Journal of Medicinal Plants Research Vol. 5(15), pp. 3387-3395, 4 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Evaluation of anti-diabetic activity and toxic potential of *Lycium shawii* in animal models

Hassan Sher[1]* and Mohammed N. Alyemeni[2]

[1]Department of Botany, University of Swat, Pakistan.
[2]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Accepted 21 April, 2011

The powder and decoction of *Lycium shawii* Roem and Schult (Solonaceae) aerial part are used as a folklore remedy in the treatment of diabetes by the local community in various parts of Saudi Arabia. In the present study, attempts were made to scientifically justify the alleged anti-diabetic efficacy of this plant and to evaluate its toxic potential. The 80% ethanol extract of *L. shawii* aerial parts was prepared. After evaporation of ethanol, it was freeze dried. A statistically significant blood glucose lowering effect was noticed in Long-Evans rats treated orally with 250 mg/kg ($P<0.05$) and 500 mg/kg body weight ($P<0.001$) of *L. shawii* extract. In addition, there was a significant decrease in blood glucose levels of animals treated with the extract with a simultaneous load of glucose (2.5 mg/kg). A significant ($P<0.001$) anti-diabetic effect was also observed in streptozotocin (STZ) diabetic rats. The data obtained clearly justified the claimed hypoglycemic activity of *L. shawii*. To demonstrate any toxic potential of *L. shawii* treatment, acute (24 h) and chronic (90 days) toxicity studies were conducted using mice as experimental model. Acute dosages were 0.5, 1.0 and 3 g/kg body weight (gavage) while chronic dosage was 100 mg/kg per day of the extract in drinking water. All morphological, biochemical, haematological and spermatogenic changes, in addition to mortality, body weight changes and any change in vital organs were recorded and compared with the respective control groups. Histopathological investigations were done on vital organs and compared with the control mice without treatment. *L. shawii* chronic treatment induced changes in body weight, biochemical and hematological parameters and was found to possess significant spermatatoxic potential.

Key words: *Lycium shawii*, blood glucose lowering potential, streptozotocin diabetes, toxicity biochemical and hematological effects.

INTRODUCTION

Saudi Arabia is generally an arid country with a few exceptional sub-humid regions on the south-western escarpments and is divided into three chorological units: the Saharo-Sindian, Somali-Masur, and Afro-Montane. The country has a desert climate except the province of Asir and Najran, characterized by extreme heat during the day, an abrupt drop in temperature at night, and slight, erratic rainfall. Diabetes has probably been known to medical sciences longer than any other hereditary metabolic disease. A large number of medicinal plants and plant-based remedies have been used in the treatment of diabetes in traditional medicine of different countries together with Saudi Arabia (Akthar and Iqbal, 1991; Jia et al., 2003). Diabetes is a growing epidemic around the world, which is regarded as chronic incurable condition caused by insulin deficiency that affects 10% of the population (Foster, 1994; Iraj et al., 2009). According to World Health Organization 171 million people worldwide suffered from diabetes in the year 2000 and the number of patients is increasing with different complications (WHO, 2000; American Diabetes Association, 2004). According to world ethanobotanical information about 1200 plants including *Lycium shawii*, are used in the control of diabetes (Jia et al., 2003; Tahraoui et al., 2007). In several cases, the folklore claims about hypoglycemic activity of medicinal plants were justified by scientific studies; however, no much attention is given to the toxic potential of herbal drugs (Gbolade, 2009; Singh et al., 2009; Al-Ashbanm et al., 2005). Saudi Arabia is the largest country in Middle

*Corresponding author. E-mail: hassan. botany@gmail.com.

East and the people live in both urban and rural areas. They depend on Arab Traditional System of Medicine for the health care and treatment of various diseases. The prevalence of diabetes mellitus in the general population is more than 10%. The general treatment of diabetes mellitus is based on oral hypoglycaemic drugs and/or insulin. However, according to world ethanobotanical information about 900 plants are used in the control of diabetes (Sher and Hussain, 2009). The aerial parts shoots and flowers of the plant *L. shawii*. (Solonaceae) locally called 'Gul Gaider' is used in the form of dry powder by traditional healers as anti-diabetic and hypotensive agent.

It is worth mentioning that several herbal drugs used in the cure of diabetes were found to possess significant toxicity in experimental models (Qureshi et al., 1990; Shah et al., 1991). The objective of the present study was to experimentally assess and justify the traditional anti-diabetic claim associated with *L. shawii* used by the local people in some parts of Saudi Arabia. However, there is a dearth of literature on the biological activity and toxicity of *L. shawii*. Keeping in view the prolonged use of this plant by diabetic patients, the present study was designed to investigate the hypoglycemic activity as well as toxic potential of this potent herbal drug following official procedures and the results are presented in the current communication.

