Hypoglycaemic effects of Methanolic extract of *Canscora decussata* (Schult) whole-plant in normal and alloxan-induced diabetic rabbits

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Abstract: In present study hypoglycaemic effects of the crude powdered *C. decussata* and its methanolic extract (ME) in alloxan diabetic rabbits were evaluated. The hypoglycaemic effect was measured by blood glucose, insulin level, HbA1c and his to pathology of pancreas. Glucose lowering effect of the ME was studied in diabetic rabbits. The effects of extract on blood glucose, body weight, food in take, fluid intake, OGTT were also evaluated. The results showed that 0.5,1 and 2g/kg of the powder significantly decreased blood glucose levels in normal rabbits and diabetic rabbits at the intervals checked. Oral intake of pioglitazone also reduced the levels in these rabbits. Synergistic hypoglycaemic effect of 600mg/kg of ME with different doses of insulin (2 & 3unit/kg, s/c) further reduced blood glucose levels of treated alloxan-diabetic rabbits. The oral glucose tolerance test revealed lowered area under curve values in ME treated rabbits. Treatment with ME (400 and 600 mg/kg) for 30 days showed highly significant decrease in blood glucose level by augmenting insulin secretion, HbA1cand significant increase in body weight, serum insulin levels in treated diabetic rabbits. Histopathology study showed regeneration of β-cells. These studies have, therefore, supported the traditional use of this herb in diabetic patients.

Keywords: Diabetes mellitus; *Canscora decussata;* alloxan; rabbits; exogenous insulin.

INTRODUCTION

It has been reported that diabetes is a growing threat to the general public, while the frequency is rapidly developing (Ghaffar *et al*., 2004). Currently available therapies for diabetes include insulin and various (oral as well as inject able) anti-diabetic agents such as sulphonylureas, biguanides, α-glucosidase inhibitors, meglitinides, thiazolidinediones, DPP -IV inhibitors, bile acid sequestrant, D-2 dopamine-receptor agonists, GLP-I receptor agonists, amylin analogues which are used as monotherapy or in combination to achieve better glycemic regulation, these agents have a number of serious adverse effects (Beigi, 2012). Herbal drugs are of low cost and free from adverse effects (Akhtar *et al*., 2011). The World Health Organization enumerated 21,000 plants, which are employed for medical goals around the world (Modak *et al*., 2007). One of such plant is *Canscora decussata* (Family: Gentianaceae), locally known as Sankhaholey is an indigenous medicinal plant that has been used empirically for centuries as a hypoglycemic agent in the ethnomedicinal practices (Chughtai *et al.,* 1974). Pharmacological investigation of *C. decussata* demonstrated various biological activities including anticonvulsant, immuno modulatory, antipyretic, anti-inflammatory and analgesic, diuretic, Spermicidal, hepatoprotective and gastro protective, cardiovascular, anti tuberculosis, antidepressant, nervine tonic, alternative and laxative (Madan *et al*., 1960; Bhattacharya *et al*., 1972; Dikshit *et al*., 1972; Dixit *et al*., 1971; Ghosal *et al*., 1975; Ghosal *et al.,* 1978; Shankarnarayan *et al*., 1979; Tyagi *et al*., 1990; Shah *et al*., 2000; Madan *et al*., 2002; Madan *et al*., 2003). This plant has been reported to contain triterpines, alkaloids and xanthones (Kokate *et al*., 2002). However, as far as ascertained no previous investigation was reported regarding its hypoglycaemic effects. Therefore, this study was carried out to find out some useful or novel agent or lead compound for the treatment of diabetes.

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MATERIALS AND METHODS

Plant material

Fresh whole plants of *Canscora decussata* (Schult) was collected from an urban area of Lahore (Pakistan) during the month of August, 2010 which was got identified and authenticated by the taxonomist of University of Sargodha, Sargodha. Voucher specimen No.UOS/CD/333 was deposited in the herbarium, University of Sargodha, Sargodha.

Preparation of powdered plant and its extract

The plant in sufficient quantity were washed with water to remove adhering dirt and then completely dried under shade. They were powdered with a Chinese herbal grinder and the powdered was stored in well-closed cellophane bags at 4ºC in a refrigerator till further use. The whole plant powdered material was used for preparation of

methanolic extract by cold maceration; percentage yield was 19.5%. The extract was dried with rotary evaporator.

