International Journal of Phytomedicine 7 (2015) 136-141
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Anti-diabetic potentials of *Clerodendrum inerme, Jasminum mesyni* Hance and *Callistemon citrinus* on nicotinamide-streptozotocin induced type 2 diabetic rats

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Abstract

To investigate antidiabetic potential of alcoholic leaves extract of *Clerodendrum inerme, Jasminum mesyni* Hance and *Callistemon citrinus* on nicotinamide-streptozotocin induced type 2 diabetic rats
The comparative antidiabetic activity was conducted in NAD+STZ wistar rats of either sex. The leaves extracts of *C. inerme, J. mesyni* Hance *and C. citrinus* were administered at a dose of 400 and 600mg/kg p.o. for 4 Weeks and observed for antidiabetic study. During 28 days of treatment, rats were observed weekly for hypoglycaemic activity. The glucose level of all groups were determined after 0, 7, 14, 21 days. The blood samples for glucose estimation were withdrawn by retro-orbital plexus puncture method for antidiabetic activity. All the plant extracts lowered the blood glucose level after 21 days significantly with respect to the diabetic control. However, only ethanolic extract of *C. inerme* at 400mg/kg p.o. exhibited maximum reduction of blood glucose level as compared to the *J. mesyni* Hance and *C. citrinus*.
The results demonstrated that *C. inerme* has potent antidiabetic effect as compared to extracts of another two plants.

Keywords: Antidiabetic activity, leaf extracts, wistar rats, Nicotinamide, Stereptozotocin, *Clerodendrum inerme, Jasminum mesyni* Hance, *Callistemon citrinus.*

Introduction

Diabetes mellitus is a metabolic disorder which is a leading cause of other diseases like nephropathy, neuropathy and retinopathy. It is caused due to deficiency of insulin or decrease in response of body to insulin as result of many factors like stress, hypertension and autoimmunity. Numerous synthetic drugs like sulphonylureas, biguanides and thiazolidindiones are commercially available for its treatment [1]. Due to drug dependence and various side effects associated with synthetic antidiabetic drugs there is a interest growing in Ayurvedic system of medicine for treatment of diabetic patients. Numerous plants are reported in literature to possess antidiabetic activity.

During last decade there has been an exponential growth in use of herbal products for treatment of various types of diseases [2]. In general, treatment involving herbal drugs spans a long duration of time. In contrast to general oldage 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.

Clerodendrum inerme, Jasminum mesyni Hance and Callistemon citrinus(Figure 1) are used for treatment of diabetes mellitus in traditional system of medicine in India.

Figure 1- *Clerodendrum inerme* (A), *Jasminum mesnyi* Hance (B) and *Callistemon citrinus* (C)

C. inerme belonging to family Verbenacae has been used as antidiabetic agent in folklore medicinal system of India [3]. It is reported to have antibacterial, hepatoprotective, anticarcinogenic, uterine and intestine stimulating properties [4]. The various constituents characterized in its leaves include phenylethanoid glycoside, neo-clerodane diterpenoids antiviral proteins (CIP-29 and CIP-34) and three iridoid glucoside (Inerminoside A1, C and D [5]. 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 [6]. The major constituents present in this plant include β-sitosterol, -amyrin, β-glucoside flavonoids, constituents include rutin and secoiridoid glucosides (9–hydroxyjasminoside, 9-hydroxy jasminosidic acid, Jasmoside and jasminoside) [7]. C. citrinus belonging to family Myrtaceae is woody aromatic tree widely distributed in the wet tropics, specially Australia, South America and tropical Asia. It is mainly used as an ornamental plant [8,9]. The major constituents present in C. citrinus include oxygenated monoterpenes, monoterpene hydrocarbons, 1,8 cineole, –pinene, β–pinene, –terpinene and –terpineole. Its leaves are employed as substitute of tea to have a delightful and refreshing flavor [10,11]. 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.

Materials and methods

Plant material

The leaves of *C. inerme, J. mesyni* 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.

Animals

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 (Sonipat, India) (Reg No. 585/02/CPCSEA) under controlled environmental conditions (25±2^{o}C) 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.