MATERIALS AND METHODS

Plant collection and processing

The plant material was collected from Riyadh area of (Saudi Arabia) and the voucher samples (V.No: LS-2-009) were kept on record. The ethanobotanical information was obtained by conducting interview sessions held in different parts of Riyadh. The decoction of *L. shawii* aerial parts was found to be an accepted preparation taken by diabetic patients. In addition, it was used for common complains such as, giddiness, nausea, dizziness, and feeling of heaviness which were common symptoms of hypertension and hyperglycemia. It was interesting to notice that the plant material was also available in the local markets of Saudi Arabia and openly sold in some shops of herbal drugs dealers. Although having no information about the biological activities and toxicity of *L. shawii*, all believed its use is safe.

The collected plant material was air dried in shade and powdered by using electric grinder. Later, it was extracted with 80% ethyl alcohol and 20% water. The solvent was evaporated at reduced pressure at 40°C using Buchi Rotavapor R-114, and to thus obtained ethanol free concentrate 100 ml water was added. The crude extract was freeze-dried and kept in refrigerator for the conduct of different experiments. The total yield obtained was ~ 20% of the original material.

Preliminary phytochemical screening

L. shawii was subjected to preliminary phytochemical screening tests to verify the presence of alkaloids, flavonoids, terpenoids, reducing sugars, saponins, glycosides, polysaccharides, and tannins according to the standard procedures defined earlier (Islam et al., 2009). The following reagents and chemicals were used: alkaloids with Dragendroff's reagents, flavonoids with metallic magnesium plus HCl, saponins with the ability to produce foam, reducing sugars with Fehling's reagent, glycosides with Liberman's test, tannins with ferric chloride, and polysaccharides with iodine solution.

Experimental animals

The anti-diabetic activity testing was performed in male Wistar rats (weighing 240 to 300 g) maintained under standard laboratory conditions. In each group eight randomly selected animals were used. The freeze dried extract dissolved in water was injected i.p. (intra-peritoneal) or given p.o. (orally) in 500 mg/kg dose level (Tanira et al., 1988). For acute and chronic toxicity studies Swiss albino mice (home bred) aged 6 to 7 weeks, weighing 22 to 26 g, and fed on Purina Chow diet and water *ad libitum* were used in the present study. The animals were maintained under standard temperature, humidity, and automated 12 h light/dark cycle. The animals were randomly assigned to different controls and treatment groups separately. The official protocol for animal experiments was followed throughout the study.

Hypoglycemic activity in rats

Anti-diabetic activity was evaluated by different procedures using rats as experimental models (Parasad et al., 2009). In an initial experiment, a dose of 500 mg/kg body weight (p.o.), was found effective to cause a highly significant ($P<0.001$) decrease in blood glucose levels of rats in the treatment group (Table 1). This dose level was use throughout the current study.

(1) Non-diabetic normal rats model: The extract of *L. shawii* aerial part was evaluated for its hypoglycemic activity using 18 h fasted normal rat as an experimental model. The treatment groups were as follows:

Group 1: Control group (Treatment given: 1 ml/kg water, p.o.)
Group 2: Control group (Treatment given: 1 ml/kg water, i.p.)
Group 3: Positive control group (Treatment: Glibenclamide 0.2 mg/kg, p.o.)
Group 4: Positive control group (Treatment: Glibenclamide 0.2 mg/kg, i.p.)
Group 5: Treatment group (*L. shawii* extract 500 mg/kg body weight, p.o.)
Group 4: Treatment group (*L. shawii* extract 500 mg/kg body weight, i.p.)

In each case, blood samples for glucose levels were drawn by puncture from the tail immediately before administration and 20, 60, 120, 240, and 360 min after treatment.
(2) Chronically hyperglycemic rats were obtained by i.p. injection of 150 mg/kg of alloxan dissolved in distilled water (Trovato et al., 1993). After eight days of treatment, the hyperglycemic rats were selected (having plasma glucose level 2 to 8 g/l) and were used in the study. The aforementioned described experimental protocol was as followed. Glibenclamide (p.o.) and (i.p.) was used as a standard drug for reference.
(3) Glucose-loaded rats were treated orally with 0.25 g/kg glucose after 12 h fasting, however, the animals had free access to water. In each case, before feeding the extract or vehicle, the animals were kept for a minute in a desiccator saturated with ether vapours for anesthesia. The extract (0.5 g/kg body weight in water) treatment was given orally with a direct stomach-feeding syringe, which was simultaneously followed by glucose (2.5 g/kg body weight in 10 ml water) treatment. The control group rats were treated with equal volume of the vehicle. Blood samples were collected after 5, 10, 20, 40, and 60 min after treatment and analyzed for glucose levels.

