

Pharmacological Screening of *Cassia sophera* for Hypoglycemic Activity in Normal and Diabetic Rabbits

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SUMMARY

Changes in blood glucose level of normal and alloxan diabetic rabbits were studied after oral administration of various doses of powder from the seeds of Cassia sophera and its aqueous and methanolic extracts. A significant hypoglycemic response was observed in normal as well as alloxan diabetic animals as a result of administration of seed powder and its both extracts.

INTRODUCTION

Active principles from many indigenous plants have been used to treat a number of human ailments in preference to the synthetic drugs as the forners are frequently considered to be less toxic^{1,2,3}. The synthetic hypoglycemic drugs like Sulphonylurea and Biguanides are associated with side effects such as severe and prolonged hypoglycemia, dilutional hyponatremia, lactic acidosis, gastrointestinal disturbances and hypersensitivity reactions⁴. Further great cautions are required for their use in patients with cardiovascular disorders or in cases where hypoglycemia could be particularly dangerous which may include pregnant women and elderly patients⁵.

Pharmacological screening of medicinal plants for safer and more effective agents, therefore remained an area of active research. Medicinal properties of different parts of *Cassia sophera* have been reported^{6,7}. The present study was undertaken to investigate the effect of *Cassia sophera* on blood glucose levels in normal and alloxan induced diabetic rabbits, following oral administration of the seed powder and its aqueous and methanolic extracts.

MATERIALS AND METHODS

Plant material

Cassia sophera seeds obtained from the local

market were powdered finely at low temperature and the powder was stored in well closed glass bottles at 4°C in the refrigerator. The powder was subjected to aqueous and methanolic extractions. Aqueous extract was prepared by the cold maceration of seed powder⁸ and the extract obtained was dried at 40°C (yield 13% w/v). Methanolic extract was prepared by continuous extraction technique using Soxhlet apparatus followed by evaporation at 40°C (yield 1.4%, w/v).

Experimental animals

One hundred and twelve (112) apparently healthy rabbits weighting 1000-15000 g were taken from the Animals House of the Department of Pharmacology, University of Agriculture, Faisalabad. The animals were kept on Rabbit feed prepared by the Department of Nutrition, University of Agriculture Faisalabad and were also supplied green fodder and fresh water *ad libitum*. Rabbits were divided randomly into two main groups. Group 1: Comprised normal (non-diabetic) rabbits and Group 2: Diabetic (Alloxan treated) rabbits.

Fifty six (56) of group 1 were further divided into 7 subgroups of 8 animals each. Subgroup A of the normal group serving as control, received 20 ml of 2% gum tragacanth solution in water only. The animal in subgroups B, C and D were administered orally 2, 3 and 4 g/kg BW seed powder suspended in 20 ml of 2% gum tragacanth solution respectively, using feeding polythene tube connected to a 20 ml

record syringe. The animals in subgroups E and F received aqueous and methanolic extracts equivalent to 4 g/kg body weight dose of the seed powder. The animals in subgroup G were administered acetohexamide 500 mg/kg body weight orally.

Fifty six (56) rabbits of Group-II who were injected alloxan monohydrate (150 mg/kg BW), exhibited, after a day, blood glucose level of 399-500 mg/100 ml as an evidence of being diabetic due to alloxan administration. These animals were also further divided into 7 (H, I, J, K, L, M and N). Subgroup of 8 animals each. Animals in subgroup H received 20 ml of 2% gum tragacanth solution in water only just like subgroup A of the control rabbits. The animals in subgroups I, J, K were administered 2, 3 and 4 g/kg BW of the seed powder respectively just like subgroups B, C and D of the normal rabbits. The animals in subgroups L and M received aqueous and methanolic extract respectively equivalent to 4 g/kg BW dose of seed powder while animals in subgroup N were administered acetohexamide (500 mg/kg BW.).

Analysis

Blood collected from marginal ear vein of each rabbit was analyzed for its glucose level at different time interval using glucose oxidase method^P.

RESULTS

Effect of various doses of *Cassia sophera* seed powder, its aqueous and methanolic extract and acetohexamide on blood glucose level in normal rabbits at 0, 2, 4, 8, 12 and 24 hours time intervals is shown in Table 1. A significant fall ($P < 0.05$) in blood glucose level (92.63 ± 0.97 and 89.13 ± 0.90) was observed at 8 and 12 hour time intervals after the administration of 2 g/kg BW in normal rabbits as compared with the control group at the corresponding interval (95 ± 1.44 and 94 ± 1.61). With a dose of 3 g and 4 g/kg BW. the lowering of glucose level was highly significant at 8 hours (83.19 ± 1.96 and 76.66 ± 0.5) and at 12 hours (84.56 ± 0.91 and 72.50 ± 0.9) P value was found to be less than 0.01 as compared with the control group. For a fall in glucose level at 4 hours interval P value was found to be less than 0.05.

