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Research Article

Pharmacognostic and anti-hyperglycemic evaluation of *Lantana camara* (L.) var. aculeate leaves in alloxan-induced hyperglycemic rats

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ABSTRACT

Lantana camara (L.) var. aculeate (family: Verbenaceae) commonly called as wild sage is large scrambling evergreen, strong smelling shrub with stout recurred prickles; leaves opposite, often rugose, scabrid on both sides. A survey on recent literature, it is clear that diabetes is a challenging metabolic disorder to mankind. Currently many researchers have been envisaged on medicinal plants to establish more effective antidiabetic drug. We had put some effort in this direction to strengthen the research. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Lantana camara* (L.) for the selected area for diabetes. It was also our aim to establish the correct identity of the selected plant through pharmacognostic study. Ash value, extractive value, loss on drying and fluorescence analysis was also carried out to characterise the crude drug. Oral administration of the methanol extract of *Lantana camara* (200 and 400 mg/kg body weight) leaves in alloxan-induced diabetic rats, showed significant (P<0.01) reduction in the blood glucose concentration in dose dependent manner. Treatment with extract (400 mg/kg) decreased blood glucose level to 121.94 mg/dl. Body weight significantly (P<0.05) increased to normal after treatment with extract and it was found effective in oral glucose tolerance test as it decreased the elevated level of glucose after one hour. The biochemical parameters like triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein were investigated and extract was found effective against diabetes induced hyperlipidemia. Present study demonstrated that methanol extract of *Lantana camata* (L.) leaves exhibit promising anti-hyperglycemic activity against alloxan-induced diabetic rats.

Keywords: antidiabetic activity; pharmacognostic evaluation; *Lantana camara*; methanol extract.

INTRODUCTION

Lantana camara (L.) var. aculeate (family: Verbenaceae) commonly known as unnicceti (Tamil), pulikampa (Telugu) and caturang (Hindi) is a significant weed commonly found throughout India. Traditionally the plant is used as diaphoretic, carminative, antispasmodic, tonic and useful in the treatment of tetanus, vitiated conditions of vata, epilepsy, gastropathy. A decoction of fresh roots is a good gargle for odontalgia and this is used by hill tribes for all types of dysentery. Powdered leaves are used for cuts, wounds, ulcers, and swellings. An infusion of the leaves is good for bilious fever, eczema and eruptions. The fruits are useful in fistula, pustules, tumours and rheumatism (Anonymous 1992; Ghisalberti 2000; Anonymous 2006; Kashyapa 2006). Different parts of the plant reported for pharmacological activities like antilymphocytic and immunosuppressive, hepatoprotective, thrombin inhibitory, termiticidal, antimotility, antifilarial, *in vitro* cytotoxic and antimicrobial activity (Garg et al., 1997; Misra et al., 1997, 2007; Noble et al., 1998; Raghu et al., 2004; Rajesh et al., 2006; Fagbounka et al., 2008) investigated. Wound healing and antidiabetic potential of hydroalcoholic and water extract of *Lantana camara* leaf has been reported, though the effect of extract on diabetes induced hyperlipidemia was not investigated (Dash et al., 2001). Chemical investigation showed the presence of essential oils in leaves and flowers. Previous studies have reported the presence of sesquiterpenes like curcumenes and safrole; triterpenes such as lantadenes A and B; iridoid glycosides; flavonoids like quercetin derivatives and steroids like β-sitosterol, campesterol, stigmasterol, β-sitosterolglucoside, oligosaccharides in the plant (Sharma et al., 1988, 1989; Ghisalberti 2000; de Mello et al., 2003).

Diabetes mellitus is one of the most common endocrine disorders, characterized by hyperglycemia and encountered as a major public health problem and affects almost 5% of the population (Taylor 1999). It is

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estimated that about 143 million people worldwide suffering from diabetes, comparatively five times more than the estimates ten years ago. This number may likely to double by the year 2030. Therefore, the human population worldwide seems to be in the midst of an epidemic of diabetes (Tiwari and Rao 2002). Diabetes is commonly classified into Type 1 or insulin-dependent diabetes mellitus (IDDM) and Type 2 or non-insulin-dependent diabetes mellitus (NIDDM). Presently available synthetic drugs for Type 2 diabetes have a number of limitations as they can produce severe adverse effects and high rates of secondary failure (Xie et al., 2002). As a complementary or alternative approach, herbal medicines with hypoglycemic activities are increasingly being used in the treatment of diabetes to confer less side effects and compatible with physiological system.