Table 1. Blood glucose lowering potential of *L. shawii* extract at different dose levels in normal rats to select the suitable dose for current investigation.

Treatment (Dose)	Route	N	Blood glucose level (mg/dl) Mean ± S.E.M.	
			0 h	3 h
Control (Distilled water)	p.o	7	89.0 ± 3.0	88.0 ± 3.3
Glibenclamide (0.2 mg/kg)	p.o.	7	90.0 ± 3.3	60.0 ± 2.5**
L. shawii (125 mg/kg)	p.o.	7	90.0 ± 3.5	78.0 ± 4.7
L. shawii (250 mg/kg)	p.o.	7	87.0 ± 3.7	75.0 ± 4.5*
L. shawii (500 mg/kg)	p.o.	7	88.0 ± 3.3	65.0 ± 3.5**

*P<0.05, **P<0.001 (Student *t*-test), treatment group was statistically compared with the control group.

Blood collection and analysis

The blood was collected from the tail of animals in both the control as well as treatment groups, at zero hour before treatment and at defined intervals after treatment. The blood samples were processed by centrifugation and stored at -20°C till analyzed. A standard curve for the estimation of glucose was prepared and concentration was calculated by extrapolating the standard curve. Blood glucose level was estimated by using GOD-PAP glucose-oxidase method (Aslan et al., 2007).

Toxicity studies in mice

Acute toxicity

A total of 20 mice were randomly allotted to one control and 3 treatment groups (5 mice in each group). The drug (*L. shawii* extract) in each case was suspended in 0.1% CMC. The suspended extract was administered orally in three doses, namely: 0.5, 1.0, and 3 g/kg body weight. The toxic symptoms observed were autonomic responses, motor activity and CNS excitation, etc. The animals were observed for 24 h for all signs of toxicity and mortality. Acute treatment with 0.5 g/kg was found to cause significant lowering in blood glucose levels in the treatment groups as compared to the control; hence, this dose was selected as the pharmacologically active dose.

Chronic toxicity

A total of 20 male and 20 female mice were randomly allotted to the control and the extract-treated groups separately (10 male and 10 female animals kept separately in each group). *L. shawii* extract was given in drinking water. The dose selected was 100 mg/kg/day, which is 1/5 of the pharmacologically active dose (Tanira et al. 1988). The treatment was continued for a period of 3 months following W.H.O. protocol (W.H.O. 2000). The animals were observed for all external general symptoms of toxicity, body weight changes, and mortality daily, up to the end of the experiment to analyze the impact of treatment. The average pre- and post-treatment body weights, vital organ weights, and viscera of the chronically treated animals were compared with the control group. The blood was analyzed for white blood cells (WBC), red blood cells (RBC), and haemoglobin using Contraves Digicell 3100H (Zurich). The blood biochemistry was performed by using Bohringer kits.

Furthermore, the chronically treated male animals were also analyzed for spermatogenic dysfunction using the sperm abnormality test, which is considered a reliable parameter for assessing germ cell mutagenicity and carcinogenicity. The caudae epididymides and the vas deferens from the same animals were dissected out and transferred to a centrifuge tube containing 3 ml Krebs Ringer bicarbonate buffer as described earlier. The sperm suspension was filtered through an 80 µm silk mesh to remove tissue fragments and 0.5 ml of 1% eosin Y was added to each tube. The contents were thoroughly mixed and the slides were made by placing one drop of the solution on a slide and spread by three passes of another slide. Coded slides were examined for the following abnormalities of the sperm head: amorphous, flat head, microcephali, megacephali and swollen achrosome.

Histopathological procedures

Tissue samples of liver, heart, testis, spleen, lungs, and kidney were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using an American Optical Rotary Microtome, sections of thickness about 5 µm were cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes.

Statistical analysis

The different parameters studied during evaluation of anti-diabetic activity testing in rats and toxicity studies in mice, were subjected to statistical analyses by: Chi-square test, Student's *t*-test, ANOVA, Newman-Keuls test.

RESULTS

The results of the present study on the hypoglycemic activity in rats and toxicity studies in mice are presented in Tables 1 to 11. A single dose treatment with *L. shawii* extract (125, 250, 500 mg/kg) was found to induce statistically significant decrease in blood glucose levels of normal rats. A similar decrease in blood glucose level was also observed upon treatment with the standard anti-diabetic drug glibenclamide. Thus confirming the hypoglycemic potential of *L. shawii* extract (Table 1) and 500 mg/kg dose was selected for further studies.