Chemicals and diagnostic kits

Glucose oxidase kits (Accu Check, Abbott Laboratories, USA), alloxan monohydrate (Research Organics, USA), methanol (Merck Chemical Co., Germany), gum acacia (UniChem, Germany), insulin (Regular Humulin, Lilly, USA). The drug pioglitazone was a generous gift from MAAN GEE distributors, Sargodha.

Animals

Rabbits of either sex, weighing about 1000-1500g were used in this study. All rabbits were housed at animal house, University of Sargodha in stainless cages under controlled conditions of humidity, temperature 22±2°C and 12 h-light-dark cycle. Animals were fed green fodder and water *ad libitum*. The experimental protocol was approved by the Institutional Animals Ethical committee (Reg. No. 570/09/A/UOS).

Grouping of animals

Hypoglycemic effects of ME of Canscora decussata whole plant in normal rabbits

Rabbits were divided into five groups of six animals each, normal control, ME treated (three groups) and pioglitazone treated. Group 1 animals served as untreated normal controls and were administered orally 20mL of 2% gum acacia solution into water only. Groups 2, 3 and 4 were given orally 0.5, 1.0 and 2g/kg body weight of ME suspended in 2 per cent gum acacia aqueous solution, respectively. Group 5 animals were treated orally with 3mg/kg body weight of pioglitazone.

Hypoglycemic effects of ME of Canscora decussata whole plant in alloxan- induced diabetic rabbits

In this study, rabbits were fasted overnight and divided into five groups of six animals each normal control, ME treated (three groups) and pioglitazone treated. Group 1 animals served as untreated diabetic controls and were administered orally 20mL of 2% gum acacia solution into water only. Groups 2, 3 and 4 were given orally 0.5, 1.0 and 2g/kg body weight of ME suspended in 2 per cent gum acacia aqueous solution, respectively. Group 5 animals were treated orally with 3mg/kg body weight of pioglitazone.

Induction of experimental diabetes

After overnight fasting, rabbits were made diabetic by i.v injection of fresh solution of 150mg per kg body weight of alloxan monohydrate (Akhtar *et al*., 2002). After three days blood glucose level of surviving rabbits was measured and rabbits with blood glucose levels between 250-300 mg/dl were used for further study (Olajide *et al.,* 1999; Shani *et al.,* 1974).

Administration of plant extracts

The test plant suspensions were administered orally to each animal by using a stomach tube and disposable syringe. Pioglitazone was also administered orally after suspending in 2% aqueous gum acacia aqueous solution.

Biochemical analysis

Blood glucose levels were determined by the glucose oxidase method using Accu Check advantage II Roche Chemicals, Switzerland while blood samples were collected from the jugular vein. Serum insulin levels were measured by the micro plate ELISA method using a commercial kit (Diasorin, Saluggia, Italy) (Pari and Latha, 2002) HbA1c levels were estimated by an autoanalyser (Hitachi 912, Germany).

Histopathology of pancreas

The whole pancreas from each animal was removed after sacrifing the animal and washed on ice-cold saline immediately. A portion of pancreatic tissue was fixed in 10% formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5m and the sections were stained with haematoxylin and eosin (Strate, 2005).

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. The statistical analysis was carried out using t-test and one-way analysis (ANOVA). Statistical $P<0.05$ was considered to be significant**.**

RESULTS

Experiments with single dose administration

Hypoglycemic effects of powdered CD whole plant in normal and diabetic rabbits

Blood glucose levels after single oral administration of 0.5,1 and 2 g of powder and pioglitazone at a dose of 10 mg/kg are shown in (table. 1). Oral administration of 0.5,1 and 2g of powder and 10 mg pioglitazone produced significant ($P<0.05$ or $P<0.001$) reduction in blood glucose level at checked intervals in normal and diabetic rabbits but level of normal and diabetic rabbits treated with acacia does not show any significant ($P>0.05$) alteration when compared with zero hr level.

Hypoglycemic effects of ME of C. decussata (equivalent to 200,400 and 600 mg/kg of powder) in alloxan-induced diabetic rabbits

Blood glucose levels after single oral administration of ME at a dose of 200,400 and 600 mg/kg are shown in fig. 1.ME treated rabbits also exhibit significant reduction in blood glucose level at checked intervals with highly significant of ME from 2^{nd} to 24^{th}hr with maximum dose 600mg as in fig. 1. However DC group showed no significant reduction in blood glucose level.

