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Acute and sub-acute toxicity study of *Clerodendrum inerme*, *Jasminum mesnyi* Hance and *Callistemon citrinus*

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ABSTRACT

Objective: To study acute and sub-acute toxicity study of *Clerodendrum inerme* (*C. inerme*), *Jasminum mesnyi* (*J. mesnyi*) Hance and *Callistemon citrinus* (*C. citrinus*). **Methods:** The acute toxicity test was conducted in Swiss albino mice. The extracts of *C. inerme*, *J. mesnyi* Hance and *C. citrinus* was administered in single dose of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g/kg and observed for behavioral changes and mortality, if any. In sub-acute toxicity study, Wistar rats of either sex were administered 1/5th of the maximum tolerated dose, *p.o.* for 4 weeks. Rats were observed weekly for any change in their body weight, food and water intake during 28 d of the treatment. At the end of 28 d, blood samples of the rats were collected for hematological and biochemical study. **Results:** In acute toxicity study, all four extracts of three plants were found to be well tolerated up to the dose of 2 000 mg/kg. These produced neither mortality nor any change in the behavior in mice. In sub-acute toxicity study, all four extracts of three plants at the LD_{50} dose level did not produce any significant alteration in hematological and biochemical parameters in rats. **Conclusions:** The results demonstrated that there is a wide margin of safety for the therapeutic use of each of the four extracts of three plants. The findings also corroborated the traditional use of these extracts.

1. Introduction

During last decade there has been an exponential growth in use of herbal products for treatment of various types of diseases[1]. In general, treatment involving herbal drugs spans a long duration of time. In contrast to general old age myth that herbal drugs are safe and do not have toxic effects, These drugs may cause some moderate to severe side effects due to complex nature of their chemical compositions. Hence, there is a need to establish safety to herbal drugs through validated scientific toxicity studies or protocols.

Clerodendrum inerme (*C. inerme*), *Jasminum mesnyi* (*J. mesnyi*) Hance and *Callistemon citrinus* (*C. citrinus*) (Figure 1) are used for treatment of diabetes mellitus in traditional system of medicine in India. *C. inerme* belonging to family Verbenacae has been used as antidiabetic agent in folklore medicinal system of India. It is reported to have antibacterial, hepatoprotective, anticarcinogenic, uterine and intestine stimulating properties. The various constituents characterised in its leaves include phenylethanoid glycoside, neo-clerodane diterpinoids antiviral proteins (CIP-29 and CIP-34) and three iridoid glucoside (Inerminoside A1, C and D[2-4]. *J. mesnyi* Hance belonging to family Oleaceae is an evergreen shrub having bright yellow flowers. It is native of China and grown in Indian gardens[5]. The major constituents present in this plant include β-sitosterol, α-amyrin, β-glucoside flavonoids, constituents include rutin and secoiridoid glucosides (9-hydroxyjasminoside, 9-hydroxy jasminosidic acid, Jasmoside and jasminoside)[6,7]. *C.*

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citrinus belonging to family Myrtaceae is woody aromatic tree widely distributed in the wet tropics, especially Australia, South America and tropical Asia. It is mainly used as an ornamental plant. The major constituents present in *C. citrinus* include oxygenated monoterpenes, monoterpene hydrocarbons, 1,8 cineole, α-pinene, β-pinene, α-terpinene and α-terpineole. Its leaves are employed as substitute of tea to have a delightful and refreshing flavor[8,9,10]. However, its medicinal uses are not reported widely and its constituents are being investigated for herbicidal properties and for potential in human medicine. Despite their traditional use in treatment of diabetes mellitus, there is no systematic study on exploration of antidiabetic potential of these plants. Further, diabetes mellitus, being a chronic disease, needs a long term treatment and chronic consumption of these herbs may cause mild to severe side or toxic effects. However, there is no report on toxicity evaluation of these

Figure 1.*C. inerme* (A), *J. mesnyi* Hance (B) and *C. citrinus* (C).