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical (USA) and NAD was purchased from Sisco Research Lab. Pvt Ltd (India). Glucose estimation kits were procured from Bayer's Diagnostics Ltd (India). The other chemicals were obtained from S.D. fine chemicals limited (India). Glibenclamide was procured from Panacea Biotech Ltd (India) as gift sample. All solvents used were of LR grade obtained from E. Merck (India).

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 270g) and extracted with petroleum ether to remove fatty constituents and chlorophyll [12,13]. 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 [14].

Induction of experimental diabetes

Diabetes mellitus was induced in STZ treated wistar rats by intraperitoneal injection of STZ (50mg/kg body weight), freshly dissolved in cold 0.1mol/l citrate buffer solution, after half hour administration of NAD (120 mg/kg body weight), rats became diabetic within 72h after STZ administration. Diabetes was allowed to develop and stabilize in these NAD+STZ-treated rats over a period of 5–6 days. Diabetic (hyperglycemic) rats were fasted for 16-h, but still allowed free access to drinking tap water throughout. At the end of the 16-h fasting period was taken as 0 day [15,16].

Acute and subacute toxicity study

The acute toxicity test was conducted in Swiss albino mice. The ethyl acetate, chloroform, ethanolic and aqueous extracts of *C. inerme, J. mesyni* 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 [17,18]. In subacute toxicity study, Wistar rats of either sex were administered 1/5 of maximum tolerated dose, p.o. for 4 Weeks. During 28 days of treatment, rats were observed weekly for any change in their body weight, Food and water intake. At the end of 28 days, rats blood were collected for hematological and biochemical study [19].

Antidiabetic activity of extracts

Animals were divided into 15 groups (I-XV) of 6 animals each at dose level of 400 and 600 mg/kg p.o. The group I was served as normal control while hyperglycemia was induced in the other groups. Briefly the animals were injected with streptozotocin (50 mg/kg i.p.) half an hour after administration of NAD (120mg/kg i.p.).

After 1 hour, the animals were fed on glucose water and normal diet for 2 days. The glucose water was replaced with normal water and animals were given free access to feed and after 7 days the blood glucose level in each animal was determined. The hyperglycaemic rats were treated with glibenclamide (0.5 mg/kg p.o) [20] as well as extracts (400 mg/kg p.o.) using 2% gum acacia solution as vehicle daily over a period of 21 days. The group II served as diabetic control where animals received the vehicle. The group III was treated with glibenclamide(0.5 mg/kg p.o). The groups IV-VII were treated respectively, with ethyl acetate, chloroform, ethanolic and aqueous extracts of *C. inerme* while the groups VIII-XI were treated respectively with ethyl acetate, chloroform, ethanolic and aqueous extracts of *J. mesyni* Hance and the groups XII-XV were treated respectively with ethyl acetate, chloroform, ethanolic and aqueous extracts of *C. citrinus*. During the study, animals had free access to water and feed. However, feed was withdrawn about 12 hour prior to sampling for blood glucose estimation. The glucose level of all groups was determined after 0, 7, 14, 21 days of the commencement of the treatment. The blood samples for glucose estimation were withdrawn by retro-orbital plexus puncture method [21,22].

Statistical analysis

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

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. mesyni* Hance (*JM*), and *C. citrinus* (*CC*)

Extract	*C. inerme* (*CI*) % Yield	*J. mesyni* Hance (*JM*)% Yield	*C. citrinus* (*CC*)% Yield
Pet. Ether	2.96	4.02	5.32
Ethyl Acetate	3.81	3.75	8.61
Chloroform	2.1	9.81	0.1
Ethanol	22.8	8.61	9.21
Water	10.12	15.2	13.54

Acute toxicity

Each extract was found to be non-toxic upto a dose of 2 g/kg in mice. The 1/5th of LD_{50} of each extract was taken as its MTD for subsequent sub-acute toxicity studies.

Sub-acute toxicity study

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). These results suggested that the selected herbs can be used for treatment of chronic diseases without exhibiting any side/toxic effects [23,24].

