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THE MECHANISM OF HYPOGLYCEMIC AND ANTIDIABETIC ACTION OF HYDROALCHOLIC EXTRACT OF CASSIA FISTULA Linn. IN RATS

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ABSTRACT

The present study was performed to identify the mechanism of action of hydroalcholic extract of *Cassia fistula* leaf in blood glucose lowering effect. The extract was tested for hypoglycemic, oral glucose tolerance and antidiabetic effect in two dose levels, 200 and 400 mg/kg, using glibenclamide (0.5 mg/kg) as standard. Other *in vivo* models included glucose uptake studies in psoas muscle, intestinal glucose absorption and liver glycogen content. Also the extract was studied for its antioxidant activity in *in vitro* (DPPH and NBT) and *in vivo* (lipid peroxidation and GSH level) models. The extract was found to be hypoglycaemic, glucose tolerant and antidiabetic ($p<0.01$) in alloxan induced diabetic model. The extract increased the uptake of glucose in psoas muscle, decreased the absorption of glucose in rat intestine and increased the glycogen level in liver. In case of the antioxidant activity, the extract showed good free radical scavenging activity in both the *in vitro* models, reduced the lipid peroxidation and increased the level of glutathione in liver and kidney.

Keywords: *Cassia fistula*, antidiabetic activity, psoas muscle, intestinal glucose absorption, liver glycogen

1. INTRODUCTION

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by defective regulation of carbohydrate, fat and protein or in other words it is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometime ketonemia. The incidence of Diabetes mellitus is on the rise worldwide[1].

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Free radicals have been implicated in causation of diabetes. Drugs that can scavenge free radicals have potential in ameliorating these disease processes[2].

Cassia fistula Linn a semi-wild Indian Labernum (also known as the Golden Shower) beside being an ornamental plant, different parts of this plant have been demonstrated to possess several medicinal values such as hypoglycemic[3], hepatoprotective[4], antitumour, hypocholesterolaemic[5], wound healing[6] antibacterial activity[7], antipyretic and analgesic properties[8]. *Cassia fistula* is part of food material of certain tribal in India[9]. Based on the ancient practices and traditional uses of this plant as antioxidant and antidiabetic, the present study was undertaken to support the

traditional use and to identify its mechanism of action.

2. MATERIALS AND METHODS

2.1 Chemicals. All the Chemicals and reagents were of analytical grade procured from Loba chem., Mumbai, except glibenclamide from torrent pharmaceutical pvt Ltd., Mumbai, GOD – POD kit from Span diagnostics Ltd., Surat, DPPH from Sigma, Mumbai, Alloxan from Spectrochem Pvt Ltd., Mumbai, Easy Gluco and its strips from Dr. Morepen, New Delhi.

2.2 Plant material. Leaves of *Cassia fistula* L. was collected from Mandsaur (M.P. - India) in Sep 2007. The plant was botanically authenticated and voucher specimen was deposited in the Herbarium of BRNCP with voucher no. BRNCP/C/005/2006. The plant was shade dried and the dried leaves of the plant were coarsely powdered.

2.3 Preparation of extracts. Powdered leaves were weighed and filled in Soxhlet apparatus for extraction. The solvent used was hydroalcholic i.e. 70% ethanol and 30% water, and extraction was carried out for 72 h. The solvent was evaporated and % yield was calculated (14.61 %). Qualitative chemical evaluation was performed to identify the type of constituents present in the extract.

2.4 Experimental animals. Wistar albino rats weighing between 100 – 150 gm of either sex were obtained from B.R.N.C.P., Mandsaur animal house with IAEC clearance no. 76/MPh/07. These animals were used for the experiment. The animals were stabilized for 1 week; maintained in standard condition at room temp; 60 ± 5% relative humidity and 12 h light dark cycle. They had been given standard pellet diet and water *ad-libitum*. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal out put. All the extracts were administered with glass syringe and microsuction canula no. 18. The initial and final body weights were also recorded.

2.5 Acute toxicity. The acute toxicity study was carried out in adult female albino rats by "fixed dose" method of OECD Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and the next day, extract of the plant *Cassia fistula* (suspended in 0.5 % w/v sodium CMC) was administered orally at a dose of 2000 mg/kg for 5 female animals. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for the next 24 hour and finally for mortality after 24 hour till 14 days.