Several studies had shown that phytoconstituents like sesquiterpenes, saponins and triterpenes may prove beneficial in the treatment of diabetes. Pharmacognostic study gives the preliminary information regarding the plant, it is important for correct identification of the plant and knowledge of their constituents or adulterants. Therefore aim of this study was to investigate the pharmacognostical character and anti-hyperglycemic activity associated with hyperlipidemia of *Lantana camara* L. leaves.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan monohydrate was purchased from Sigma-Aldrich, USA. Glibenclamide was obtained from Cadila Health Care Ltd. Ahmedabad. Cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and triglycerides kits were procured from Agappe diagnostic, Kerala. All other chemicals used in this experiment were purchased from Sigma-Aldrich, USA and all the chemicals used were of analytical grade.

Plant material

The plant was collected from Komarapalayam region of Tamil Nadu state, India, in the month of February 2009. The herbarium was authenticated by Botanical Survey of India (BSI), Ministry of Environment and Forests, Government of India, Coimbatore and a voucher specimen (No.BSI/SC/5/23/08-09/Tech.1781) was deposited in the BSI, Tamil Nadu, India. The leaves of *Lantana camara* were dried under shade and then made into a coarse powder. The powder was passed through sieve No.40 and stored in an air tight container at 25°C.

Pharmacognostic evaluation

Pharmacognostical evaluation of *Lantana camara* leaves was carried out by determining ash value, extractive value, loss on drying and fluorescence analysis. Ash values such as total ash, acid insoluble ash and water soluble ash were determined as per standard procedures given in Indian pharmacopoeia. Extractive values like alcohol soluble and water soluble extractive value were also performed using the procedure described by Kokate 1985. Loss on drying is the loss in weight in percentage w/w determined by means of the standard procedure. Fluorescence analysis was carried out by observing the fluorescence characteristic of the powdered leaves of *Lantana camara* in daylight and UV light and also by treating the drug powder with different chemical reagents (Brain and Turner 1975; Kokate 1985; Anonymous 1996; Kokate et al., 2000; Mukherjee 2002).

Preparation of plant extract

Coarsely powdered *Lantana camara* leaves were subjected to solvent extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (40-60°C), methanol and water. The extract was dried under reduced pressure using rotary flash evaporator and yields were found to be 5.00%, 12.50% and 11.00% w/w respectively with reference to the air dried plant material.

Preliminary phytochemical investigation

The individual extracts like petroleum ether, methanol and water were subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins, triterpenoids (Yarnalkar 1991; Khandelwal 2004).

Animals

Wistar albino rats of either sex weighing between 150-250 g were used. They were maintained under standard laboratory conditions at a temperature of 23±2°C, with 12 h light - dark cycle, and relative humidity (50±10%). The animals were fed with standard food pellets (Hindustan Lever Ltd, India) and water *ad libitum*. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Anti-hyperglycemic activity

The overnight fasted rats were injected intraperitoneally alloxan monohydrate (150 mg/kg) dissolved in sterile normal saline. Blood glucose was measured after 72 hours using one-touch glucometer. Only alloxanised hyperglycemic animals (blood glucose levels of 200–300 mg/dl) were used for further studies. Animals were divided into 5 groups (each contain six animals). Group I served as normal control (saline), Group II served as diabetic control received alloxan monohydrate alone, Group III received glibenclamide (5 mg/kg) and served as standard. Two tests Group IV and V received 200 and 400 mg/kg methanol extract of *Lantana camara* (MELC) leaves respectively. Standard drug and extract were prepared in 0.5% carboxy methyl cellulose

suspension as vehicle and administered orally, treatment for Group III – V was continued for 14 consecutive days, blood was collected on 0^{th} (before treatment), 7^{th}, 14^{th} day to investigate different biochemical parameters (Dhawan et al., 1996; Babu et al., 2002; Hatapakki et al., 2005).