The extract of *L. shawii* exhibited a remarkable hypoglycemic activity 20 min after oral (p.o.) and intraperitoneal (i.p.) administration in normal rats. The blood glucose levels reached a mean value of 0.56 g/l and 0.52 g/l after 2 h respectively as compared to 0.95 g/l

Table 2. Effect of *L. shawii* extract oral (p.o.) and intraperitoneal (i.p.) treatment on plasma glucose levels of normal rats.

Treatment (Dose)	Route	Plasma glucose level (g/l) and time after treatment (min) (Mean ± S.E.M.)					
		0	20	60	120	240	360
Control (water)	p.o.	0.98 ± 0.05	0.98 ± 0.07	0.97 ± 0.07	0.95 ± 0.09	0.95 ± 0.05	0.98 ± 0.06
Control (10 ml/kg)	i.p.	0.99 ± 0.04	0.97 ± 0.05	0.92 ± 0.08	0.93 ± 0.11	0.93 ± 0.08	0.95 ± 0.05
Glibenclamide (0.2 mg/kg)	p.o.	0.97 ± 0.06	0.74 ± 0.08*	0.55 ± 0.08***	0.60 ± 0.06**	0.77 ± 0.15	0.85 ± 0.06
Glibenclamide (0.2 mg/kg)	i.p.	0.95 ± 0.08	0.79 ± 0.06*	0.66 ± 0.09**	0.62 ± 0.08**	0.72 ± 0.05*	0.83 ± 0.09
L. shawii (500 mg/kg)	p.o.	0.94 ± 0.07	0.72 ± 0.06*	0.64 ± 0.16**	0.56 ± 0.09***	0.85 ± 0.15	0.80 ± 0.05*
L. shawii (500 mg/kg)	i.p.	0.98 ± 0.06	0.68 ± 0.08**	0.55 ± 0.18***	0.52 ± 0.15***	0.70 ± 0.07**	0.78 ± 0.16

*P<0.05, **P<0.01, ***P<0.001 (ANOVA and Newman-Keuls test), in each group 8 rats were used, treatment groups were compared to the respective control groups.

Table 3. Effect of *L. shawii* extract treatment on plasma glucose levels of alloxan-diabetic rats.

Treatment (Dose)	Route	Plasma glucose level (g/l) and time after treatment (min) (Mean ± S.E.M.)					
		0	20	60	120	240	360
Control (water)	p.o.	2.77 ± 0.30	2.60 ± 0.15	2.75 ± 0.45	2.27 ± 0.40	2.40 ± 0.33	2.90 ± 0.44
Control (10 ml/kg)	i.p.	2.72 ± 0.22	2.62 ± 0.22	2.65 ± 0.58	2.30 ± 0.45	2.32 ± 0.40	2.95 ± 0.42
Glibenclamide (0.2 mg/kg)	p.o.	2.92 ± 0.16	2.12 ± 0.20	1.55 ± 0.35**	0.95 ± 0.10***	1.45 ± 0.15***	1.80 ± 0.25**
Glibenclamide (0.2 mg/kg)	i.p.	2.98 ± 0.01	2.33 ± 0.29	1.38 ± 0.44*	1.65 ± 0.24**	1.50 ± 0.25**	1.87 ± 0.30*
L. shawii (500 mg/kg)	p.o.	2.90 ± 0.06	2.25 ± 0.37	1.60 ± 0.37*	1.20 ± 0.10***	1.10 ± 0.10***	0.90^{a} ± 0.15***
L. shawii (500 mg/kg)	i.p.	2.88 ± 0.10	1.92 ± 0.15*	1.25 ± 0.20**	0.86 ± 0.20***	1.28 ± 0.22***	1.22^{a} ± 0.15***

Treatment groups were compared to the respective control groups, *P<0.05, **P<0.01, ***P<0.001 (ANOVA and Newman-Keuls test), treatment groups were compared to glibenclamide group, (^{a}P<0.05), in each group 8 rats were used.

Table 4. Blood glucose levels in glucose-loaded rats before and after administration of *L. shawii* extract.

Treatment (Dose)	Route	Plasma glucose level (g/l) and time after treatment (min) (Mean ± S.E.M.)					
		0	5	10	20	40	60
Control (0.25 g/kg glucose)	p.o.	1.00 ± 0.06	1.30 ± 0.08	1.55 ± 0.15	1.67 ± 0.10	1.45 ± 0.08	1.30 ± 0.10
L. shawii (500 mg/kg)	i.p.	0.98 ± 0.08	1.20 ± 0.02	1.47 ± 0.10	1.48 ± 0.09*	1.35 ± 0.07***	1.10 ± 0.13**

*P<0.05, **P<0.01, ***P<0.001 (ANOVA and Newman-Keuls test), the treatment group was statistically compared with the control group, in each group 8 rats were used.

observed in the control group (Table 2). After 4 h of treatment (i.p.) the effect of *L. shawii* was significant as compared to the control.

