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Research Article

EVALUATION OF *IN VIVO* ANTI-DIABETIC ACTIVITY OF ROOT OF CRATAEVA MAGNA LOUR., ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The purpose of the study is to evaluate the anti-diabetic effect of the ethanolic extract of root of *Crataeva magna* Lour., on streptozotocin-induced diabetic rats.

Methods: Streptozotocin (45 mg/kg) was used to induce the diabetes mellitus in rats. Afterward, the diabetic rats will be divided into four groups Group I rats served as diabetic control rats, Group II rats were induced diabetes and treated with standard drug through oral intragastric tube, Group III and Group IV were treated with ethanolic extract (200 and 400 mg/kg). Glibenclamide as reference was to evaluate the effect of the extract.

Results: The administration of extract decreased the fasting blood glucose and body weight as well as increased level of total cholesterol and triglycerides which and decreased and HDL level. SGPT and SGOT level were significantly reduced in treatment group. Blood urea and creatinine were a significant difference in blood urea and creatinine.

Conclusion: The extract of *C.magna* Lour. exhibited significant anti-diabetic activity evident from blood glucose level, body weight, serum cholesterol profile, SGOT, SGPT, serum creatinine, and serum urea level.

Keywords: *Crataeva magna*, Root, *In vivo*, Acute toxicity, Anti-diabetic, Biochemical parameters.

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INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia is associated with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. It is a heterogeneous disorder. The central disturbance in DM is an abnormality in insulin production or action or both [2]. In Greek medicine diabetes means "A melting of the flesh and limbs into urine. The patients never stop making water but the flow is incessant, their mouth becomes parched and their body dry" [3].

Crataeva magna (*C. magna*) belonging to family Capparidaceae is a multipurpose tree that is also used to increase appetite and for the treatment of various diseases such as rheumatism and nephrotoxicity, arthritis, and urinary disorders. It is a small wild or cultivated tree native to India, China, Bangladesh, Sri Lanka, Burma, Thailand, Cambodia, Laos, Indonesia, and Malaysia. *C. magna* is a small tree with a much branched head is found to be distributed mainly in the warmer (tropical) parts of the world. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorder, kidney bladder stones, fever, vomiting, and gastric irritation. Leaves are deciduous three foliolate, petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, and pale beneath [4].

METHODS

Plant collection and identification

Dried entire root of *Crataeva magna* Lour. was collected from the forest around Kutralam hills, Tirunelveli District, Tamil Nadu (India), in the month of October. It was authenticated by Prof. V. Chelladurai, Research Officer-Botany (Scientist-C) (Rtd), Central Council for Research in Ayurveda and Siddha, Palayamkottai, Tirunelveli District, Tamil Nadu, Govt. of India.

Preparation of plant extract

The powdered plant material (50g) was extracted by hot continuous Soxhlet extraction method. The plant material was extracted with ethanol (99.9% v/v), 500 ml for 2 days in a percolator.

Procedure

Weighed about 50 g of dried powdered root and transferred into thimble for packing. While packing, the content was wetted with ethanol and then poured until the siphon tube was filled. A piece of porcelain was added into the round bottom flask to avoid bumping effect. It is a process of continuous extraction method in which the solvent can be circulated through the extractor several times. The vapors from the solvent are taken to the condenser and the condensed liquid is returned to the extract for continuous extraction. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

In vivo anti-diabetic study

Experimental animals

The present study was conducted as per CPCSEA/IAEC approval no:1917/ReBi/S/16/CPCSEA/25.10.2016 and 7/AEL/IAEC/MMC, Date:12.9.2017. The Wistar rats used for this study were procured from Animal Experimental Laboratory, Madras Medical College, Chennai-03, India.

Toxicity study

Acute toxicity study was designed as per the OECD guidelines (423).

In vivo anti-diabetic evaluation

The anti-diabetic activity of *Crataeva magna*Lour. was evaluated in diabetic Wistar rats. Diabetes was induced by intra-peritoneal injection of streptozotocin (45 mg/kg body weight) kept for 48 h. The anti-diabetic effect of plant extract was compared with Standard drug Glibenclamide.

Induction of diabetes

Rats were rendered diabetic by a single intra-peritoneal injection of freshly prepared STZ (45 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) in the volume of 1 ml/kg body weight using sterile 25G needle. Diabetes was identified in rats by moderate polydipsia and marked polyuria. After 48 hours of STZ administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose level ranging between 200 and 300 mg/dl were considered diabetic and used for the experiments.