When the aqueous and methanolic extracts or acetohexamide drug were administered to the normal rabbits, a significant lowering of blood glucose level was observed at 8 hours and 12 hours

interval as compared with the control group at corresponding time interval. At 8 hours interval, glucose level after aqueous and methanolic extracts and acetohexamide administration, was found to be 83.88 ± 1.22 , 76.38 ± 1.12 and 76.69 ± 1.48 , respectively, while the values were 81.63 ± 0.86 , 72.56 ± 0.96 and 84.69 ± 0.86 at 12 hours time interval with above treatment.

Almost similar response was seen in alloxan diabetic rabbits with the administration of seed powder or its aqueous or methanolic extracts but quantitatively of lesser magnitude as shown in Table 2. However, no hypoglycemic response was observed as a result of acetohexamide administration. Maximum hypoglycemic response to seed powder intake appeared at 12 hours time interval and the blood glucose level was found to be 315.00 ± 6.32 for 2 g/kg BW, 308.91 ± 7.79 for 3 g/kg BW and 300.09 ± 5.85 for 4 g/kg BW, whereas it was 335.71 ± 8.15 for the control group.

Methanolic extract was more effective in lowering blood glucose level comparative to aqueous extract. With methanolic extract glucose level at 4, 8 and 12 hours time intervals was found to be 315.38 ± 3.71 , 311.41 ± 2.99 and 304.78 ± 7.5 , respectively, whereas with aqueous extracts, the values at corresponding time intervals were 319.50 ± 2.59 , 313.00 ± 2.9 and 301.13 ± 5.61 . In the control group the glucose level at 4, 8 and 12 hours was found to be 339.63 ± 8.25 , 339.41 ± 8.52 and 335.71 ± 8.15 , respectively and was appearing significantly higher than that seen in the groups receiving methanolic and aqueous extracts.

DISCUSSION

The present data reveals that seed powder from *Cassia sophera* and its aqueous and methanolic extracts produce hypoglycemia in normal as well as in alloxan diabetic rabbits (Tables 1 and 2). From the hypoglycemic response in normal and diabetic rabbits in our study, it appears that the seed powder from *Cassia sophera* and its extracts exert direct effect on blood glucose level similar to insulin^S. Sulphonylureas such as acetohexamide on the other hand are believed to act indirectly by stimulating beta cells of the pancreas to release more insulin¹⁰, hence they fail to produce hypoglycemia in alloxan diabetic rabbits having beta cells completely destroyed.

A dose dependent hypoglycemic response in

Hypoglycemic Activity in Normal and Diabetic Rabbits

Table 1: Effect of various doses of *Cassia sophera* seed powder, its aqueous and methanolic extracts and acetohexamide on he blood glucose level (mg/100 ml) in normal rabbits. Each value is the Mean \pm SEM of 8 observations taken at 0, 2, 4, 8, 12 and 24 hours time intervals.

| Animals | Time interval (Hours) | | | | | |
|---|-----------------------|------------------|--------------------|--------------------|--------------------|-------------------|
| | 0 | 2 | 4 | 8 | 12 | 24 |
| Group A (Control) | 95.13 \pm 1.48 | 95.00 \pm 1.16 | 94.44 \pm 1.91 | 95.00 \pm 1.44 | 94.00 \pm 1.61 | 94.63 \pm 1.57 |
| Group B (on 2 g/kg BW seed powder) | 97.63 \pm 1.97 | 95.69 \pm 1.39 | 94.25 \pm 1.54 | 92.63 \pm 0.97* | 89.13 \pm 0.90* | 97.19 \pm 1.79 |
| Group C (on 3 g/kg BW seed powder) | 99.31 \pm 1.76 | 96.00 \pm 2.13 | 87.75 \pm 1.98* | 83.19 \pm 1.96** | 84.13 \pm 0.90** | 97.19 \pm 1.89 |
| Group D (on 4 g/kg BW seed powder) | 92.50 \pm 2.30 | 88.33 \pm 1.00 | 83.33 \pm 1.44* | 76.66 \pm 0.50** | 72.50 \pm 0.90** | 91.25 \pm 1.90 |
| Group E (on aqueous extract equivalent to 4 g/kg BW of seed powder) | 98.31 \pm 1.19 | 87.88 \pm 0.98 | 84.94 \pm 0.80* | 83.88 \pm 1.22* | 81.63 \pm 0.86* | 89.94 \pm 1.36 |
| Group F (on methanolic extract equivalent to 4 g/kg BW seed powder) | 90.13 \pm 2.59 | 89.06 \pm 1.21 | 85.00 \pm 0.96 | 76.38 \pm 1.12** | 72.56 \pm 0.96** | 89.81 \pm 1.44 |
| Group G: (on Acetohexamide 500 mg/kg BW) | 89.75 \pm 1.24 | 74.38 \pm 1.46 | 74.81 \pm 0.91** | 76.69 \pm 1.48** | 84.69 \pm 0.86* | 86.00 \pm 0.88* |