The standard glibenclamide also induced a similar hypoglycemic activity in rats (Table 2). Both glibenclamide as well as the extract treatment showed some glucose lowering activity up to 6 h after treatment. *L. shawii* extract treatment in diabetic rats induced a significant decrease in glucose levels as compared with the rats in the control groups. A maximum decrease in plasma glucose levels was achieved after 2 h of p.o. and i.p. administration (Table 3). In a glucose tolerance test, the plasma glucose levels were increased in glucose-loaded rats. *L. shawii* extract (i.p.) treatment significantly reduced the glucose level at 20, 40, and 60 min to achieve the normal basal levels (Table 4).

Toxicity studies in mice

Keeping in view the appreciable anti-diabetic potential of *L. shawii* during current study and its use, the acute and chronic toxicity experiments were considered essential. Acute and chronic toxicity experiments were conducted in mice and the results obtained are illustrated in Tables 4 to 11.

Effect of acute treatment

No alarming signs of toxicity could be seen when mice were treated with *L. shawii* extract. However, mild increase in respiration and excitation were noticed in the mice treated with 3 g/kg dose level with *L. shawii* extract.

Table 5. Quantitative data on the mortality induced in mice on chronic treatment with ethanol extract of *L. shawii*.

Treatment and dose 100 mg/kg (3 months)	N		Mortality						Total dead animals		Lethality (%)	
			0-30 days		31-60 days		61-90 days		M	F	M	F
	M	F	M	F	M	F	M	F				
Control	10	10	0	1	0	0	0	0	0	1	0	10
L. shawii	10	10	0	0	1	1	2	1	3	2	30	20

*P < 0.05 (Chi-square test). M = male mice, F = female mice.

Table 6. Effect of chronic oral treatment with *L. shawii* extract on body weight changes in mice [a].

Treatment and dose 100 mg/kg (3 months)	Pre-treatment average body weight ± S.E.		Post-treatment average body weight ± S.E. [b]	
	Male	Female	Male	Female
Control (water)	24.4 ± 1.4	23.9 ± 1.2	32.0 ± 1.0**	31.7 ± 1.5**
L. shawii	24.8 ± 1.5	24.4 ± 1.3	27.2 ± 1.6*[c]	27.6 ± 1.2*[c]

Significant relative to pre-treatment values: *P < 0.05, **P < 0.01 (Student's *t*-test), [a] ten male and ten female mice were used in each group, [b] the average weight was calculated based on the number of surviving animals, [c] the average weights of mice in the treatment group were compared with the respective control group mice (P<0.05).

Table 7. Effect of chronic oral treatment with *L. shawii* extract on the water intake of mice.

Treatment and dose 100 mg/kg (3 months)	Pre-treatment average daily water intake ml ± S.E.		Post-treatment daily average water intake ml ± S.E. [b]	
	Male	Female	Male	Female
Control (water)	4.0 ± 0.2	3.9 ± 0.3	6.6 ± 0.3*	6.4 ± 0.5*
L. shawii	4.2 ± 0.4	4.0 ± 0.3	5.7 ± 0.7	5.0 ± 0.5

*P < 0.05, (Student's *t*-test), significant relative to pre-treatment values.

Table 8. Effect of chronic oral treatment with *L. shawii* extract on organ weights (per 100 g body weight) of mice [a].

Treatment and dose 100 mg/kg (3 months)	Average organs weight (per 100 g body weight)						
	Heart	Lungs	Liver	Kidney	Spleen	Testis	Seminal vesicles
Control	0.47 ± 0.02	0.77 ± 0.10	5.65 ± 0.27	1.52 ± 0.04	0.56 ± 0.08	0.69 ± 0.02	0.87 ± 0.13
L. shawii	0.46 ± 0.03	0.80 ± 0.19	5.76 ± 0.14	1.66 ± 0.07	0.53 ± 0.09	0.54 ± 0.03*	0.82 ± 0.15

* P < 0.05 (Student's *t*-test), [a] the tabular values represent the mean ± SEM of five randomly selected animals.

Table 9. Effect of chronic oral treatment with *L. shawii* extract on the haematological parameters in mice [a.]

Treatment and dose (100 mg/kg, 3 months)	WBC x 10^3 (N/ml)	RBC x 10^6 (N/ml)	Hemoglobin (%)
Control	5.50 ± 0.70	7.78 ± 0.22	11.92 ± 0.48
L. shawii	6.62 ± 0.85	8.26 ± 0.34	12.30 ± 0.55

P > 0.05 (Student's *t*-test), [a] the tabular values represent the mean ± S.E.M. of five randomly selected animals.