Hypoglycemic activity of ME of C. decussata whole plant with different doses of insulin in alloxan-induced diabetic rabbits

As fig. 2, showed that diabetic rabbits treated with 6 units of insulin/kg b.w 2 and 3 units of insulin plus 600 mg/kg of extract significantly (P<0.001) reduced blood glucose level within 1-hr of drug administration. The administration of 600mg/kg extract also produced highly (P<0.001) hypoglycemic effects at 1, 2, 3 and 4hr intervals.

Oral glucose tolerance test (OGTT)

Fig.3 shows the oral glucose tolerance for ME and pioglitazone (600and 3mg/kg, respectively). After glucose load, It was observed that diabetic control rabbits showed higher AUC glucose values. Administration of ME (600mg/kg) showed significantly (P<0.001) lower AUC glucose values compared to zero hr value. But, pioglitazone significantly (P<0.05) lower AUC glucose values from 120min to onward

Experiments with the repeated dose administration (duration of treatment 30 days)

Effect of extract on fasting blood glucose levels and on serum insulin level

Fig. 4 clearly showed that ME highly significantly (P<0.001) reduced the blood glucose level of diabetic rabbits treated for 30 days while pioglitazone significantly reduced at 15 days and highly significant at 30th day. There was no significant change in blood glucose levels of group-1treated with 2% aqueous gum acacia solution while fig. 5 showed that it significantly increase the insulin level of the diabetic treated rabbits as compared to diabetic group in dose dependent manner.

Effect of* ME *on weight, food and water intakes of rabbits

Table 2 showed that in extract and pioglitazone treated groups these parameters stabilized as compared to diabetic control group.

Histological examination

Histopathological observations showed improvement i.e. regeneration of β-cell in the rabbits treated with ME for 30 days as compared to diabetic control and relatively improvement also observed in pioglitazone treated rabbit (fig. 6).

Experiment with the repeated dose administration (duration of treatment 90 days)

Effect of Me of C. decussate on haemoglobin (Hb) and glycosylated haemoglobin (HbA1c) levels in diabetic rabbits

ME significantly (P<0.05) increased the Hb level while significantly (P<05) decreased the HbA1c level in diabetic treated rabbits during three-month study as compared to diabetic control group (fig. 7).

Phytochemicals constituent of methanolic extract of Canscora decussata

The phytochemicals study showed that methanolic extract contain alkaloids, tannins, saponins, cardiac glycoside, flavonoid, reducing sugar, steroids and terpenoids (Venkatesh *et al*., 2008).

DISCUSSION

The current study was carried out to determine the hypoglycemic activity of *CD* in normal as well as alloxan diabetic rabbits and was observed that administration of 0.5,1 and 2g/kg of *C. decussata* to both type of rabbits produced significant effect at the time intervals checked. Previously similar effects of different medicinal plants including *Berberis aristata* have been reported (Akhtar *et al*., 2011). Standard drug pioglitazone produced highly significant (P<0.001) decrease of blood glucose both in normal and diabetic rabbits. Hypoglycemic effect of ME was also studied in diabetic rabbits (fig. 1). The ME produced the maximum decrease in blood glucose level (fig. 1). The 600mg/kg of extract also produced synergistic effects with insulin hypoglycaemic (fig. 2). These findings clearly showed that there is some active principle (s) in ME of *CD* that possess insulin like effect. The results were also in line with previous study (Ahmad *et al*., 2009). In the present study, the oral glucose tolerance tests showed that the ME lowered AUC value after 30 min (fig. 3) while in diabetic control group after glucose load, the glucose levels remained elevated even after 5 hours. Similar findings have also been reported with the administration of the *Caralluma sinica* in streptozotocin-induced diabetic rabbits (Habibuddin *et al*., 2008). In addition, the administration of the ME was found to prevent an increase in blood glucose levels may be probably due to the restoration of insulin response (table 2). The administration of the ME at 600mg/kg dose continuously for 30 days highly significantly (fig.4) lowered blood glucose levels. Similarly, pioglitazone significantly reduced blood glucose levels after 30 days. Alloxan destroys β-cells of the islets of Langerhans, resulted in massive reduction in insulin release (Li *et al.,* 2002). In our study, we observed a significant increase in the insulin level (fig. 5) of alloxan-diabetic rabbits after treated with ME (400 mg and 600 mg/kg) continuously for 30 days, the increase in levels of insulin has indicated that the hypoglycaemic action exerted by the extract of *C. decussata* may be due to the pancreatic release of insulin from the existing β-cells of islets of Langerhans i.e. direct insulinotropic effect. The significant and continuous hypoglycemic effects of the ME in diabetic rabbits can also be due to increased glucose utilization in peripheral tissues, as proposed for the hypoglycemic effect produced by the administration of ethanol extract the root extract of *Plumbago zeylanica* to diabetic rats (Olagunju *et al*., 1999). The phytochemical studies of ME have showed the presence of alkaloids, tannins, carbohydrate, terpenes,