*P < 0.05 as compared with the control group.

**P < 0.01 as compared with the control group.

Table 2: Effect of various doses of *Cassia sophera* seed powder, its aqueous and methanolic extracts and acetohexamide on he blood glucose level (mg/100 ml) in normal rabbits. Each value is the Mean \pm SEM of 8 observations taken at 0, 2, 4, 8, 12 and 24 hours time intervals.

| Animals | Time interval (Hours) | | | | | |
|---|-----------------------|--------------------|---------------------|---------------------|---------------------|--------------------|
| | 0 | 2 | 4 | 8 | 12 | 24 |
| Group H (Control) | 343.54 \pm 9.00 | 339.91 \pm 8.19 | 339.63 \pm 8.25 | 339.41 \pm 8.52 | 335.71 \pm 8.15 | 338.79 \pm 9.19 |
| Group I (on 2 g/kg BW seed powder) | 345.00 \pm 7.94 | 340.09 \pm 8.53 | 334.47 \pm 7.09 | 320.03 \pm 8.16* | 315.00 \pm 6.32* | 338.91 \pm 9.00 |
| Group J (on 3 g/kg BW seed powder) | 362.97 \pm 7.31 | 358.96 \pm 7.31 | 352.44 \pm 7.69 | 344.58 \pm 7.81 | 308.91 \pm 7.79** | 356.84 \pm 6.53 |
| Group K (on 4 g/kg BW seed powder) | 373.13 \pm 16.74 | 367.84 \pm 15.77 | 357.91 \pm 13.58 | 325.38 \pm 5.58* | 300.09 \pm 5.85** | 370.00 \pm 15.61 |
| Group L (on aqueous extract equivalent to 4 g/kg BW of seed powder) | 332.06 \pm 7.89 | 320.13 \pm 8.02 | 319.50 \pm 2.59 | 313.00 \pm 2.90 | 301.13 \pm 5.61** | 386.63 \pm 5.88* |
| Group M (on methanolic extract equivalent to 4 g/kg BW seed powder) | 365.34 \pm 5.16 | 343.22 \pm 5.57* | 315.38 \pm 3.71** | 311.41 \pm 2.99** | 304.78 \pm 7.15** | 358.41 \pm 5.96 |
| Group N (on Acetohexamide 500 mg/kg BW) | 360.59 \pm 7.13 | 336.94 \pm 3.02 | 330.00 \pm 1.79 | 352.00 \pm 3.65 | 335.38 \pm 3.38 | 350.56 \pm 4.80 |

*P < 0.05 as compared with the control group.

**P < 0.01 as compared with the control group.

alloxamide diabetic rabbits similar to the one seen in normal rabbits was observed as a result of administration of Cassia sophera seed powder and its extract. The hypoglycemic response began 4 hours after each treatment, reached its maximum at 12 hours and disappeared at 24 hours time interval.

Certain medicinal plants have high content of minerals such as chromium, potassium, manganese, copper, zinc, calcium, phosphorus etc. It is possible that Cassia sophera too has hypoglycemic properties associated in some way with such minerals which act as cofactors for a number of enzymes involved in different biochemical pathways^{11,12}. No visible signs and symptoms of toxicity appeared in experimental animals after the administration of Cassia sophera seed powder or its aqueous and methanolic extracts. The animals were kept under observation upto seven days for this purpose. The present study signifies a sufficient potential in Cassia sophera as anti-diabetic agent. Further work on the isolation of active principle and its exact mechanism of action may prove it a valuable therapeutic agent.

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