2.6 Selection of doses. For the assessment of hypoglycemic and antidiabetic activity, two dose levels were chosen in such a way that, one dose was approximately one tenth of the maximum dose during acute toxicity studies and a high dose, which was twice that of one tenth dose (200mg/kg and 400mg/kg).

2.7 Assessment of hypoglycemic activity of extract. Four groups of fasted rats, six rats in each, were used. Group first (N1) was treated with normal saline and was considered as a normal control group and group second (N2) was treated with glibenclamide 0.5 mg/kg and considered as standard group. Group third and fourth (N3 and N4) were given orally *Cassia fistula* leaf extract 200mg/kg and 400mg/kg of body weight respectively. Blood samples were collected at 30, 60, 120, 180 and 240 min after administration of extract [1].

2.8 OGTT (Oral glucose tolerance test) in normoglycaemic rats. Four groups of fasted rats, six rats in each, were used. Group O1 was treated with normal saline and considered

as a normal control group and O2 was treated with glibenclamide 0.5 mg/kg, considered as a standard group. While the third group (O3) and fourth group (O4) were given orally *Cassia fistula* leaf extract 200mg/kg and 400mg/kg of body weight respectively. Glucose solution 2 gm/kg was given orally to all the groups after 30 min of extract administration. Blood samples were collected at 30, 60, 120 and 180 min after administration of extract[10].

2.9 Assessment of antidiabetic activity of extract in alloxan treated rats. Animals were fasted for 24 hours and a single intra peritoneal injection of freshly prepared alloxan monohydrate (120 mg/kg dissolved in 0.9% saline) was injected. After that the animals were left aside for 4 h and then 20% glucose solution was placed in the cages for 24 h. The diabetes was confirmed by estimation of blood glucose level (BGL) at 3rd day. Rats having blood glucose level more than 250 mg/dl were used for study (Barry et al., 1997). A normal group (none diabetic) (D1) was added in the study that was treated only with normal saline. D2 was treated with normal saline, diabetic group. D3 was treated with glibenclamide 0.5 mg/kg and the last two groups (D4 and D5) were given orally *Cassia fistula* leaf extract 200mg/kg and 400mg/kg, respectively. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3rd, 7th and 10th day[1].

Collection of blood and determination of blood glucose. Blood samples from rats were collected from the tail vein under light anaesthesia. Blood glucose levels were determined by Dr Morepen's glucometer using specified strips and levels were expressed in mg/100 ml of blood.

Determination of glycogen level in Liver. On the 11th day of diabetic study all groups of animals were sacrificed. Livers and kidney were taken and washed with saline and stored in chilled condition until used. Liver tissues were homogenized in hot ethanol (10 ml) at tissue concentration of 100 mg /ml. Centrifuged at a rate 8000 X g for 20 min. 20 µl of it was used for initial glucose level estimation. The residue was collected and allowed to dry over water bath. Then 5 ml of KOH (10%) and 6 ml perchloric acid (52%) were added and left aside for 20 min at 0°C. The collected material was then centrifuged at 8000 X g for 15 min. From collected supernatant 20 µl of supernatant was removed for final estimation of glucose level. The glucose was estimated by glucose estimation kit at 500 nm (Pushparaj *et al.,* 2001; Maiti *et al.,* 2006). The Glucose conc. was calculated as

Glucose conc. (mg/dl) = AT / AS X 100

Where;

AT means Absorbance of test

AS means absorbance of standard.

Uptake of glucose in rat psoas muscle tissue. Psoas muscle was isolated from two anaesthetized adult rats and placed immediately in KRB solution containing 11.1 mM glucose. Muscle tissue was cut into pieces of equal mass, about 0.250 g and preincubated for 5 min in CO_2 incubator under 95% O_2, 5% CO_2 atmosphere. Four sets (in triplets) including muscle tissue (P1) alone, muscle tissue (P2) with insulin (25mU/l), muscle tissue (P3) with both insulin (25mU/l) & extract (200 □ g) and muscle tissue (P4) with extract (200 µg) alone were incubated for 2.5 h in CO_2 incubator. Aliquots of 25 µl were removed from incubation mixture at 0, 30, 60, 90, 120 and 150 min and changes in glucose concentration were measured spectrophotometrically at 500 nm[11].