Experimental design

The animals were divided into four groups of six animals each. Group I rats served as diabetic rats, Group II rats were induced diabetes and treated with standard drug glibenclamide (2 mg/kg body weight) through oral intragastric tube, Group III animals were induced diabetes and treated with ethanolic root extract of *C. magna* (200mg/kg body weight), and Group IV rats were treated with ethanolic root extract of *C. magna* only 400 mg/kg body weight. The extract was given daily through oral gastric tube for a period of 30 days.

The fasting glucose and body weight of all animals were recorded at the beginning of the study. The blood glucose was checked by one-touch glucometer throughout the study in the experiments, 24 rats were divided into four groups of six animals each.

Group I: Streptozotocin (45mg/kg.i.p) induced diabetic control rats received 5% CMC.

Group II: STZ-induced diabetic rats received Standard Glibenclamide 2mg/kg p.o for 30 days

Group III: STZ-induced diabetic rats received 200mg/kg ethanolic extract of *Crataeva magna* Lour. for 30 days.

Group IV: STZ-induced diabetic rats received 400mg/kg ethanolic extract of *Crataevamagna* Lour. for 30 days.

All rats body weight was measured before the induction of diabetes and on 1, 15, and 30 days of treatment. Blood glucose level was measured on 1st, 15th, and 30th days of the study period by tail tip cutting method. At the end of the experiment on 30th day, 2 ml of blood was collected by retro-orbital bleeding from all animals under anesthesia for estimation of biochemical parameters [5].

Biochemical parameters

The blood samples were centrifuged at 3000 rpm for 5 min using REMI (412 LAG) cooling centrifuge. The serum was kept at –80°C until analyzed. Levels of total cholesterol [6], triglycerides (TGL) [7] and HDL [8], Serum Glutamate Oxaloacetic Transaminase (SGOT) [9], Serum Glutamate Pyruvic Transaminase (SGPT) [10], Serum creatinine [11], and urea [12] were determined with an analytical instrument (Hitachi 911, Japan).

Statistical analysis

The values were represented as Mean±SEM. Data were analyzed using one-way ANOVA followed by Dunnett's test for the comparison of means. The statistical tool applied for the analysis was GraphPad prism software version 7.3. P values < 0.05 were considered to be significant.

RESULTS

Toxicity study

Acute toxicity study revealed that the Ethanolic extract of root of *Crataeva magna* Lour., was relatively nontoxic up to 2000 mg/kg/b.w, p.o indirectly pronouncing safety profile of the extract.

DISCUSSION

In the present study, after the administration of extract (200 and 400 mg/kg), the fall in fasting blood glucose was evident at the end. Fall in fasting blood glucose levels was progressively noticed till the end of the study. When the reduction of blood glucose levels with 200 mg/kg and 400 mg/kg was compared, there was a statistically significant reduction in blood glucose levels ($p<0.0001$) exhibited with 400 mg/kg of the Ethanolic root extract of *Crataeva magna* Lour. (Table 1 and Fig. 1). Diabetic induced rats showed significant reduction in body weight. After the administration of *Crataeva magna* Lour., root extracts (200 and 400 mg/kg.p.o) and Glibenclamide (2mg/kg.p.o) to the diabetic rats restored the changes in the body weight (Table 2 and Fig. 2).

The increased level of total cholesterol and triglycerides which were decreased and HDL level was increased significantly, after the treatment of glibenclamide and ethanolic extract of root of *Crataeva magna* Lour., (Table 3 and Fig. 3). The SGOT and SGPT level were significantly reduced in the treatment groups (Table 4 and