Estimation of mean body weight and biochemical parameters

Mean body weight was measured during treatment on weekly basis. Blood samples were collected from tip of rat tail and blood glucose levels were estimated using one touch glucometer (Glucocheck, New Delhi) on 0^{th}, 7^{th} and 14^{th} day. Serum was also analyzed after 14 days treatment for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) by using standard commercial diagnostic kits (Agappe Diagnostics, Kerala) following the manufacturer's instruction in a semi auto analyzer (Mispa Excel Chemistry Analyser, Mumbai).

Estimation of oral glucose tolerance test (OGTT)

After 14 days of drug treatment, fasting blood samples were taken from all five groups followed by glucose (3.5 g/kg) administration. Blood samples were collected at 30, 60, 90, 120, 150 and 180 min intervals after the administration of glucose. Percentage reduction in blood glucose level was estimated in comparison with control group (Sridevi et al., 2000).

Statistical analysis

Values were expressed as mean ± standard error mean (S.E.M) and analyzed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test, $P< 0.05$ were considered statistically significant.

RESULTS

Pharmacognostic evaluation

Ash value, extractive value and loss on drying were determined and results were given in Figure 1. Results showed 8.50, 2.00 and 3.67% total ash, acid insoluble ash and water soluble ash respectively. Extractive values like alcohol soluble and water soluble extractive value were found 5.80 and 4.52% and loss on drying was found 4.33%. Fluorescence characteristics of the powdered leaves after treatment with different chemical reagents don't show any characteristic fluorescence behaviour.

Preliminary phytochemical screening

All the extracts namely petroleum ether (40-60°C), methanol and aqueous were tested with various chemical reagents and results are presented in the Table 2. Phytochemical investigation showed the presence of saponins, glycosides, carbohydrates, tannins, flavanoids, steroids and triterpenoids in methanol extract; saponins, glycosides, carbohydrates, tannins and flavanoids were found in the aqueous extract; tannins, steroids and triterpenoids in petroleum ether extract.

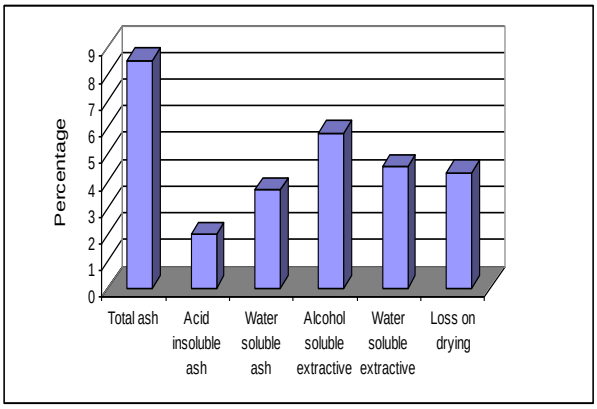

Figure 1: Data for ash values, extractive values and loss on drying of powdered *Lantana camara* leaves

Table 1: Data showing preliminary phytochemical screening of the leaf extracts of *Lantana camara* Linn.

Phyto constituents	Petroleum ether extract	Methanol extract	Aqueous extract
Alkaloids	-	-	-
Saponins	-	+	+
Glycosides	-	+	+
Carbohydrates	-	+	+
Tannins	+	+	+
Flavonoids	-	+	+
Steroids	+	+	-
Triterpenoids	+	+	-
Fixed oils and fats	-	-	-

Effect of methanol extract of *Lantana camara* leaves on mean body weight and blood sugar

Preliminary phytochemical investigation showed the presence of various phytoconstituents in methanol extract in comparison to other extracts therefore antidiabetic activity of methanol extract was evaluated.

Table 3 represents the mean body weight of control and experimental animals on 0, 7 and 14 days of treatment. Induction of alloxan results in the reduction of mean body weight, which was prevented by methanol extract of *Lantana camara* after 14 days treatment in dose dependent manner.