Glucose absorption through intestine: An intestinal glucose absorption technique. Three groups of three rats in each were taken, overnight-fasted and anaesthetized with

sodium pentobarbitone (20 mg/kg). An incision was made in abdomen (1 cm), intestine was pulled out and canulated as per intestinal perfusion technique. Perfusion solution was composed of the following (gm/l): 7.37 NaCl, 0.20 KCl, 0.065 NaH_2PO_4. $6H_2O$, 1.02 $CaCl_2$, 0.6 $NaHCO_3$ and 54.0 Glucose at pH 7.5. The system was set at a constant temperature of 37°C and perfusion rate was 0.5 ml/min. The perfusion time was 30 min. The experiment was repeated with normal rats i.e. untreated group (Ia 1), extract treated group (Ia 2) with perfusion solution 5 mg/ml of *Cassia fistula* leaf extract and Standard group (Ia 3) treated with acarbose with perfusion solution 5 mg/ml[12].

2.10 Antioxidant activity

DPPH (α, α- diphenyl β-picryl hydrazyl) free radical scavenging activity. DPPH scavenging activity was measured by the spectrophometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of 0.973. Decrease in the absorbance in presence of sample extract at different concentration (10-125 µg/ml) was noted after 15 min and absorbance was measured at 517 nm[13].

N.B.T superoxide scavenging activity. This was determined by the NBT (Nitro blue tetrazolium) reduction method. The assay was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system. The reaction mixture contains EDTA (402 mg in 10 ml phosphate buffer), riboflavin (5 mg in 25 ml phosphate buffer), nitro blue tetrazolium (NBT- 5 mg in 5 ml phosphate buffer) and various concentrations of extract with phosphate buffer (pH 7.6) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min and absorbance was measured at 560 nm before and after illumination. The percentage inhibition of superoxide generation was measured by comparing the absorbance values of control and those of the test compound[14].

$$\% \text{ Reduction} = \frac{\text{Control absorbance- Test absorbance}}{\text{Control absorbance}} \times 100$$

Determination of GSH level in liver and kidney. Liver (200 mg) and kidney (400 mg) of animals under diabetic study were homogenized in 8.0 ml of 0.02m EDTA in an ice bath. This was then stored in ice bath till utility. 5 ml of Aliquots of homogenates were taken and mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50% TCA. This mixer was then centrifuged at 3000 X g for 15 min. 2.0 ml of supernatant was mixed with 4.0 ml of 0.4M Tris buffer, and 0.1 DTNB was added with vigorous shaking. The absorbance was taken at 412 nm against a reagent blank with no homogenate within 5 min of addition of DTNB[1].

Determination of lipid peroxidation level in liver and kidney. Liver and kidney of animals under diabetic study were separated and washed with chilled NaCl (0.9%). 0.5 gm of organs were taken and homogenized with 5 ml of 0.25 M sucrose. The sample was centrifuged at 1000 X g for 10 min and then 2000 X g for 30 min. 1% of homogenate was taken from each group separately and incubated with 5 ml of $FeCl_2$ (0.5 mM) and H_2O_2 (5ml); 20 mg/ml of extract and 0.5 mg/ml of glibenclamide were added in the respective groups; and incubated for 60 min at 37°C. The absorbance was read at 535 nm as measurement of MDA a lipid peroxidized product. The percentage inhibitory effect was calculated as

$$\text{Inhibitory rate} = [1 - (A_1 - A_2) / A_0] \times 100$$

Where A_0 was the absorbance of control {without extract} and A_1 was the absorbance

of the extract group, A_2 was the absorbance without liver homogenate[13].

Statistical analysis. The data were expressed as mean ± SEM. The data were analyzed by one way analysis of variance (ANOVA) followed by "Dunnet's t-test" (Graph pad prism 4.0). *p* value less than 0.05 was considered as statistically significant.

3. RESULT

Acute Toxicity studies. Acute toxicity studies on female rats showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days and during the study no noticeable responses were seen in the rats. This indicated that it does not contain any type of toxicity and is safe.

Effect of extract on blood glucose levels of normal rats. Leaves extract at doses, 200 mg/kg and 400 mg/kg, decreased glucose level till the 4th h. The effect of extract was dose dependent (Table 1).