Antidiabetic activity of extracts

The antidiabetic activities of various extracts of *C. inerme, J. mesyni* Hance and *C. citrinus* at dose level of 400 mg/kg p.o. are given in Table.2 and Figure 2. and at dose level of 600mg/kg p.o. are given in Table.3 and Figure. 3. All the extracts of both the plants lowered the glucose level after 28 days significantly with respect to the diabetic control. However, only ethanolic extract of *C. inerme* at 400mg/kg p.o exhibited maximum reduction but still less than glibenclamide.

Table 2. Effect of various extracts of *C. inerme* and *J.mesyni* Hance and *C.citrinus* on blood glucose level in NAD+STZ induced diabetic rats at dose of 400mg/kg p.o.

	Blood glucose level(mg/dl)			
Groups (400mg/kg p.o.)	0 days	7 days	14 days	21 days
Group I(Normal control)	94.5±4.89	94.83±3.33	89.5±5.25	91.17±5.26
GroupII(Diabetic control)	298±8.08	296.3±7.07	312.2±9.35	315.3±10.43
Group III(Glibenclamide)	280.3±9.99	221.2±9.82**	198.3±8.91**	127.7±9.40**
C. inerme extract Group IV(Ethyl acetate)	292.7±15.08	285.3±15.89	265.7±14.86	242.8±14.09**
Group V(Chloroform)	297.3±24.95	286.8±20.10	277.8±13.68	255.5±13.18**
Group VI(Ethanolic)	301.2±10.88	275.3±8.34	236±9.22**	173.5±8.26**
Group VII(Aqueous)	300.5±7.66	292.3±7.89	271.3±8.14	225.2±7.07**
J.mesyni Hance Group VIII (Ethyl acetate)	306.7±26.11	261.8±22.41	257.8±19.91*	245±19.35**
Group IX (Chloroform)	292.3±14.52	281.8±10.22	249.2±9.88**	208.7±10.36**
Group X (Ethanolic)	290±25.94	273.5±21.50	237±21.69**	198.2±17.70**
Group XI (Aqueous)	299.3±8.12	285.0±9.00	261.3±9.02	231.3±6.21**
C.citrinus extract Group XII(Ethyl acetate)	301.5±7.97	299.5±6.61	292.0±5.83	283.8±5.46
Group XIII(Chloroform)	302.5±7.73	299.8±7.12	291.2±7.56	280.5±8.31
Group XIV(Ethanolic)	298.0±4.35	284.0±4.59	263.7±6.23**	245.2±6.60**
Group XV(Aqueous)	301.0±5.22	288.0±5.18	271.3±6.93**	252.2±8.03**

*Values are given as mean ±S.E.M. from six rats in each group.

