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ANTI-DIABETIC ACTIVITY;

SCREENING OF CASSIA SOPHERA FOR ANTIDIABETIC ACTIVITY IN NORMAL AND ALLOXAN TREATED DIABETIC RABBITS.

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ABSTRACT… Objectives: The study was designed to evaluate the hypoglycaemic effects of Cassia sophera's powdered seeds and its different fractions. **Study Design:** An experimental study. **Setting:** Faculty of Veterinary, University of Agriculture, Faisalabad, Pakistan. **Period:** November 2014 to February 2017. **Methodology:** Initial experiments were designed to find out the hypoglycaemic effects of Cassia sophera powdered seeds in increasing doses i.e. 2, 3, 4g/kg body weight at 0, 2, 4, 8, 12 and 24 hours intervals. The effects of methanolic and aqueous extracts equivalent to 4g/kg body weight of Cassia sophera seeds were also studied in both normal and diabetic rabbits. **Results:** At 4 hours interval, 2grams/kg body weight of grinded seeds results no significant decrease in mean blood level of glucose. At 8 and 12 hours intervals, a significant decrease while at 4, 8 and 12 hours a highly significant reduction were observed with both 3g/kg and 4g/kg body weight. With methanolic and aqueous extracts equivalent to 4g/kg body weight, normal rabbits showed significant decrease i.e. with P value less than 0.05 at 8 and 12 hours. In addition, the methanolic extract of Cassia sophera seeds produced better hypoglycaemia as compared to aqueous extract. The effects of these extracts were comparable to Acetohexamide. The same study was also done in alloxan-induced diabetic rabbits and it was noticed that Cassia sophera powdered seeds caused significant decrease in blood glucose level at increasing doses 2, 3 and 4gram/kg of body weight and highly significant reduction (with P value less than 0.001) in blood level of glucose was noticed with methanolic extracts at 4 hours interval which continued upto 8 hours while treatment with aqueous extract with Cassia sophera seeds showed highly significant decrease at 12 hours. **Conclusion:** It is concluded that powdered seeds of Cassia sophera constitutes more than one type of hypoglycaemic elements, which have exerted a significant and consistent hypoglycaemic effects in normal and alloxan treated diabetic rabbits.

Key words: Anti-diabetic, Hypoglycaemic, Cassia Sophera.

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INTRODUCTION

Diabetes mellitus is a syndrome with altered metabolism and inappropriate glycaemic control due to either a deficient insulin secretion (Type 1 diabetes) or a combination of insulin deficiency and insulin resistance (Type 2 diabetes). There are some medicinal plants, which have been reported to be effective in diabetes globally and use traditionally as anti-diabetic agent.[1]

Diabetes is a major public health problem worldwide. About 382 million people were suffering from diabetes in 2013. It is proposed that by 2035, this figure is going to increase up to 592 million. Most people with diabetes live in low- and middle-income countries.[2] In Pakistan, the prevalence of diabetes is 3.5% for women and 6.0% for men in urban while 2.5% for women and 6.9% for men in rural areas. In urban versus rural areas, the Inappropriate glucose tolerance is 6.3% for men and 14.2% for women against 6.9% for men and 10.9% for women respectively.[3]

Interestingly many of the indigenous medicinal plants have been traditionally used to treat hyperglycaemia and Diabetes Mellitus.[4] These include Citrullus colocynthis, Artemisia, Opuntia streptacantha, Trigonela foenum

gracum, Momordica charantia, Polyglasenega, Gymne-masylestre, Allium sativum andaloe. However, few plants have received scientific or medical scrutiny.[5] Information available about compounds obtained from different botanical families which are responsible for hypoglycaemic activity i.e. glycoside isolated from families like Caesalpiniaceae, Papaveraceae, Ranunculaceae, Rhamnaceae and Scrophulariaceae, glycans of Ranunculaceae and Granmineae. In addition, certain triterpenes from Ranunculaceae family also got hypoglycaemic activity.[6] Peptides of Papolionceae, saponins of Malvaceae and alkaloids of Zygophyllaceae, Apocyanaceae, Papaveraceae and Rhamnaceae found to be effective in decreasing blood level of sugar.[7] However, search for newer anti-diabetic plants continues.