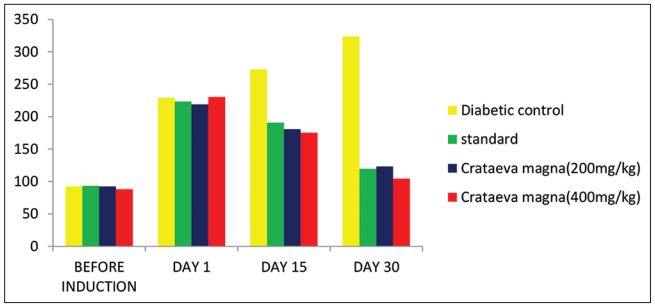

Fig. 1: Graphical representation of mean blood glucose level

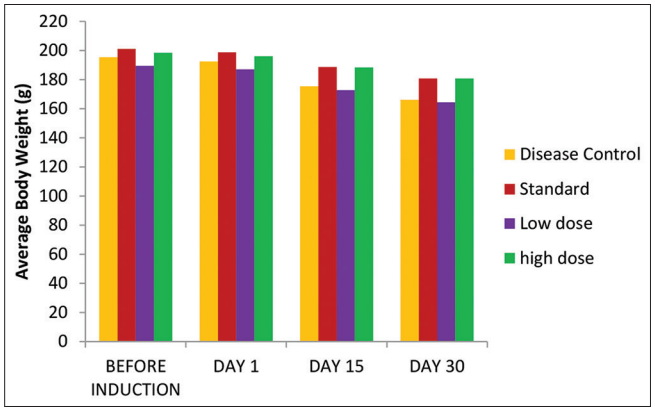

Fig. 2: Graphical representation of effect on body weight

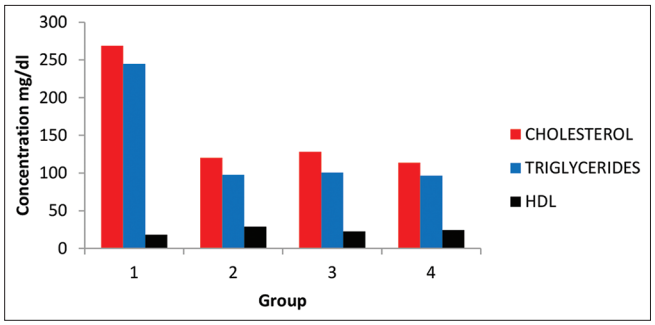

Fig. 3: Graphical representation of effect on lipid profile levels

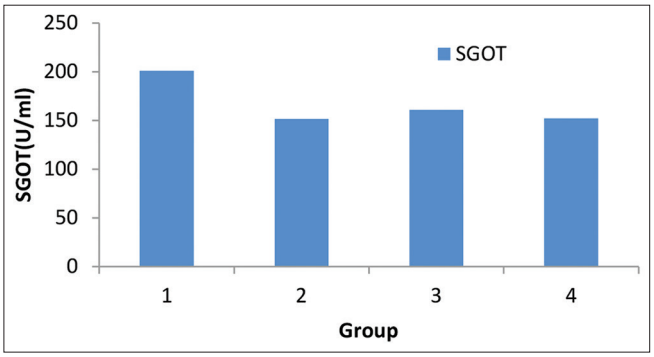

Fig. 4: The graphical representation of SGOT levels

Table 1: Estimation of whole blood glucose level

Group	Treatment	Whole blood glucose (mg/dl)			
		Normal (before induction)	1st day	15th day	30th day
I	Diabetic control STZ (45 mg/kg)	92.33±2.18	229.33±5.94	273.16±6.70	323.83±5.89
II	Glibenclamide (2 mg/kg)	93.33±2.76	223.33±11.60^{ns}	190.83±7.11****	119.5±3.54****
III	*Crataeva magna* L, extract 200 mg/kg	92.33±2.41	218.83±6.39^{ns}	180.66±7.10****	123.16±3.73****
IV	*Crataeva magna* L, extract 400 mg/kg	88.16±2.84	230.33±7.06^{ns}	175.33±5.02****	104.50±1.64****

The values are expressed as mean±SEM (n=6). The data's were analysed by using one-way ANOVA followed by Dunnett's multiple comparison. *P* values ****p≤0.0001, ***p≤0.001, **p≤0.01, *p≤0.1, ^{NS}p>0.05 compared with disease control.

Table 2: Estimation of body weight

Group	Treatment	Average body weight (g)			
		Normal	1st Day	15th Day	30th Day
I	Diabetic control STZ (45 mg/kg)	198.6±1.42	195.3±1.97	178±3.30	167.66±1.96
II	Glibenclamide (2 mg/kg)	202±2.92	200.33±2.80^{ns}	192.33±3.55**	185±3.21***
III	*Crataeva magna* L extract 200 mg/kg	201.3±1.8	199±1.91^{ns}	190.33±1.08**	182.66±1.68*
IV	*Crataeva magna* L extract 400 mg/kg	208±2.30	206±2.30**	197.66±1.81***	190.66±1.60***

Table 3: Estimation of serum lipid profile

Group	Treatment	Cholesterol	Triglycerides	HDL
I	Diabetic control STZ (45 mg/kg)	268.85±2.48	244.71±4.99	18.08±0.55
II	Glibenclamide (5 mg/kg)	120.24±0.87****	97.60±0.64****	28.88±0.57****
III	*Crataeva magna* Lour., extract (200mg/kg)	128.11±1.23****	100.60±1.13****	22.39±0.41****
IV	*Crataeva magna* Lour., extract (400mg/kg)	113.64±1.08****	96.49±0.61****	24.53±0.36****