2. Methods and materials

Swiss albino mice (25-30 g) and Wistar albino rats of either sex (150-250g) were procured from Lala Lajpat Rai University of Veterinary & animal Sciences, Hisar and housed in Animal House of Hindu College of Pharmacy (Sonepat, India) (Reg No. 585/02/CPCSEA) under controlled environmental conditions (25±2) oC with natural light/dark cycle. The animals were allowed free access to food (standard pellet diet, Golden feed, Delhi, India) and water and acclimatized for at least a week before the commencement of the experiment. All experiments were duly approved by the Institutional Animal Ethics committee.

The leaves of *C. inerme*, *J. mesnyi* Hance and *C. citrinus* were collected from healthy plants in Sonipat(India) in July 2009. The leaves were authenticated by Dr. H.B. Singh (Scientist F and Head Raw Materials Herbarium and Museum, NISCAIR, Delhi) with a Voucher number - Niscair/RHMD/Consult/-2009-10/1241/45.

2.1. Chemicals and reagents

The chemicals and solvents required for chemical evaluation and extracts were procured commercially from E. Merck (Mumbai, India), s.d. fine chemicals (Mumbai, India) and C.D.H. Private Limited, (New Delhi, India). Soxhlet extraction assembly of 2 L capacity (Borosil, New Delhi, India) was used for extraction of plant material. The extracts were concentrated on rotary vacuum evaporator (Hi - Con, New Delhi, India)

2.2. Preparation of extracts

The leaves were dried at room temperature under well-ventilated shade by spreading them uniformly. The dried leaves were sorted, powdered, weighed (about 270 g) and extracted with petroleum ether to remove fatty constituents and chlorophyll. The marc was then subjected to successive solvent extraction with different solvents viz. Ethyl acetate, Chloroform, Ethanol and Water in soxhlet apparatus using about 800 mL of each solvent. Each extract was dried under vacuum and percent yield was calculated as % w/w with respect to total weight of dried leaves taken for extraction[13,14].

2.3. Acute toxicity study

The acute toxicity studies were carried out on Swiss albino mice of either sex weighing 25-30 g by the method described by Miller and Tainter[15,16]. The toxicity study was conducted at six doses (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g/kg) of each of ethyl acetate, chloroform, ethanolic and aqueous extracts of *C. inerme*, *J. mesnyi* Hance and *C. citrinus*. The animals were divided into different groups with 6 animals in each group and fasted overnight. Each extract was administered orally at each of the six dose levels. The animals were observed for first 12 h for any toxic symptoms and for 24 h for any mortality. The number of animals dying during the period was noted. The LD50 was calculated by the method of Miller and Tainter. The percent mortality was calculated followed by calculation of the corrected percent mortality using the formula:

For 0% death = $100 \times (0.25/n)$

For 100% death = $100 \times [(n-0.25)/n]$

Where, n is the number of animals in each group. Corrected percent mortality was then transformed into probit values[17,18]. A graph of percent mortality (in probits) was plotted against log dose. The dose corresponding to probit 5 was read to be LD50.

2.4. Sub-acute toxicity study

Healthy Wistar albino rats of either sex weighing (150-250 g) were divided into different groups with 6 rats in each group. The control group received vehicle alone and the other groups received maximum therapeutic dose (MTD) of ethanolic, aqueous, ethyl acetate and chloroform extracts of *C. inerme*, *J. Mesnyi* Hance and *C. citrinus* for 28 d. The MTD was calculated from LD50 determined through acute toxicity study. The animals were monitored for body weight, mortality, food and water intake daily. After 28 days, all animals were fasted overnight and anaesthetized with ether[19,20]. The blood samples were collected in heparinised tubes for determining haematological parameters such as haemoglobin, RBC count, WBC count, blood urea, creatinine, SGOT, SGPT and blood sugar level[21,22,23].

2.5. Statistical analysis

All results were expressed as Mean ± Standard error. Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's test. The results were regarded as significant at $P<0.05$.

3. Results

The % yield of each extract of each of the selected plants is given in Table 1. Ethanol extract of *C. inerme* was obtained with maximum yield (22.8%) whereas chloroform extract of *C. citrinus* was obtained in minimum amount (0.1%).