** Statistical significance vs. diabetic control ($P < 0.05$)

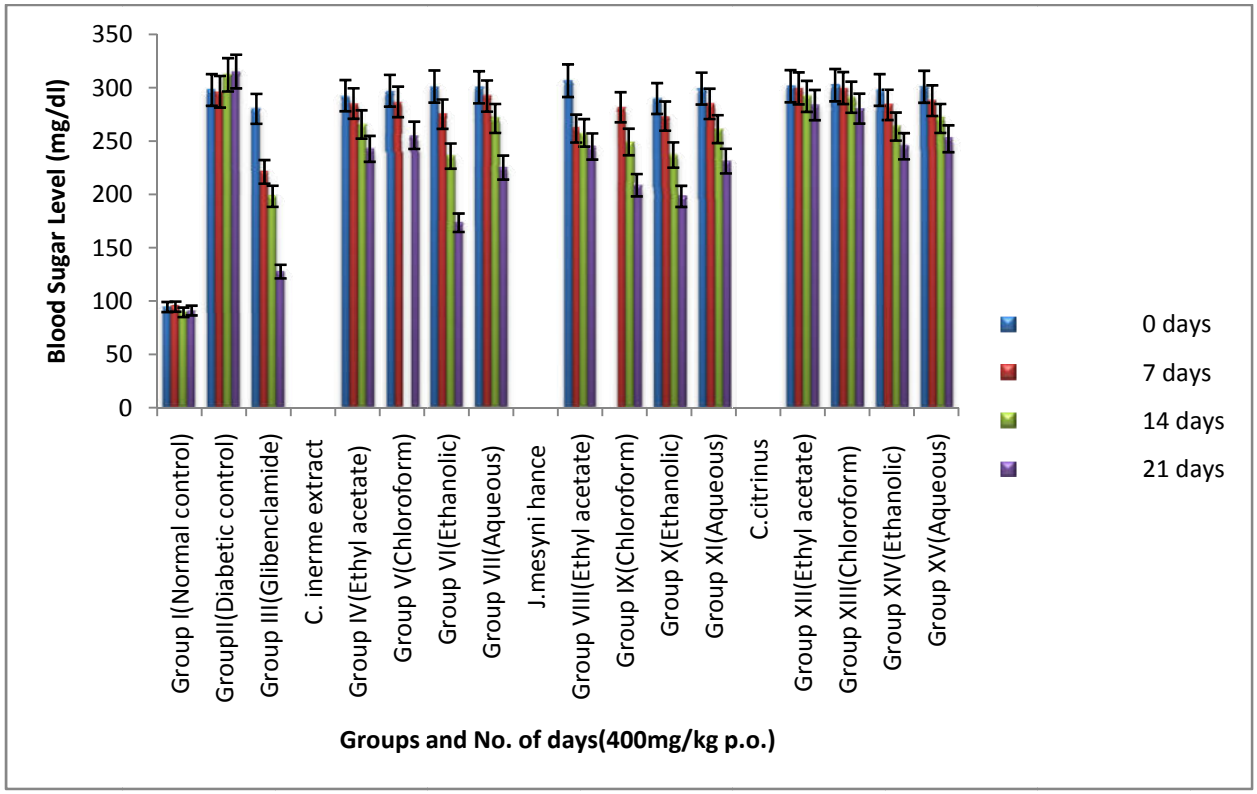

Figure 2. Antidiabetic activity of various extracts of *C. inerme* and *J. mesyni* Hance and *C.citrinus* at 400mg/kg p.o.

Table 3. Effect of various extracts of *C. inerme* and *J.mesyni hance* and *C.citrinus* on blood glucose level in NAD+STZ induced diabetic rats at dose of 600mg/kg p.o.

Groups (600mg/kg p.o.)	Blood glucose level(mg/dl)			
	0 days	7 days	14 days	21 days
Group I(Normal control)	94.5±4.89	94.83±3.33	89.5±5.25	91.17±5.26
Group II(Diabetic control)	302±8.08*	303.3±7.07*	312.2±9.35*	325.3±10.43*
Group III(Glibenclamide)	280.3±9.99	221.2±9.82**	198.3±8.91**	127.7±9.40**
C. inerme extract Group IV (Ethyl Acetate)	314.5±8.25	274.3±3.19	285.3±7.31	279.2±8.93 **
Group V (Chloroform)	289±7.51	279.8±7.67	265.8±7.02 **	260.2±6.83 **
Group VI(Ethanolic)	294.8±6.49	296±8.82	218.3±4.95 **	187.7±6.14**
Group VII(Aqueous)	317.7±9.97	282.3±8.39	254.5±3.93 **	230±5.53 **
J.mesyni Hance Group VIII (Ethyl acetate)	315.0±9.02	297.3±9.40	270.7±6.27 **	262.5±6.56 **
Group IX (Chloroform)	312.7±6.69	292.7±7.57	274.2±7.54 **	261.2±9.07 **
Group X(Ethanolic)	310.7±6.65	293.5±5.14	262.7±5.80 **	223.0±9.01 **
Group XI(Aqueous)	313.8±9.03	285.5±6.99	264.8±6.81 **	253.5±11.87 **
C.citrinus extract GroupXII(Ethyl acetate)	301.8±7.62	295.5±6.60	287.8±6.03	277.7±5.00 **
Group XIII(Chloroform)	309.3±7.49	296.7±7.61	291.7±7.45	287.5±9.19
Group XIV (Ethanolic)	297.7±7.01	286.0±8.41	261±6.52 **	246.7±5.64 **
Group XV(Aqueous)	305.7±5.49	295.2±6.77	280.8±7.79	264.2±8.27