reducing sugar, saponins, cardiac glycosides, tannins and flavonoid. It has been reported that flavonoid and terpenes possess anti-diabetic actions (Marles and Farnsworth, 1995). It have been reported that flavonoid cause proliferation of pancreatic β-cells leading to increase secretion of insulin as the mechanism by which they reduced hyperglycemia caused by streptozotocin in diabetic rats (Mahesh and Menon, 2004; Sri-Balasubashini *et al.,* 2004), while tannins such as tannic acid have been reported to stimulate glucose utilization and inhibited the adipocyte differentiation (Liu *et al.,* 2005). It is conceivable, therefore, that the hypoglycaemic effect would be exercised by ME due to these ingredients i.e. tannins and flavonoid. Since in diabetes, excess glucose produced in the body reacts with Hb to form HbA1c which is used as marker to determine the degree of protein glycation during diabetes mellitus. Thus we also conducted a three months study in diabetic rabbits which showed that ME of the test plant significantly reduced the HbA1c level while the Hb level of diabetic

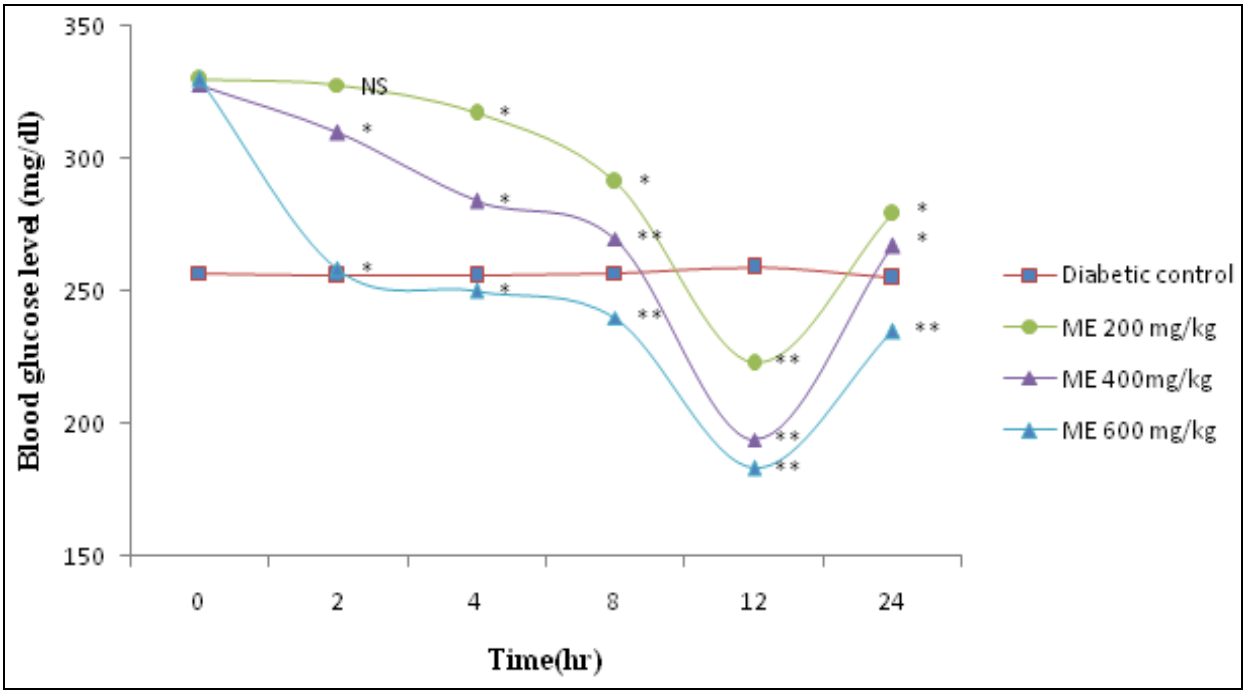

Fig. 1: Effect of ME in diabetic rabbits. Where, ME= Methanolic extract.
Values are statistically significant at *=P<0.05, **=P<0.001 compared to zero hr.