In our study, Cassia sophera was used for its hypoglycaemic potential. Cassia sophera grows in India and also in Sindh, Pakistan. Its seeds have been used since time immemorial in ethno medicine for many varied medicinal purposes including respiratory disorders, common cold, asthma and osteoarthritis.[8] The root is useful in thirst, urinary discharges; cures tumours, skin disease and asthma. The leaves are effective in ulcers, leprosy and skin ailments. The flowers shows beneficial effects in sore throat, urinary discharges and diabetes mellitus. The seeds are used in ophthalmic diseases, diabetes mellitus and dysentery.[9] The hypoglycaemic activity of the seeds of this plant has been evaluated and compared with an oral hypoglycaemic agent acetohexamide in both normal and diabetic rodents. Further to explore the hypoglycaemic effects by different fractions, its methanolic and aqueous extracts equivalent to 4gram/kg of body weight powdered seeds producing maximum response were also administered to normal as well as alloxan treated diabetic rabbits.

MATERIALS AND METHODS

An experimental study was carried out at Faculty of Veterinary, University of Agriculture, Faisalabad, Pakistan from November 2014 to February 2017.

Preparation of Aqueous Extract

The seeds were made to fine powder with a grinder at room temperature and stored in well-closed bottles at 4°C. Hundred grams of powdered seeds were kept for maceration in distilled water, agitated occasionally.[9] After passing through fine filter, extract was dried in Petri dish at 40°C.[10]

Preparation of Methanolic Extract

Soxhlet apparatus was used to prepare methanolic extract. Powdered form of seeds (100 grams) were put into1000ml of methanol and the extract obtained was allowed to evaporate at 40°C. Before instillation to rabbits, the dried methanol extract was dissolved in distilled water.

Experimental Animals

Healthy male rabbits of Oryctologus cunniculus species weighing 1000-1500g were selected in this study. Before starting the experiments, animals were kept under close observation for one week in animal house. The animals were fed upon fresh green fodder and clean water.

Preparation of Alloxan-Diabetic Rabbits

Rabbits weighing 1-1.5kg were selected and alloxan monohydrate was injected in a dose of 150mg/kg body weight to make them diabetic. The dose/animal was weighed, dissolved in distilled water and then injected by a tuberculin syringe slowly intravenously.[20] Eight days after injection, blood glucose levels of the surviving rabbits were determined by glucose oxidase method.[13] Rabbits with blood glucose level of 300-500mg/100ml were taken as diabetic and enrolled in our study.

Grouping of Rabbits

One hundred and twelve rabbits were randomly divided into two major groups i.e. Normal Non-diabetic and Alloxan treated diabetic rabbits:

Group I: Normal (Non-diabetic) Rabbits

There were fifty-six rabbits in Group 1, which were further divided into following eight sub-groups.

Subgroup A_1, an untreated control group, received 20ml 2% gum tragacanth solution in water only while subgroups B_1, C_1 and D_1 were administered

with 2, 3 and 4grams/kg body weight of Cassia sophera's powdered seeds orally.

Subgroup A_3, an untreated control, received 20ml 2% gum tragacanth solution while B_3, C_3 and D_3 were administered 4g/kg of body weight of methanolic extract of Cassia sophera seeds, 4g/kg of body weight aqueous extract of Cassia sophera seeds and 500mg/kg of body weight oral dose of Acetohexamide respectively.

Group II: Diabetic (Alloxan-Treated) Rabbits

There were fifty-six rabbits in GroupII, which were further divided into following eight sub-groups.

Subgroup A_2, an untreated control, received 20ml 2% gum tragacanth solution in water only while B_2, C_2 and D_2 were administered 2, 3 and 4grams/kg body weight of Cassia sophera's powdered seeds orally.

Subgroup A_4, an untreated control, received 20ml 2% gum tragacanth solution while subgroups B_4, C_4 and D_4 were administered 4g/kg of body weight of methanolic extract of Cassia sophera seeds, 4g/kg of body weight aqueous extract of Cassia sophera seeds and 500mglkg of body weight oral dose of Acetohexamide respectively.