During the acute toxicity studies, no mortality was observed up to 3 g/kg dose level, indicating that the extract was less toxic in the given dose levels. There was a significant (P<0.01) fall in glucose levels of mice in all

Table 10. Effect of chronic oral treatment with *L. shawii* extract on the biochemical parameters in mice [a].

Parmaters	Control	*L. shawii*
Blood glucose (mg/dL)	88.00 ± 2.42	52.15 ± 3.35**
ALT/GPT (U/L)	22.09 ± 5.68	27.69 ± 4.87*
AST/GOT (U/L)	58.55 ± 5.54	66.75 ± 6.85*
CK-MB	135.62 ± 16.33	134.66 ± 10.98
Creatinine (µmol/L)	28.38 ± 5.05	29.59 ± 4.48
Urea (µmol/L)	8.74 ± 1.84	9.56 ± 1.57

*P < 0.05, **P < 0.01, (Students *t*'-test), [a] Five animals were used in each group, treatment groups were compared with the control group.

Table 11. Effect of *L. shawii* extract on the quality of epididymal spermatozoa after chronic treatment in mice.

Group	Total sperms screened	Percent sperm head abnormalities (Mean ± S.E.)						Abnormal sperms (%)
		Swollen achrosome	Amorphous	Microcephali	Megacephali	Rotated head	Flat head	
Control	5348	0.51 ± 0.07	0.45 ± 0.11	0.07 ± 0.03	0.05 ± 0.03	0.25 ± 0.08	0.05 ± 0.04	1.33 ± 0.24
L. shawii	5600	2.79 ± 0.81**	2.10 ± 0.15**	0.09 ± 0.03*	0.48 ± 0.06*	1.16 ± 0.22**	0.26 ± 0.07*	3.13 ± 1.30**

*P < 0.05, **P < 0.01 (Student' *t*-test).

the treatment groups as compared to the control. The visceral condition and all other biochemical and hematological indices were normal and comparable to the control.

Effects of chronic treatment

During chronic toxicity studies two male mice were found with fore-limb inflammation and snout alopecia at the end of 31 to 60 days of treatment with *L. shawii* extract. Two more male mice developed inflammation in the fore-limbs as well as in the hind limbs during 61 to 90 days of treatment. In the survival studies (Table 5) the lethality in the treatment groups was found higher as compared to the control group, however, it was statistically not significant.

Effects on body weight

During the chronic treatment with *L. shawii* extract, the body weight gain was arrested in both male and female treatment groups as compared to control group. The slowness of growth was evident by body weights and was significant at P<0.05 in male as well as female mice (Table 6).

Effect on water intake

There was a significant increase (P<0.05) in water intake of animals in both male and female control groups. However, increase in water intake in the treatment groups was statistically not significant indicating toxic effect due to weight arrest caused by the treatment (Table 7).

Effects on vital organ weights

In the present study, prolonged treatment for 90 days had minimal effect on organ indices of animals. The gross visceral condition of all male and female mice in the treatment groups was normal and comparable to the respective control mice. However, in the male treatment group there was a significant decrease (P<0.05) in the weight of testes as compared to the control (Table 8).

Effects on hematological and biochemical parameters

All the hematological parameters of both male and female mice remained within normal range and were comparable to the control groups after chronic treatment (Table 9). On the other hand, the biochemical studies revealed a significant decrease (P<0.01) in blood sugar levels and increase (P<0.01) in ALT/GPT and AST/GOT levels of animals in the treatment groups as compared to the control (Table 10). It is worth mentioning that both during acute as well as chronic toxicity studies, blood glucose lowering effect was observed as compared to the

respective control group mice (Table 10).

Effects on sperm morphology

Treatment of male mice with *L. shawii* extract during the current study for 90 days clearly increased ($p<0.01$) sperm morphological abnormalities as compared to the respective control group (Table 11). The indices screened for the morphological abnormalities, namely: swollen achrosomes, amorphous, microcephali, megacephali, rotated head and flat head showed significant increase in all these indices as compared to the control male mice, indicating spermatotoxic properties of *L. shawii* extract.