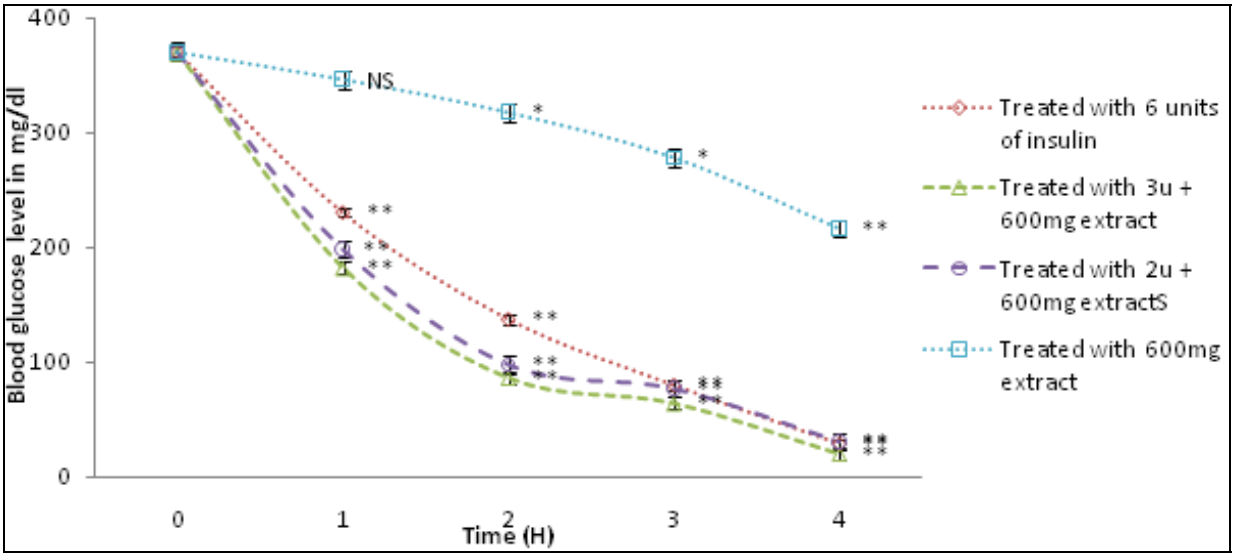

Fig. 2: Hypoglycemic activity of methanol extract with different doses of insulin in diabetic rabbits, Values are statistically significant at *=P<0.05, **=P<0.001 compared to zero hr.

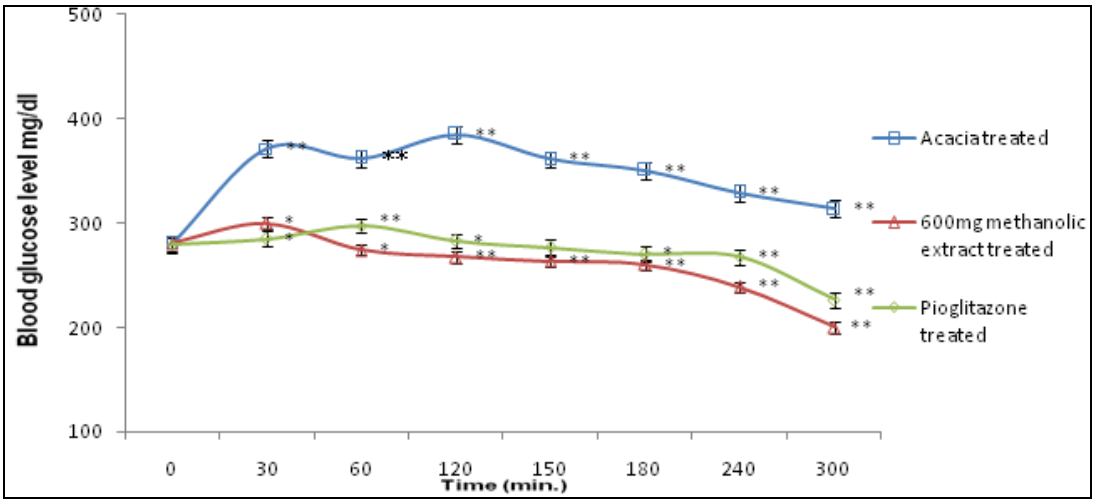

Fig. 3: Effect of ME on oral glucose tolerance test (mg/dL ±SEM) of alloxan -diabetic rabbits. Values are statistically significant at *=P<0.05, **=P<0.001 compared to zero level value.