Statistical Analysis

Data has been expressed as mean ± SEM while student's T test was used to calculate the hypoglycaemic effects and to compare the efficacy of various doses of the study plant at different time intervals.

RESULTS

Effects of Cassia sophera powdered seeds on blood level of glucose in normal rabbits

As shown in Table-I, Gum tragacanth did not affect the blood levels of glucose. Comparing results of subgroups B_1, C_1 and D_1, 4g/kg body weight dose is more effective than other doses used. This is obvious from the results that in 4g/kg body weight dose after 2 hours intervals, there was significant decrease in blood glucose levels ($t_{(14)} = 2.45$; $p<0.05$) compared to that of 3g/kg body weight dose. In similar comparisons between 4g/kg vs 3g/kg body weight at 4 hours intervals ($t_{(14)} = 6.62$; $p<0.001$), 8 hours interval ($t_{(14)} = 2.65$; $p<0.05$), 12 hours interval ($t_{(14)} = 6.62$; $p<0.001$) there was very significant decrease in glucose levels with 4g/kg body weight dose. At 24 hours interval mean blood glucose levels increase but they were non-significantly less than that in 3g/kg body weight dose.

Effects of Cassia sophera powdered seeds on blood level of glucose in Alloxan treated diabetic rabbits

As shown in Table-II, comparing results of subgroups B_2, C_2 and D_2, the mean blood level of glucose at 24 hours interval raised nearly equivalent to that at time zero. At 8 hours time interval 2g/kg body weight vs 3g/kg body weight shows non-significant increase in mean blood glucose levels in the later case. However, at this time interval (8 hours) 3g/kg body weight vs 4g/kg body weight shows highly significant decrease in later group ($t_{(14)} = 14.38$; $p<0.001$). Both 3g/kg body weight ($t_{(14)} = 2.28$; $p<0.05$) and 4g/kg body weight groups ($t_{(14)}=2.21$; $p<0.05$) showed significant decrease at 12 hour compared to 8 hour blood glucose levels.

Effects of Methanolic and Aqueous extracts of Cassia sophera seeds on blood level of glucose in Normal rabbits

Table-III shows that efficacy of methanolic extract of Cassia sophera seeds is better than aqueous extract in lowering the blood level of glucose. This fact is more evident at 8 hours interval ($t_{(14)} = 3.20$; $p<0.05$) and 12 hours intervals ($t_{(14)} = 4.98$; $p<0.05$). In comparison with aqueous extracts at 2 and 4 hours intervals, the blood glucose levels were significantly low with Acetohexamide treatment.

Effects of Methanolic and Aqueous extracts of Cassia sophera seeds on blood level of glucose in Alloxan treated diabetic rabbits

As displayed in Table-IV, methanolic extract has better results than metformin in developing hypoglycaemia. At 4 hours interval, there was highly significant decrease in blood levels of glucose with methanolic extract as compared to metformin ($t_{(14)} = 3.34$; $p<0.001$). Similarly, methanolic extract caused significant reduction

in glucose level at 8 hours intervals ($t_{(14)}$ = 6.11; $p<0.001$) and 12 hours interval ($t_{(14)}$ = 2.90; $p<0.05$) compared to that of metformin was observed.

Time interval (Hours)	20ml 2% gum tragacanth	Cassia sophera powdered seeds (g/kg body weight)		
	A_1	B_1 2	C_1 3	D_1 4
0	95.13 ± 1.48	97.63 ± 1.97	99.31 ± 1.76	92.50 ± 2.3
2	95.00 ± 1.16	95.69 ± 1.39	96.00 ± 2.13	88.33 ± 1.0
4	94.44 ± 1.91	94.25 ± 1.54	87.75 ± 1.98	83.33 ± 1.4
8	95.00 ± 1.44	92.63 ± 0.97	83.19 ± 1.96	76.66 ± 0.5
12	94.00 ± 1.61	89.13 ± 0.90	84.56 ± 0.91	72.50 ± 0.9
24	94.63 ± 1.57	97.19 ± 1.79	97.44 ± 1.89	91.25 ± 1.9