Table 1. Effect of extract on blood glucose level of normal rats

Group	Blood glucose level (mg/dl)					
	0 min	30 min	60 min	120 min	180 min	240 min
N 1	91.40 ± 1.03	90.60 ± 0.68	90.00 ± 1.00	91.20 ± 0.86	89.80 ± 1.07	91.20 ± 0.58
N 2	87.00 ± 2.91	82.80 ± 3.38*	78.40 ± 1.96*	71.00 ± 2.30*	61.20 ± 1.99 *	50.00 ± 2.74*
N 3	97.00 ± 1.51	90.20 ± 1.08*	80.20 ± 1.77*	71.40 ± 1.78*	63.00 ± 2.00*	76.40 ± 3.79*
N 4	99.00 ± 1.70	95.80 ± 1.16*	92.40 ± 1.03**	88.20 ± 1.39*	82.40 ± 1.57*	77.80 ± 1.66*

N 1- normal control; N 2- standard; N 3 & N 4 -*Cassia fistula* leaf extract 200mg/kg and 400mg/kg respectively, N=6, *p < 0.01, **p < 0.05 vs normal control, ANOVA followed by Dunnett's test, Values are expressed in mean ± SEM

Effect of extract in glucose – hyperglycaemic animals (Oral glucose tolerance test, OGTT). The groups treated with leaves extract showed initially increase in glucose level upto 30 min then it gradually decreased, the level was maintained at 120 min i.e. the glucose level reached the normal level and maintained upto 180 min. Where as glibenclamide maintained glucose level at normal range through out the study period (Table 2).

Table 2. Effect of extract in glucose induced hyperglycaemic animals

Group	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	120 min	180 min
O 1	85.67 ± 1.45	135.30 ± 4.80	116.71 ± 4.67	98.37 ± 2.96	92.67 ± 1.45
O 2	90.33 ± 2.33	95.33 ± 1.20*	86.00 ± 1.53*	83.67 ± 1.45*	81.67 ± 0.88*
O 3	89.67 ± 1.76	131.30 ± 2.40*	106.70 ± 2.03*	94.67 ± 1.20*	83.67 ± 1.42 *
O 4	88.39 ± 1.51	128.64 ± 4.23*	101.19 ± 3.52*	94.81 ± 4.92*	82.13 ± 2.18*

O 1- normal control; O 2- standard; O 3 & O 4 -*Cassia fistula* leaf extract 200mg/kg and 400mg/kg respectively, N=6, *p < 0.01 vs normal control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Antidiabetic activity. Leaves extract at a dose of 400 mg/kg showed a decrease in glucose level highly and brought the blood glucose level at near normal on 7th day of diabetes. Leaves extract at a dose of 200 mg/kg also showed significant effect. The activity was found dose dependant (Table 3). Body weight of rats showed a decline initially but after 10 days of treatment, the treated leaves extract groups showed increase in body weight.

Table 3. Effect of extract in alloxan induced diabetic animals

Group	**Blood glucose level** (mg/dl)			
	0 DAY	**3 DAY**	**7 DAY**	**10 DAY**
D 1	82.40 ± 1.63	83.04 ± 2.30	82.20 ± 1.96	81.00 ± 1.30
D 2	88.22 ± 3.05	381.82 ± 13.03	490.40 ± 15.32	492.19 ± 12.63
D 3	85.42 ± 1.75	390.88 ± 26.96*	80.40 ± 4.729*	80.20 ± 3.23*
D 4	76.60 ± 1.75	420.00 ± 28.12*	238.80 ± 29.46*	99.00 ± 7.98*
D 5	87.41 ± 2.54	404.65 ± 14.63*	182.80 ± 26.09*	94.20 ± 9.33*

D 1- normal control; D 2- diabetic control; D 3- standard; D 4 & D 5- *Cassia fistula* leaf extract 200mg/kg and 400mg/kg respectively, N=6, *p < 0.01 vs diabetic control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Estimation of glycogen level in liver. Rat liver showed an increase in level of glycogen of both glibenclamide treated and leaves extract treated group at both the dose levels. The increase in Glycogen level in liver of animal treated with the leaf extract was lesser than glibenclamide (Table 4).

Effect of extract on uptake of glucose in rat psoas muscle tissue. On estimation of uptake of glucose in rat Psoas muscle, results showed an increase in the glucose level but when compared with insulin treated rats it was in significant range i.e. it was about 118.81 as compared to 113.51 mg/dl of insulin and 113.54 of insulin + extract (Table 5).