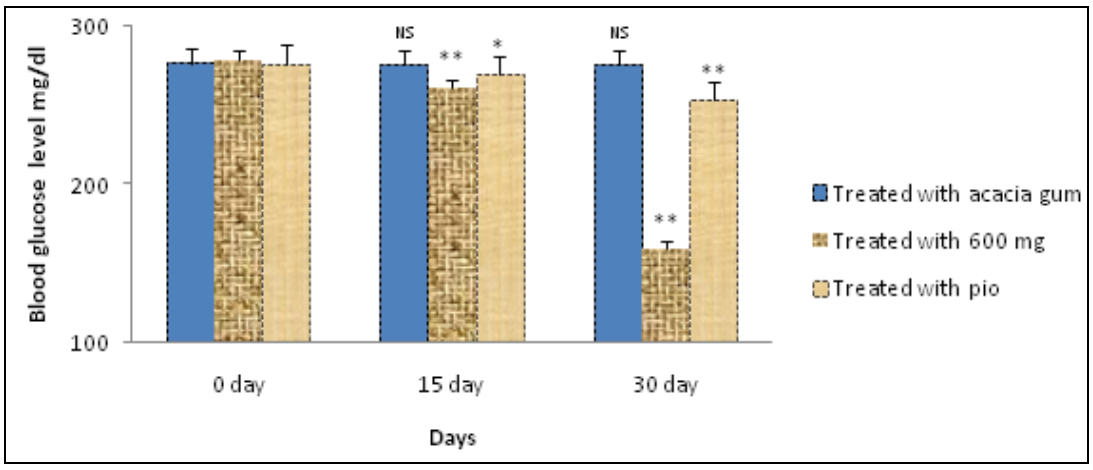

Fig. 4: Blood glucose level of diabetic rabbits in (mg/dl ± SEM) on 0,15and 30th days. Values are statistically significant at *=P<0.05, **=P<0.001 compared to 0 day.

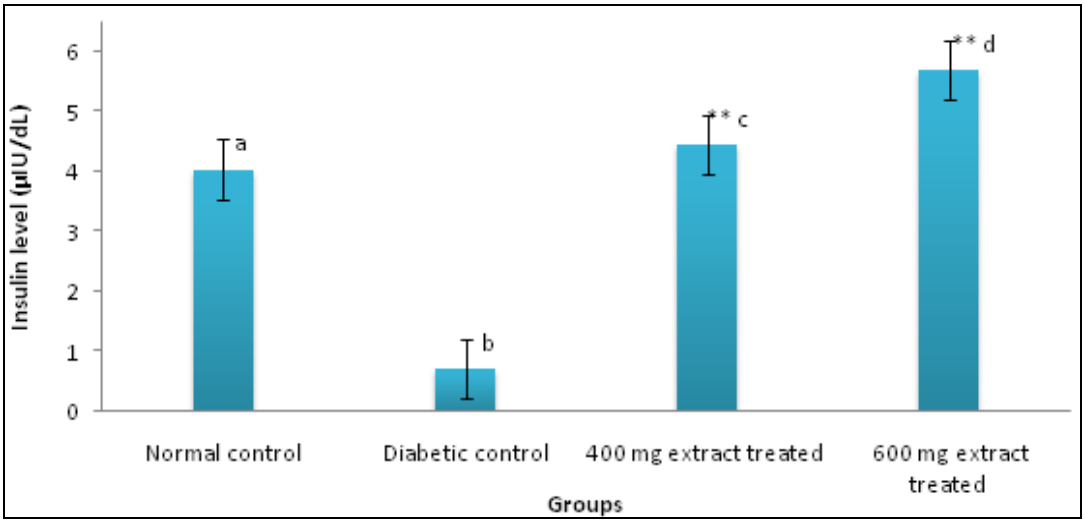

Fig. 5: Insulin levels (µIU/dl± SEM) of normal and extract treated groups after 30 days. Values are statistically significant at *=P<0.05, **=P<0.001 compared to diabetic group.