Blood glucose level in normal and experimental animals was tested on 0, 7 and 14 days of drug treatment. Methanol extract significantly decreased the elevated blood sugar level in dose dependent manner. MELC at a dose of 400 mg/kg, decreased blood sugar level to 121.94 mg/dl after 14 days treatment, which was found significant ($P<0.01$) and antidiabetic activity is comparable to that of the standard drug glibenclamide (Table 4).

Table 2: Effect of *Lantana camara* leaves (methanol extract) on mean body weight

Drug Treatment	Mean body weight (g)		
	0th day	7th day	14th day
Normal Control	190.67± 6.19	194.66± 6.61	200.33± 7.32
Diabetic Control	181.24± 4.98[a]	170.16± 5.81[a]	161.83± 5.20[a]
Diabetic + MELC (200 mg /kg)	168.12± 5.24[b]	169.83± 6.03[b]	177.67± 5.88[b]
Diabetic + MELC (400 mg/kg)	164.83± 5.09[b]	167.93± 4.07[b]	180.00± 4.66[b]
Diabetic + Glibenclamide (5 mg/kg)	172.50± 9.57[a]	177.50± 9.49[a]	187.33± 7.69[a]

Table 3: Effect of methanol extracts of *Lantana camara* leaves on mean blood glucose level in alloxan induced diabetic rats

Drug Treatment	Mean blood glucose concentration (mg/dl)		
	0th day	7thday	14th day
Normal Control	89.23± 3.43	89.56± 2.52	90.06± 2.27
Diabetic Control	289.34± 4.52[a]	264.78± 5.13[a]	259.74± 5.19[a]
Diabetic + MELC (200 mg /kg)	287.33± 6.29	215.26± 4.19[c]	143.57± 3.04[c]
Diabetic + MELC (400 mg/kg)	274.78± 4.06	191.41± 5.70[c]	121.94± 4.07[b]
Diabetic + Glibenclamide (5 mg/kg)	292.03± 7.13	186.62± 5.06[c]	118.63± 3.91[b]

Data are expressed as mean ± S.E.M for six animals in each group. Values are statistically significant at [a]$P<0.01$, [b]$P<0.05$ compared to respective control group.

Effect of methanol extract of *Lantana camara* leaves on different biochemical parameters

Table 5 shows the level of serum lipoproteins such as TC, TG, LDL, HDL and VLDL. Serum TC, TG, LDL and VLDL levels were significantly elevated and HDL level was decreased in diabetic group when compared with control group animals. Treatment with MELC has reversed the diabetes induced hyperlipidemia. A significant percentage reduction ($P<0.01$) of TC, LDL, TG and VLDL level and increase in HDL level was observed after the treatment with MELC. Extract produced dose dependent effect as MELC (400 mg/kg) brought down the level of TC, LDL, TG, VLDL and HDL to normal.

Effect of methanol extract of *Lantana camara* leaves on OGTT

After two hours of oral glucose administration, OGTT test has been carried out and results are tabulated in Table 6. Peak increase in blood glucose concentration was observed after 90 min and found to reduce after 120 min in diabetic animals but drug treated animals showed a significant decrease in blood glucose concentration after 1 h of the treatment when compared with diabetic control rats.

DISCUSSION AND CONCLUSION

The pharmacognostical studies made on the leaves of *Lantana camara* like determination of ash value, extractive value, loss on drying and fluorescence analysis of crude drug powder was performed, these observations will help in the botanical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulterants. Extractive value is an important tool to check quality and variation in chemical constituents of the drug. Ash value reflects the presence of inorganic salts in the plant part, loss on drying was useful to detect the net weight of a substance after drying at a specified temperature or under reduced pressure. Pharmacognostical evaluation designed to detect and check adulteration and exhausted drug, absence of other parts of the plant, presence of an abnormal proportion of extraneous mineral matter and adulteration like starch or stone cells (Kar 2005; Jahan et al., 2008).

The decreased mean body weight in diabetic rats is due to excessive break down of tissue protein. Treatment with MELC improved mean body weight significantly which may be due to preventive effect of muscle wasting in hyperglycemic condition. MELC produced dose dependent anti-diabetic effect, MELC at a dose of 400 mg/kg produced significant ($P<0.01$) antihyperglycemic activity. Transient hyperglycemia can be produced by an oral glucose tolerance test, extract also proved effective in oral glucose tolerance test as it decreased the elevated blood glucose level after one hour as like that of standard drug glibenclamide, study proved the antidiabetic potential of the methanol extract of *Lantana camara* leaves.