Table 4: Estimation of serum SGPT, SGOT, urea and creatinine

Group	Treatment	SGPT (U/ml)	SGOT (U/ml)	UREA (mg/dL)	CREATININE (mg/dL)
I	Diabetic control	106.64±0.75	201.32±0.90	51.11±0.56	1.45±0.02
II	Glibenclamide (2 mg/kg)	82.73±0.76****	151.73±1.19****	43.64±0.73****	0.72±0.01****
III	*Crataeva magna* Lour., (200 mg/kg)	87.33±0.72****	161.05±1.46****	41.57±0.50****	0.94±0.005***
IV	*Crataeva magna* Lour., (400 mg/kg)	81.77±0.66****	152.40±0.96****	41.23±0.52****	0.87±0.17**

The values are expressed as mean±SEM, n=6. The data's were analyzed using one-way ANOVA followed by Dunnett's multiple comparison. p values ****p≤0.0001, ***p≤0.001, **p≤0.01, *p≤0.1, ^{NS}p>0.05 compared with disease control

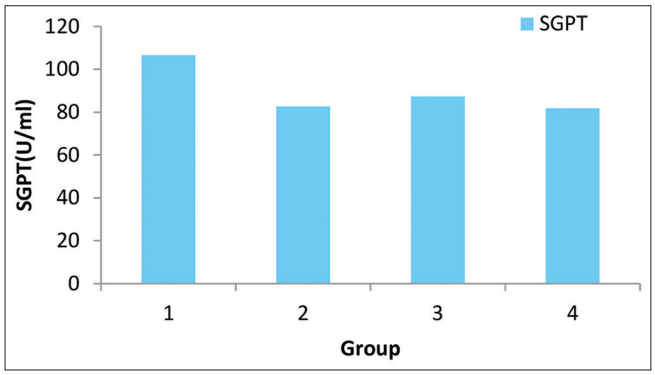

Fig. 5: The graphical representation of SGPT level

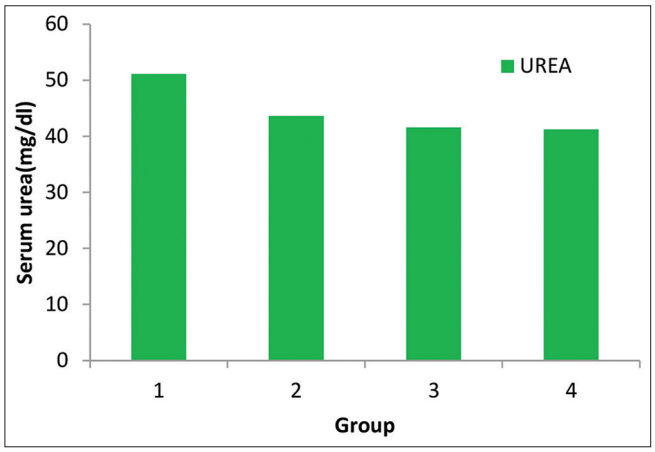

Fig. 6: The graphical representation of serum urea levels

Figs. 4 and 5). Moreover, SGOT and SGPT levels also act as indicators of liver function and restoration of these parameters to normal levels indicate normal functioning of liver.

Blood urea and creatinine were elevated in Group I (Diabetic control). When comparing the Groups II, III, and IV with Group I, there was a significant difference in blood urea and creatinine (Table 4 and Figs. 6 and 7). This shows that in the groups which received *Crataeva magna* Lour., ethanolic root extract there was no significant increase in urea and creatinine levels. The data of this present studies may suggested that ethanolic extract of root of *Crataeva magna* Lour., has beneficial effects in management of diabetes mellitus holding the hope for anti-diabetic drugs, based on this extract in the near future.

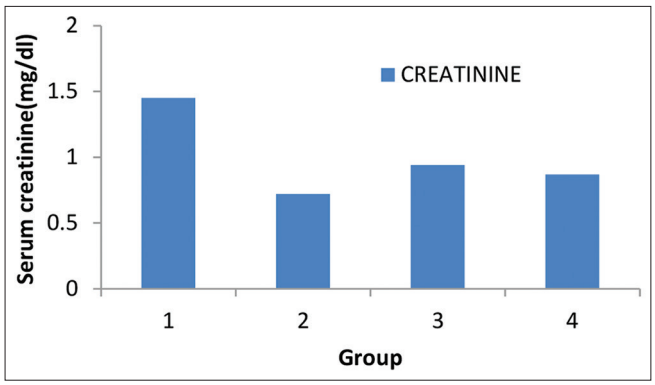

Fig. 7: The graphical representation of serum creatinine level

CONCLUSION

From the above findings, it was concluded that ethanolic extract of root of *Crataeva magna* Lour. possess antidiabetic activity. The mechanism by which it elucidates its anti-diabetic potential has to be determined.

AUTHOR'S CONTRIBUTION

All the authors contributed equally in research.

CONFLICT OF INTEREST

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