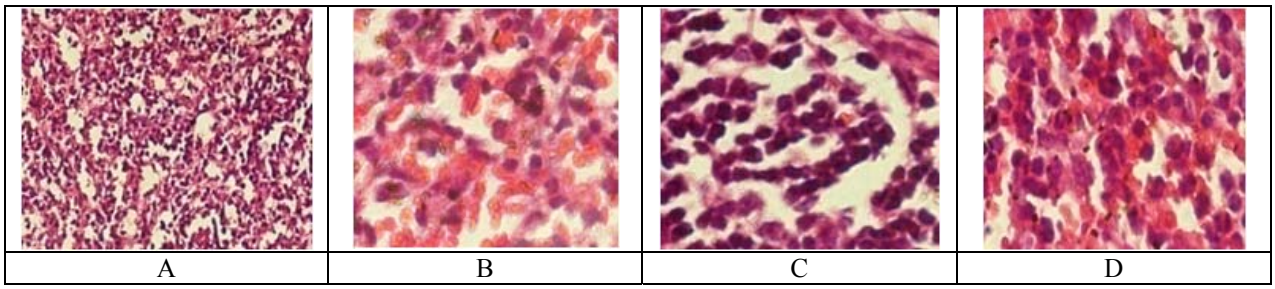

Fig. 6: Histology of pancreas in experimental rabbits after 30 days of treatment (A) Normal control-showing no hemorrhages (B) Diabetic control-showing massive destruction and scattered red blood cell and hemorrhage. (C) Diabetic + ME (600 mg)-showing no hemorrhages relatively normal pancreas. (E) Diabetic + Pio. (3mg)-less hemorrhage than diabetic control

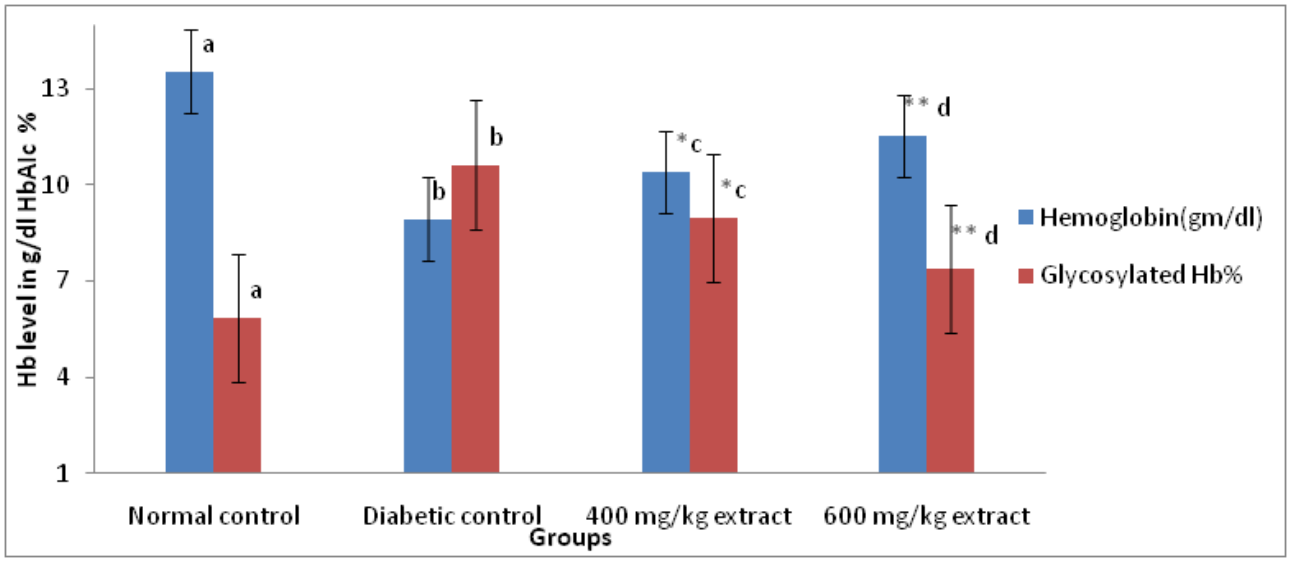

Fig. 7: Hb and HbA1c levels in normal, diabetic and diabetic treated rabbits after 90 days Values are statistically significant at *=P<0.05, **=P<0.001 compared to diabetic group.

Table 1: Mean blood glucose level of rabbits in mg/dl ± SEM at various time intervals after oral administration of 2% gum acacia aqueous solution, and powdered *C. decussata* in 0.5g, 1g and 2g/kg dosing.