Table 1.
Successive extract of leaves of *C. inerme* (CI), *J. mesnyi* Hance (JM), and *C. citrinus* (CC)
Extract

Extract	Yield (% w/w of dried plant material)		
	C. inerme (CI)	*J. mesnyi* Hance (JM)	*C. citrinus* (CC)
Pet. Ether	2.96	4.02	5.32
Ethyl Acetate	3.81	3.75	8.61
Chloroform	2.10	9.81	0.10
Ethanol	22.80	8.61	9.21
Water	10.12	15.2	13.54

3.1. Acute toxicity

It was carried out to determine LD_{50} of each extract of *C. inerme*, *J. mesnyi* Hance and *C. citrinus*. Each extract was found to be non-toxic upto a dose of 2 g/kg in mice. The LD_{50} of each extract is given in Table 2. The 1/5th of LD_{50} of each extract was taken as its MTD for subsequent

Table 2.
LD50 and MTD of the ethyl acetate (EA), chloroform(C), ethanolic (E) and aqueous (A) extracts of *C. inerme* (CI), *J. Mesnyi* Hance (JM) and *C. citrinus* (CC).

Extract	LD_{50} (mg/kg)	MTD(mg/kg)	Extract	LD_{50} (mg/kg)	MTD(mg/kg)	Extract	LD_{50} (mg/kg)	MTD(mg/kg)
EACI	4 000	800	EAJM	4 000	800	EACC	4 000	800
CCI	3 600	720	CJM	3 500	700	CCC	4 200	840
ECI	3 600	720	EJM	4 000	800	ECC	3 200	640
ACI	4 300	860	AJM	4 800	960	ACC	4 000	800

Table 3.
Haematological and biochemical parameters of animals during sub-acute toxicity study.