Table 4. Effect of extract on glycogen level in liver of diabetic rats

Group	D 1	D 2	D 3	D 4	D 5
Glycogen level (mg/gm wet tissues)	137.18 ± 3.24	117.05 ± 2.85	210.25 ± 2.45*	198.73 ± 2.29*	195.54 ± 4.62*

D 1- normal control; D 2- diabetic control; D 3- standard; D 4 & D 5- *Cassia fistula* leaf extract 200mg/kg and 400 mg/kg respectively, N=6, * p < 0.01 vs diabetic control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Table 5. Effect of extract on uptake of glucose in rat Psoas muscle tissue

Group	**Glucose level** (mg/dl)				
	0 min	**30 min**	**60 min**	**120 min**	**180 min**
P1	100.00 ± 4.77	108.44 ± 2.38	105.71 ± 2.27	101.62 ± 1.80	98.33 ± 1.88
P2	100.50 ± 3.65	101.63 ± 3.93	102.11 ± 1.38	113.52 ± 1.88	113.51 ± 1.38
P3	100.00 ± 2.70	95.84 ± 1.38*	107.88 ± 1.80*	112.50 ± 1.80*	113.54 ± 0.88**
P4	99.49 ± 4.17	98.94 ± 1.86*	99.48 ± 1.38*	101.62 ± 1.80*	118.81 ± 0.90*

P 1- normal control; P 2- standard; P 3- test standard (insulin with extract) & P 4- *Cassia fistula* leaf extract, N=6, *p < 0.01, **p > 0.05 vs standard control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Effect of extract on absorption of glucose in rat intestine. The glucose absorbed by the leaves extract at 200mg/kg is 9.82 % as compared to 5.43% of acarbose treated rats, when the level was compared with normal range (17.55%) it was almost half of the glucose level. The result showed that the extract had decreased the intestinal glucose absorption and is a better substitute for the miscellaneous antidiabetic drugs (Table 6).

Effect of extract in free radicals. Invitro antioxidant activity of *Cassia fistula* leaves showed 84.94% free radical scavenging activity and 77.45 % superoxide scavenging activity at a conc. of 125 µg/ml (Table 7).

Inhibition of lipid peroxidation in rat liver homogenate. Leaves extract at a dose of 200 mg/kg p.o. showed 61.52 % reduction of lipid peroxidation as compared to 41.82 % reduction by glibenclamide treated groups Leaves extract 200 mg/kg showed even more protection against ROS as compared to 400 mg/kg (Table 8).

Table 6. Effect of extract on absorption of glucose in rat Intestine

Group	Absorbance (nm)	Conc. absorbed (mg/dl)	% of glucose absorbed
Ia 1	0.057 ± 0.001	89.06 ± 1.804	17.55 ± 1.65
Ia 2	0.065 ± 0.002	102.15 ± 2.901	5.43 ± 2.739*
Ia 3	0.062 ± 0.001	97.39 ± 4.064	9.817 ± 3.763*

Ia 1- normal control; Ia 2- *Cassia fistula* leaf extract; Ia 3 – standard, N=6, *p < 0.01 vs normal control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Table 7. Antioxidant activity of *Cassia fistula* (invitro)

Conc. (µg/ml)	DPPH		NBT	
	Absorbance	% Reduction	Absorbance	% Reduction
10	0.882 ± 0.004	9.47 ± 0.564	0.766 ± 0.002	8.41 ± 0.273
20	0.804 ± 0.003	17.36 ± 0.356	0.671 ± 0.005	19.79 ± 0.626
30	0.719 ± 0.007	26.11 ± 0.791	0.611 ± 0.009	26.94 ± 1.192
40	0.634 ± 0.013	36.17 ± 0.176	0.575 ± 0.009	31.24 ± 0.402
50	0.510 ± 0.007	47.55 ± 0.774	0.478 ± 0.005	42.92 ± 0.621
100	0.254 ± 0.006	73.45 ± 0.384	0.343 ± 0.008	59.17 ± 1.028
125	0.146 ± 0.010	84.94 ± 1.028	0.189 ± 0.003	77.45 ± 0.368

Table 8. Malondialdehyde (MDA) % inhibition and glutathione (µmole/gm) **(GSH) levels in liver and kidney in Alloxan-induced-diabetic rats after 10 days treated with *Cassia fistula* leaves extracts**