Time Intervals	0hr	2hr	4hr	8hr	12hr	24hr
Normal Rabbits						
Acacia	99±0.25	98±0.71^{NS}	97±0.56^{NS}	98±0.83^{NS}	95±0.91^{NS}	100±0.71^{NS}
0.5g	96±0.75	94±0.50^{NS}	97±1.28^{NS}	80±1.04**	68±1.60**	88±0.56*
1.0g	98±0.71	83±1.10**	80±1.04**	70±0.80**	63±1.87**	75±1.26**
2.0g	99±0.25	82±0.83**	79±0.66**	77±1.13**	57±0.66**	72±1.21**
Pio	97±0.56	75±0.70**	69±0.91**	56±0.75**	67±0.56**	65±0.83**
Diabetic Rabbits						
Acacia	257±0.91	256±0.97^{NS}	256±0.97^{NS}	257±0.91^{NS}	259±0.91^{NS}	255±1.21^{NS}
0.5g	258±1.04	255±0.83^{NS}	253±1.53*	251±1.28*	225±0.87**	253±1.36*
1.0g	258±1.04	238±1.71**	224±1.21**	220±1.04**	205±0.91**	245±1.04**
2.0g	260±1.01	226±1.53**	220±0.94**	200±1.01**	174±1.26**	240±0.80**
Pio	263±1.26	242±1.13**	196±1.67**	190±0.66**	162±0.71**	175±1.69**

Values are given as mean ± SEM for 6 rabbits in each group; experimental groups are compared with zero hr level. Values are statistically significant at *=P<0.05, **=P<0.001, ^{NS}=Not significant.

Table 2: Effect of methanolic extract on weight (kg ±SEM), food intake (g ±SEM) and water intake (ml ±SEM) of normal and diabetic rabbits at $0^{th}$and 30^{th} day of drug administration

Groups	Dose	Body weight (kg)		Food intake (g)		water intake (ml)	
		initial	final	initial	Final	Initial	Final
Normal control	2% acacia	1.54±0.01	1.55±0.01	19.9±0.72	20.6±0.38	20.4±0.39	22.5±0.62
Diabetic control	2% acacia	1.55±0.01	1.13±0.10	13.4±0.43	18.1±0.51	19.7±0.78	25.12±0.96
Diabetic +Extract	600 mg	1.56±0.01	1.54±0.01*	18.3±0.56	18.0±0.36*	19.1±1.32	19.3±0.95*
Diabetic + Pioglitazone	3 mg	1.59±0.01	1.58±0.01*	19.0±0.55	18.98±0.55*	20.01±0.45	19.99±0.77*

* P<0.05, ** P<0.001vs. Diabetic control

rabbits was increased in graded dose manner (fig. 7). Previously, it has been reported that the fruits *Citrullus Colocynthis* reduced HbA1c levels thereby increasing Hb levels in streptozotocin-induced diabetic rats (Jayaraman *et al.* 2009). The advanced glycated end product (AGE) is associated with severe diabetic complications (Mc Cance *et al*, 1993. Sell *et al,* 1992). The ME of the plant may be advantageous to reduce diabetic complications. Proteolysis, lipolysis and fluid loss during acute diabetes, contributing to weight loss (Alberti and Zimmet, 1998). The ME (600mg/kg) significantly reduced the blood glucose level and prevented the loss in weights of the diabetic rabbit during one-month study. It may be, therefore, suggested that the ME contained some growth factor (s) that might have stimulated the body anabolism to maintain the body weight. These findings are also supported by (Effraim *et al*., 1999; Gupta *et al.,* 2008). ME also normalized fluid intake and feed intake in the treated diabetic rabbits (table 2). Finally, histological studied conducted showed that treatment with ME cause regeneration of (β-cell) and restored normal cellular size island without hemorrhage. It is thus apparent that the hypoglycemic effect is likely due to pancreatic mechanism. Pioglitazone treated group also show improvement in pancreatic histology, which has already reported by Kanda *et al.,* (2009). The histological study further confirmed that the given extract is effective and can act in the management of diabetes. The present investigation has properly supported folkloric use of plant medicine in Eastern traditional medicine.

CONCLUSION

In view of the above-described studies, it has been found that the ME exercise lasting hypoglycemic effects in diabetic rabbits. These results suggest that the active principle (s) responsible for the hypoglycemic action may be due to stimulation of insulin release from pancreatic β-cells in normal as well as in alloxan-induced diabetic animal that has a direct insulin-like action. It seems sufficient to continue and implement additional activity-oriented fractionation and isolation of active principle (s) studies of this abundantly available indigenous medicinal plant.

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