S.No.	Parameter	Clinical Parameter	Values expressed as Mean ± SEM (n=6)											
			EACI	CCI	ECI	ACI	EAJM	CJM	EJM	AJM	EACC	CCC	ECC	ACC
1	Body Wt. (g)	Pre-treatment	174.00±7.13	176.00±5.85	ECI	169.00±4.60	171.00±9.32	158±6.44	179.00±8.18	181.00±7.27	193.00±9.48	175.00±7.26	175.00±6.34	192±6.27
		Post-treatment	175.00±5.61	172.00±4.07	162.00±6.29	166.00±2.83	178.00±8.86	162±5.91	180.00±7.01	188.00±7.67	192.00±9.71	180.00±7.53	180.00±4.89	193±4.71
2	HB (g%)	Pre-treatment	12.80±0.92	13.30±0.45	167.00±7.04	12.70±0.47	10.10±0.91	10.2±0.91	13.70±0.34	12.30±0.84	11.90±0.43	11.40±0.59	12.70±0.19	12.4±0.25
		Post-treatment	12.30±0.85	13.30±0.55	12.80±0.27	12.00±0.49	11.40±1.10	11.4±0.58	13.80±0.43	12.20±0.49	11.80±0.26	11.60±0.64	13.20±0.10	12.9±0.27
3	RBCcount($10^6/\mu$ L)	Pre-treatment	6.40±0.30	6.30±0.27	13.00±0.35	7.40±0.29	6.00±0.54	6.3±0.59	7.80±0.64	6.80±0.25	7.30±0.30	7.10±0.55	6.50±0.43	7.9±0.39
		Post-treatment	6.40±0.17	6.50±0.27	7.30±0.26	7.40±0.19	6.70±0.64	6.1±0.43	8.15±0.54	7.20±0.17	7.40±0.20	7.60±0.65	6.50±0.31	7.9±0.31
4	WBCcount ($10^3/\mu$ L)	Pre-treatment	9.30±0.36	9.00±0.16	7.20±0.17	9.30±0.29	9.75±0.67	9.95±0.5	8.70±0.23	9.12±0.27	9.35±0.30	9.30±0.19	8.40±0.19	8.67±0.22
		Post-treatment	8.90±0.27	9.90±0.32	9.10±0.50	9.80±0.35	9.45±0.65	10.78±0.58	9.40±0.24	9.20±0.14	10.80±0.14	10.80±0.32	8.52±0.19	8.87±0.23
5	Urea (mg/dL)	Pre-treatment	29.50±1.71	29.50±0.99	9.20±0.39	26.00±2.35	35.80±2.38	36.3±2.31	28.10±1.48	30.50±2.57	28.30±1.39	30.30±0.83	28.30±0.37	8.67±0.22
		Post-treatment	34.00±1.27	32.30±0.39	27.00±1.62	30.60±3.29	42.1±2.45	45.7±3.50	32.70±1.17	32.80±2.55	36.30±0.42	36.30±2.16	33.30±1.22	8.87±0.23
6	Creatinine (mg/dL)	Pre-treatment	0.80±0.10	1.10±0.20	31.00±1.75	0.70±0.14	0.90±0.11	0.87±0.10	0.70±0.19	0.70±0.18	0.87±0.12	0.95±0.06	0.95±0.06	29.6±0.77
		Post-treatment	0.90±0.02	0.80±0.17	0.90±0.08	0.70±0.17	1.50±0.14	1.60±0.18	0.80±0.10	1.10±0.17	1.02±0.04	1.40±0.07	0.90±0.04	28.3±1.42
7	SGOT	Pre-treatment	41.10±2.64	39.10±1.11	0.90±0.06	37.70±1.84	39.50±2.19	38.50±1.10	37.60±2.01	36.50±1.36	35.70±2.14	39.70±0.89	39.20±1.57	0.95±0.09
		Post-treatment	42.80±2.90	45.70±1.07	46.50±2.34	41.50±1.50	43.70±1.21	42.50±1.48	46.50±2.78	43.50±1.83	44.30±1.87	48.80±1.58	47.70±4.01	1.10±0.17
8	SGPT	Pre-treatment	40.50±3.05	32.40±3.07	56.80±3.76	31.20±2.34	34.00±2.37	32.40±1.62	30.00±0.70	33.50±2.44	30.10±0.84	32.40±1.84	24.20±0.89	39.7±1.44
		Post-treatment	45.30±2.95	36.30±4.65	34.60±2.18	33.90±1.54	38.20±2.91	34.30±1.98	37.90±1.01	38.50±2.48	37.70±1.66	37.00±2.16	28.80±0.39	42.4±1.76
9	Blood sugar	Pre-treatment	93.50±2.72	94.50±3.72	36.30±4.38	86.20±3.48	93.00±4.72	97.20±6.04	97.20±4.72	99.50±4.55	95.50±3.29	94.50±2.94	93.70±2.25	23.5±2.16
		Post-treatment	90.50±1.89	90.50±2.78	93.20±3.30	82.50±3.40	96.50±5.17	100.50±4.97	95.70±3.47	95.00±3.46	98.00±3.71	98.50±3.79	93.00±1.91	22.7±1.81
	(mg%)				90.20±2.65									96.5±4.48

sub-acute toxicity studies.

3.2 Sub-acute toxicity study

None of the extract of any of the three plants produced any mortality in animals at the MTD administered (Table 2) over 28 d. No sign of observable toxicity was detected during the experimental period. All the haematological parameters such as haemoglobin, RBC count, WBC count, urea, creatinine level, blood sugar level and biochemical parameters such as SGOT, SGPT were determined before the start of dosing (pre-treatment) as well as at the end of the study (post-treatment) (Table 3). All parameters were found within the normal range. These results suggested that the selected herbs can be used for treatment of chronic diseases without exhibiting any side/toxic effect.

4. Discussion

In conclusion, Acute and sub-acute studies on *C. inerme*, *J. mesnyi* Hance and *C. citrinus* were carried out as a prerequisite to exploration of antidiabetic potential of these plants. The LD50 of each of the ethyl acetate, chloroform, ethanolic and aqueous extracts of each plant was determined and MTD was calculated. Each extract was evaluated for its sub-acute toxicity at its MTD and was found to be non toxic. The selected plants can be further explored for their therapeutic potential in different chronic diseases.

Conflict of interest statement

We declare that we have no conflict of interest

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