*Values are given as mean ±S.E.M. from six rats in each group.
** Statistical significance vs. diabetic control ($P < 0.05$)

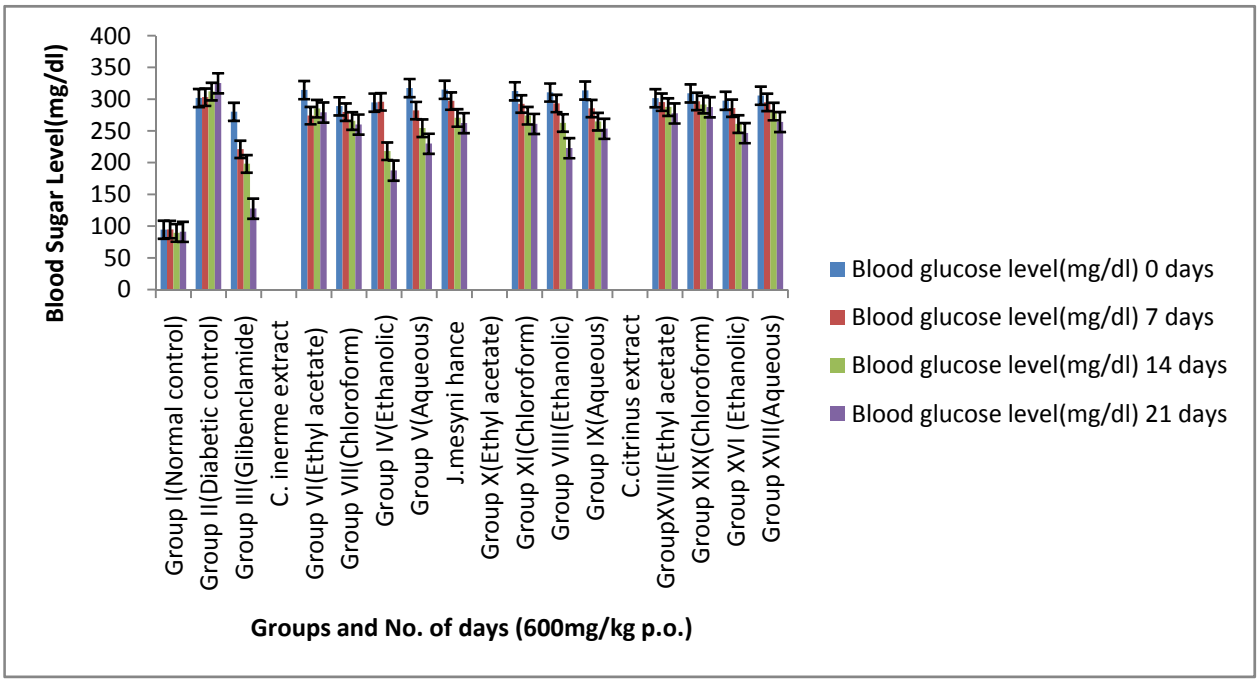

Figure 3. Antidiabetic activity of various extracts of *C. inerme* and *J. mesyni* Hance and *C. citrinus* at 600mg/kg p.o.

Discussion

Acute toxicity study was carried out to determine the LD_{50} of each extract of *C. inerme, J. mesyni* Hance and *C. citrinus*. None of the extract of any of the three plants produced any mortality in animals at the MTD administered over 28 days. No sign of observable toxicity was detected during the experimental period [25]. All parameters were found within the normal range. Further the

ethanolic extract of *C. inerme* was found to be better antidiabetic than the ethanolic extract of *J. mesyni* Hance and *C. citrinus* administered at the dose of 400 mg/kg. This may be attributed to better extraction of active constituents of *C. inerme* in ethanol solvent. Hence, we propose that ethanolic extract *C. inerme* has indicated the somewhat lesser or equivalent antidiabetic activity in comparison to glibenclamide.

Conclusion

The ethanolic extract of *C. inerme* at 400mg/kg p.o was found to be maximally antidiabetic but less potent than glibenclamide.

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