Table-I. Mean levels of blood glucose (mg/dl) in normal rabbits at 0, 2, 4, 8, 12 and 24 hour intervals, post oral administration of 20ml 2% gum tragacanth as control and Cassia sophera's powdered seeds (2, 3 and 4 g/kg body wt):

Time interval (Hours)	20ml,2% gum tragacanth	Cassia sophera powdered seeds (g/kg body weight)		
	A_2	B_2 2	C_2 3	D_2 4
0	343.54 ± 9.0	345.00 ± 7.94	362.97 ± 7.31	373.13 ± 16.74
2	339.91 ± 8.19	340.09 ± 8.53	358.97 ± 7.31	367.84 ± 15.77
4	339.63 ± 8.25	334.47 ± 7.09	352.44± 7.69	357.91 ± 13.58
8	339.41 ± 8.52	320.03 ± 8.16	344.58 ± 7.8	325.38 ± 5.58
12	335.71 ± 8.15	315.00 ± 6.32	308.91 ± 7.79	300.09 ± 5.85
24	338.79 ± 9.19	338.91 ± 9.00	356.84 ± 6.53	370.00 ± 15.61

Table-II. Mean levels of blood glucose(mg/dl) in diabetic rabbits at 0, 2, 4, 8, 12 and 24 hour intervals post oral treatment with 20ml 2% gum tragacanth as control and Cassia sophera'spowdered seeds (2, 3 and 4 g/kg body wt):

Time interval (Hours)	20ml,2% gum tragacanth	Cassia sophera powdered seeds extract equivalent to 4g/kg body weight		Acetohexamide (500mg/kg body weight)
		Methanolic	Aqueous	
	A_3	B_3	C_3	D_3
0	95.13 ± 1.48	90.13 ± 2.54	98.31 ± 1.19	89.75 ± 1.24
2	95.00 ± 1.16	89.06 ± 1.21	87.88 ± 0.98	74.38 ± 1.46
4	94.44 ± 1.91	85.00 ± 0.96	84.94± 0.80	74.81 ± 0.91
8	95.00 ± 1.44	76.38 ± 1.12	83.88 ± 1.22	76.69 ± 1.48
12	94.00 ± 1.61	73.56 ± 0.96	80.63 ± 0.86	84.69 ± 0.86
24	94.63 ± 1.57	89.81 ± 1.44	89.94 ± 1.36	86.00 ± 0.88

Table-III. Mean levels of blood glucose (mg/dl) in normal rabbits at 0, 2, 4, 8, 12 and 24 hour interval post oral treatment of 20ml 2% gum tragacanth as control, methanolic and aqueous extracts of Cassia sophera's powdered seeds (equivalent to 4g/kg body wt) and Acetohexamide(500mg/kg body wt):

Time interval (Hours)	20ml, 2% gum tragacanth	Cassia sophera powdered seeds extract equivalent to 4g/kg body weight		Metformin (500mg/kg body weight)
		Methanolic	Aqueous	
	A4	B4	C4	D4
0	343.54 ± 9.0	365.34 ± 5.16	332.97 ±7.89	360.59 ±7.13
2	339.91 ± 8.19	343.22 ± 5.57	320.13 ±8.02	336.94 ±3.02
4	339.63 ± 8.25	315.38 ± 3.71	319.50 ±2.59	330.00 ±1.79
8	339.41 ± 8.52	311.41 ± 2.99	313.00 ±2.90	352.00 ±3.65
12	335.71 ± 8.15	304.78 ± 7.15	301.13 ± 5.61	335.38 ±3.38
24	338.79 ± 9.19	358.41 ± 5.96	368.63± 5.88	350.56 ±4.80

Table-IV. Mean levels of blood glucose(mg/dl) of diabetic rabbits at 0, 2, 4, 8, 12 and 24 hour intervals post oral treatment with 20ml 2% gum tragacanth as control, methanolic and aqueous extracts of Cassia sophera's powdered seeds (equivalent to 4g/kg body wt) and Metformin (500mg/kg body wt):

DISCUSSION

In the present study, Cassia sophera was used for experimentation. The seeds of this plant was used in different forms i.e. crude extract, methanolic extract and aqueous extract to find out in which form it will be more effective in developing hypoglycaemia. Different treatment doses, i.e. 2, 3 and 4grams/kg body wt were tried with Cassia sophera, which indicated that 4g/kg body weight dose is the one that developed better hypoglycaemic effect. Methanolic extract of Cassia sophera reduced blood glucose levels after 2 hours of treatment and continued upto 12 hours. The efficacy of methanolic extract of Cassia sophera powdered seeds was more than aqueous extract. Placebo and diabetic control groups did not show appreciable difference in blood levels of glucose at different intervals.

