC count which indicates the unlikelihood of the extract to induce anemia. Insignificant change in WBC count was probably due to normal response to foreign bodies or stress associated with the chronic toxicity studies[26].

In a toxic environment, blood level of liver marker enzymes like AST, ALT, ACP and ALP are known to significantly increase[27]. These two classical enzymes are reliable indices of liver toxicity. Since in this study the enzymes showed no appreciable increase in the treated animals, it implied that the extract has no hepatotoxic effect[28].

This is further strengthened by the observation that levels of protein, albumin, direct and total albumin in these groups were not adversely affected, implying normal synthetic and excretory functions of the liver in treated group. This can be associated with the antioxidant properties of the extract and is supported by a study conducted by[29] who reported antioxidative effects of the plant in Wistar rats.

Bilirubin is formed from degeneration of hemoglobin by the action of reticuloendothelial systems throughout the body. Increased bilirubin level reflects the depth of jaundice[30]. The normal value of the hepatic biochemical parameters reveals the safety profile of the extract on liver function even on its chronic use. The normal values of the renal biochemical parameters, including urea, uric acid and creatinine suggest that the extract does not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts and hence is safe on its chronic use in various diseases. Although the plant possesses antidiabetic property, however it does not affect normal blood glucose level. The extract exerted protective effect on the lipid profile of the animals, which was evident by the unaltered values of LDL and HDL cholesterol in the treated group.

The endogenous antioxidant status after the chronic use of the extract was found to be quite equivalent to that of the normal mice. However the slight higher value of GSH was observed in the MEJS treated mice. This indicates the protective role of the extract on the endogenous antioxidant system which may be beneficial especially in case of the oxidative stress of the various disease conditions. Histological observations 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 as extract clearly[31].

Conclusion

The present investigation thus provides evidence for the total safety profile of the methanol extract of the aerial parts of *J.sambac*, suggesting its safe use in single dose treatment as well as for long term therapeutic application in case of various chronic diseases, without producing any toxic effects. Hence further phytopharmacological studies on the basis of its ethno botanical use can help to explore and establish the bioactive constituents of the extract which can be used safely for the treatment of various diseases and disorders in future.

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