DISCUSSION

The results of the evaluation of anti-diabetic potential of *L. shawii* extract treatment clearly demonstrated its hypoglycemic potential in normal rats as well as hyperglycemic rats after both oral as well as intraperitonieal administration. The hypoglycemic effect induced by *L. shawii* could be comparable to the well established hypoglycemic drug glibenclamide. In addition, *L. shawii* treatment significantly reduced the plasma glucose levels induced by glucose load treatment during glucose tolerance test. The results of the present study confirmed the validity of folklore claims about anti-diabetic properties of *L. shawii*. The preliminary phytochemical screening of *L. shawii* showed the presence of flavonoids, terpenoids, glycosides, polysaccharides, and saponins. Many compounds belonging to such groups of natural products possess diverse biological activities, however, such compounds were earlier implicated as having anti-diabetic effects (Malviya et al., 2010; Qi et al., 2010).

Besides the named classes of chemical constituents detected in *L. shawii* during current phytochemical screening, mostly *L. shawii* is known to be a rich source of biologically active clerodan, labdane, and tricyclic clerodane-type diterpenoids such as limbatolides, and tetracyclic type of diterpenes possessing anti-inflammatory, cholinesterase-inhibiting, and cytotoxic activities (Al-Musayeib et al., 2000; Demetzos et al., 2001; Gholamhoseinian et al., 2008). Furthermore, diterpenods isolated from different sources were also reported to inhibit alpha-glycosidase, urease, HIV-1 transcriptase, and proplyl endopeptidase (Jassbi, 2006). Polysaccharides are known to help protect pancreatic islets and beta cells (Jia, 2003). However, among the natural compounds, flavonoids and saponins are reported as potent anti-diabetic agents (Aslan et al., 2007; Han et al., 2008; Misra, 2009). In an earlier experiment, quercetin and quercitrin were found to possess antidiabetic activity mediated via inhibition of alpha glucosidase associated with body glucose digestion and absorption (Shu et al., 2009; Widharna et al., 2010). In addition, antioxidant potential of plants plays a vital role in the control of diabetes and its complications (Ayatollahi et al., 2009). The observed hypoglycemic activity of *L. shawii* might be attributed to an enhancement of peripheral metabolism of glucose, besides the possible increased release of insulin caused by its chemical constituents (Park et al., 2008). Furthermore, terpenoids and flavonoids of *L. shawii* are also known for their inhibitory effect on digestive enzyme alpha glucosidase. Glycoside trimming enzymes are known to play a crucial role in metabolic path ways, such as glycoprotein and glycolipids processing and carbohydrate digestion in the intestinal tract. The anti-diabetic potential of *L. shawii* extract might partially be attributed to its chemical constituents possessing glucosidase inhibitory potential (Ayatollahi et al., 2009).

The rapidly increasing incidence of diabetes mellitus worldwide is becoming a serious threat to mankind health. Several medicinal plants or naturally occurring compounds isolated from plants were found to possess anti-diabetic activity with more efficacy than oral hypoglycemic drugs products used clinically (Han et al., 2008; Rauter et al., 2009; Qi et al., 2010). However, more studies are suggested to isolate and evaluate the chemical compounds responsible for such an activity shown by *L. shawii* and to explore their mechanism of action. It is interesting to specify that in most cases toxic manifestations of anti-diabetic herbs are not well documented and need more attention before prescribing such drugs for human use. In the current investigation, *L. shawii* extract demonstrated significant anti-diabetic activity. There is no report available in the literature on its toxicity, hence it was subjected to acute and chronic toxicity assessment using mice as experimental model.

During the current acute toxicity test, no alarming signs of toxicity were seen except mild increase in respiration and excitation in the animals treated with the highest dose of 3 g/kg. None of the mice died up to 3 g/kg dose level in *L. shawii* treatment group. Histopathological investigations proved the visceral condition and all vital organs to be normal and comparable to the control. Biochemical studies revealed that there was a significant decrease in blood glucose level of mice during acute treatment with *L. shawii*. However, all hematological indices remained unchanged up to 3 g/kg dose treatment as compared to the control. During chronic toxicity studies, two male mice developed fore-limb inflammation and alopecia, while one other male mice developed inflammation in their fore-limbs as well as hind limbs in the treatment groups as compared to the control. In the female treatment group, one mice developed snout alopecia after 60 days of treatment. There were no other signs of toxicity in the female treatment group as compared to the control. These findings clearly indicated that the prolonged *L. shawii* treatment is toxic. In the

survival studies after *L. shawii* treatment for 90 days (Table 5), the lethality was statistically non-significant in the both male and female mice in the treatment groups.

However, in the treatment groups, one male and one female mouse died during 31 to 60 days of treatment, while two male and one female mouse died during 61 to 90 days of treatment. These results further indicated some toxic potential of *L. shawii* extract on prolonged treatment in male mice.