Doses of Cassia sophera have more prolonged duration of action as compared to the Acetohexamide, which has been reported to produce significant hypoglycaemic effect in human patients for about 6-8 hours.[10]

Alloxan, a selective beta cytotoxic drug has been demonstrated to produce in the treated animals all the clinical signs of human diabetes i.e. hyperglycaemia, Glycosuria, Polydipsia, Polyuria and Polyphagia and loss in body weight.[11] It has been observed that the single intravenous injection of 150mg/kg of alloxan to rabbits destroys their pancreatic β cells[12] therefore; this dose of alloxan already known to kill the β cells of the rabbits was selected for the present experiment. Thus, administration of alloxan was observed to increase the blood level of glucose in rabbits to about 3-4 times of their initial normal levels.[13] However, sulphonylurease have been demonstrated not to decrease the blood levels of glucose inalloxan-induced diabetic animals but only the insulin.[14]

Furthermore, drugs like biguanides have been reported to cause hypoglycaemia due to enhanced glycolysis and reduced hepatic gluconeogenesis.[15] However, biguanides do not causes hypoglycaemia in normal individuals because the increased peripheral utilization of glucose is compensated by increased hepatocytic glucose output. Keeping all these in mind and the data presented it may be suggested that Cassia sophera might be having certain hypoglycaemic constituents, which either stimulates the insulin release and or themselves posses some insulin-like action.[16]

Although the definite phyto chemical analysis of this plant has not yet been carried out but because of their hypoglycaemic effects both in diabetic and non-diabetic rabbits showed that they have some alkaloids in them which mimic alkaloids like vindoline and leurosine isolated from Vincarosea reported to lowers the sugar levels in normal animals only. Similarly, Charantia from Momordicacharantia possess hypoglycaemic activity.[17] Thus on the same analogy, it may be hypothesized that presently used plant contain some alkaloids and some other types of hypoglycaemic components, which act together to produce hypoglycaemia in normal rabbits by some indirect mechanism. On the contrary, in the alloxan treated diabetic rabbits, powdered seeds do not seem to enhance the insulin release, as the alloxan results in permanent destruction of β cells.[18] If it is suppose to be true then the drug should reduce the blood level of glucose to same extent in both normal and diabetic rabbits. However, this was not the case as 4g/kg dose of Cassia sophera produced a highly significant ($p<0.001$) decrease in blood level of glucose in normal non diabetic rabbits at 8 and 12 hours interval while in the alloxan treated diabetic rabbits, the decrease was only slightly significant lower at 8 and 12 hours with p value less than 0.05 after drug administration. It is that is why suggested that this substance produces not only direct insulin-like action but also exerts indirect action by enhancing the release of insulin from β cells of pancreas.

Some authors have used observational techniques to get information regarding acute toxicity and changes in behavioural pattern in treated animals.[19] During present study all the treated animals did not show any toxic effects and changes in behavioral patterns of the plants under study which were used in different forms.

Rabbits were used as mammalian model, which indicated that the use of these plants in different forms are also safe. Therefore, the present plants are safe for use by humans is also recommended.

Cassia sophera do has significant effect on controlling blood glucose levels and their use should be cost effective as well, but this is subject to availability of plant in some localities.

CONCLUSION

In conclusion, the present results provide convincing evidence about Cassia sophera's hypoglycaemic activity in mammalian model rabbits. However, the efficacy of herbal medications most commonly used as alternative therapy need to be further assessed by well designed clinical trials. Moreover, preparations of standardized medicinal herbs are urgently needed for further studies and therapy. Conventional herbal drugs for diabetes mellitus can be considered for potential adverse herbal drug interactions also.

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Be there for others, but never leave yourself behind.

– Dodinsky –

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