Insulin enhances the transcription of lipoprotein lipase which hydrolyzed triglycerides results release of intermediate density lipoprotein that convert to LDL by liver. Hyperlipidemia caused in diabetes is due to excess mobilization of fat from the adipose tissue is due to under utilization of glucose. Untreated diabetes causes hypertriglyceridemia and hypercholesteromia with the increase of LDL and VLDL which may leads various health problems. Treatment with MELC decreased cholesterol, triglycerides, LDL, VLDL and increased HDL level, showed the effectiveness of MELC to treat hyperlipidemia.

Table 4: Effects of methanol extracts of *Lantana camara* leaves on different biochemical parameters

Treatment	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TC (mg/dl)
Normal Control	76.12±4.12	43.57±1.32	39.12±4.13	15.22±0.82	88.84±6.27
Diabetic Control	184.21±7.43[a]	38.79±4.73[a]	125.41±1.21[a]	22.8±1.48[b]	242±7.38[a]
Diabetic + MELC (200 mg /kg)	103.40±4.02[c]	40.71±5.76[c]	77±3.92[c]	19.9±1.01[c]	137±0.68[c]
Diabetic + MELC (400 mg/kg)	88.42±6.90[b]	46.46±2.80[c]	41.1±0.04[*b]	17.70±1.38[b]	97.0±3.32[b]
Diabetic + Glibenclamide (5 mg/kg)	83.74±4.9[b]	49.02±1.90[b]	32.8±1.26[b]	16.74±0.98[b]	90.94±4.98[b]

Data are expressed as mean ± S.E.M for six animals in each group. Values are statistically significant at [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to respective control group.

Table 5: Shows effects of methanol extract of *Lantana camara* leaves on OGTT

Drug Treatment	Mean blood glucose levels (mg/dl)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Normal Control	86.20 ±2.58	130.11 ±4.09	125.81 ±4.05	104.33 ± 3.24	97.07 ± 2.84	82.09 ± 2.95	73.65 ± 3.42
Diabetic Control	267.59 ± 10.72[a]	335.39 ± 11.80[a]	405.62 ± 13.95[a]	446.50 ± 14.09[a]	421.81 ± 13.90[a]	415.57 ± 14.36[a]	399.50 ± 15.18[a]
Diabetic + MELC (200 mg /kg)	139.06 ± 5.12[c]	160.00 ± 3.78[c]	151.42 ± 5.25[b]	144.00 ± 3.14[b]	133.98 ± 4.02[b]	125.53 ± 3.56[b]	113.21 ± 4.33[b]
Diabetic + MELC (400 mg/kg)	126.00 ± 5.42[b]	142.07 ± 4.97[b]	131.76 ± 6.09[b]	127.84 ± 7.01[b]	120.71 ± 5.06[b]	113.79 ± 6.98[b]	101.30 ± 5.31[a]
Diabetic + Glibenclamide (5 mg/kg)	121.68 ± 5.61[b]	140.98 ± 4.57[b]	123.38 ± 4.73[b]	115.08 ± 5.47[b]	106.84 ± 3.45[b]	100.00 ± 2.89[a]	92.89 ± 3.89[a]

Data are expressed as mean ± S.E.M for six animals in each group. Values are statistically significant at [a]*P*<0.001, [b]*P*<0.01, [c]*P*<0.05 compared to respective control group.

Alloxan caused diabetes by producing selective cytotoxic effect on pancreatic cell as well as by the formation of free radicals (Jithendra et al., 2009). Different phytoconstituents like flavanoids, saponins has proven effective against diabetes (Tiwari and Rao, 2002). Preliminary study showed the presence of different phytoconstituents which may be beneficial for the antihyperglycemic activity of the extract.

In conclusion, it is stated that MELC was found effective in alloxan induced diabetic rats and plant can be a future effective medicine for the treatment of diabetes. Further studies are needed to confirm the exact mechanism of action and isolation of phytoconstituents responsible for such activity.

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