During the current chronic regimen of treatment with *L. shawii* extract, the slowness of growth was evident by body weights of animals in the treatment groups as compared to the respective control groups. Similarly, the water intake was also affected. The saponins of *L. shawii* might be held responsible for decrease in weight gain in the treatment groups (Ntambi et al., 2003; Karu et al., 2007). Our results are supported by earlier observations where different saponins caused a significant decrease in weight gain of animals mediated by inhibition of pancreatic lipase activity (Karu et al., 2007). In addition, *L. shawii* extracts are also known to cause reduction in processing and intestinal carbohydrate absorption and possess glucosidase inhibitory activity (Gholamhoseinian et al., 2008; Ebrahimpoor et al., 2009; Widharna et al., 2010). In general, plants of family Laminaceae were found to contain neo-clerodane diterpenes, cis-clerodane limbatolides, diterpenoids, triterpenes, and different flavonoids possessing growth-inhibitory properties. Thus providing support to our findings on the slow growth of mice observed after 90 days of treatment with *L. shawii* extract as compared to the control mice.

In the present study, prolonged treatment of animals caused a significant reduction ($P<0.05$) in weight of testes. All other vital organs showed no significant changes as compared to the control. Biochemical parameters showed a significant ($P<0.01$) reduction in glucose levels in the treatment groups as compared to the control indicating hypoglycemic potential of *L. shawii* treatment. In general, several plants of family Solonaceae are known for their hypoglycemic properties. As a part of current study, *L. shawii* treatment was found to possess significant anti-hyperglycemic potential in different animal models. The major chemical constituents of Solonaceae, detected in *L. shawii* were flavonoids, terpenoids, and saponins. Several natural flavonoids and saponins are known for their multi biological function including their anti-diabetic effects (Kenjiro et al., 2006; Alyemeni et al., 2011). In addition, diterpenoids and some other *L. shawii* constituents were found to inhibit alpha glucosidases and thereby reduce blood glucose levels (Widharna et al., 2010; Khiyami et al., 2011). Hence, the hypoglycemic activity of *L. shawii* is attributed to its chemical constituents such as flavonoids, saponins, and terpenoids. The hypoglycemic effect of *L. shawii* treatment was slow and sustained, and without any risk of developing severe hypoglycemia. The hypoglycemic effect observed during the current toxicity studies on *L. shawii* and the results of our earlier investigations in different experimental models used for anti-diabetic activity, add support to the traditional claim and medicinal use of *L. shawii* by diabetic patient in different countries.

In the treatment groups, there was significant reduction in ALT/GPT and AST/GOT levels in the treatment groups as compared to the control group animals, which indicated some adverse effect on the liver of mice on chronic treatment. However, there were no significant changes in creatinine, urea, and CK-MB levels as compared to the control. Some of the *L. shawii* chemical constituents having anti-inflammatory and anticancer effects may be held responsible for such activity. However, histopathological investigations revealed that the liver of animals in the treatment groups was normal and comparable to the control.

After chronic treatment with *L. shawii*, all the hematological parameters such as WBC, RBC, and hemoglobin remained within normal range and were comparable to the control. Our results proved that *L. shawii* chronic treatment was devoid of hematological toxicity.

During current study, *L. shawii* chronic treatment significantly decreased ($P<0.05$) weight of testes in the male treatment group and significantly increased ($P<0.01$) the sperm morphological abnormalities as compared to the control. In the present study, on average, the percentage of abnormal sperms increase, in the treatment group, was up to 3.13 (± 1.30)%, while in the control male mice percent abnormal sperm were 1.33 (± 0.24)%. Noxious stimuli like chemicals and radiation are known to induce detrimental genotypic changes in the spermatogenic cells. The mouse sperm morphology assay, has been used to characterize mutagenic properties of a wide variety of chemicals. However, the damage induced in the sperm morphology during the current study, reflected genetic damage in the male germ cells, either due to small deletions or point mutations, or protein abnormality. Several anticancer compounds were earlier reported to be germ cell toxicant/mutagen. The spermatotoxic properties of *L. shawii* extract observed in the present study may be attributed to some of its chemical constituents possessing antineoplastic, anti-leukemic and anticancer potential (Akthar and Iqbal, 1991).

Based on the results of present study, it is suggested that special caution must be taken when decoction of *L. shawii* or its extracts are added to special foodstuff formulated for people with diabetes. Furthermore, the results of the current study provide basic information about the toxicity of *L. shawii* that might be helpful in planning future pre-clinical experiments on this potent natural drug.

It is also worth mentioning that in our earlier experiments on herbal drugs with antidiabetic properties, for example: *Artemisia abyssinica*, *Teucrium polium* (Al-Ashban et al., 2005; Premurkumar et al., 2011), and now

L. shawii treatment, all were found to possess spermatotoxic activity and further studies are warranted on this issue.

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