Group	Liver		Kidney	
	MD (%inhibition)	**GSH**	**MDA** (%Inhibition)	**GSH**
D 1	39.80 ± 1.349	262.68 ± 3.430	31.33 ± 1.015	108.25 ± 4.113
D 2	14.00 ± 1.157	90.25 ± 3.452	18.58 ± 3.981	69.65 ± 1.609
D 3	41.82 ± 1.552*	144.59 ± 1.535*	47.51 ± 0.864*	96.30 ± 0.984 *
D 4	61.52 ± 5.019*	132.37 ± 1.392*	54.94 ± 4.257*	94.33 ± 1.145*
D 5	59.28 ± 3.207*	138.24 ± 1.265*	53.59 ± 3.049*	93.56 ± 0.892*

D 1- normal control; D 2- diabetic control; D 3- standard; D 4 & D 5-*Cassia fistula* leaf extract 200mg/kg and 400 mg/kg respectively, N=6, *p < 0.01 vs diabetic control, ANOVA followed by Dunnett's test, Values expressed in mean ± SEM

Non-protein sulfhydryl groups (cellular GSH) level in tissues. Leaves extract had increased the level of GSH in liver from 90 µmole/gm of diabetic rats to level comparable with standard 144.59. Where as GSH in kidney had increased from 69.65 µmole/gm but less than the level attained by glibenclamide i.e. 96.30 at the end of study (Table 8).

4. DISCUSSION

The present investigation reports the hypoglycaemic and antidiabetic effect of hydroalcholic extract of leaves of *Cassia fistula*. The observation and preliminary idea of the mechanism of its action reported here offer scientific explanation for the potential use of this plant for the treatment of diabetes mellitus. Experiments with doses of 200 and 400 mg/kg b wt. of extract on normal BG and OGTT in normal healthy rats produced significant fall in BG (Table 1) and improved glucose tolerance (Table 2). Extract at both the doses were found equally effective, hence the dose of 200 mg/kg may be appropriate dose for diabetic. This study also revealed that the maximum hypoglycaemic effect was produced only 1 h after administration of extract to the fasted animals. This indicates that it takes about 1 h for the active ingredient(s) or its (their) metabolites in the extract to enter into the circulation and reach target tissues to bring about hypoglycaemic effect, which is maintained for at least 2 1/2 h. In the OGTT experiment reduction in blood glucose level was started after 1 h and maintained at least for 2 h. Treatment of rats with alloxan induced diabetes for 10 days (Table 3) brought down the elevated blood glucose levels, ranging from 250 to 400 mg/dl to nearly normal range. Alloxan not only destroys the pancreatic β-cells but causes kidney damage, which is however reversible causing diabetes close to type-II in humans[15]. The elevated blood glucose levels in the diabetic animals used by us were more than 250 mg/dl, which resembles both type-II diabetes (150 to about 250 mg/dl) with partially functional pancreas as well type-I (above 300 mg/dl) with considerable amount of pancreas damaged. This shows that the *Cassia fistula* extract might be useful both in type-II and type-I diabetes, irrespective of whether the pancreas is partly functional or almost totally destroyed. This is an advantage, keeping in mind that the present-day

sulphonylurea drugs act only when there is a functional pancreas[16].

Insulin is also main regulator of glycogenesis in muscle and liver. The decrease in the glycogen level of the liver was been observed which is due to diabetic condition of experimental rats[17]. The lack in the level may probably due to inactivation of glycogen synthetase system. The treated group shows an elevation in the glycogen content of the liver this suggest that extract causes stimulation of glycogenesis process, may be Cassia *fistula* activate glycogen synthetase system.

Our studies on isolated psoas muscle indicated that the *Cassia fistula* extract enhanced the uptake of glucose in muscle tissue (Table 5) in a short time of 30 min in the absence of insulin, i.e. directly and the effect was more in the presence of insulin. Direct effect in the absence of insulin indicates that the extract has either insulin-like effect on psoas muscle (skeletal muscle) or direct stimulatory effect on the enzymes involved in the metabolism of glucose. Increase of glucose uptake in the presence of insulin suggests the possibility of increased binding of insulin to receptor in the muscle or increase in the number of insulin receptors receptors[11].

The enhanced uptake of glucose would lead to increased utilization of glucose from the blood. The extract could also inhibit uptake of glucose by the rat intestine slightly, (Table 6). Even though the inhibition is less, one has to keep in mind that the extract may also act at a different site, namely the intestine.

It is profound that diabetes mellitus is not a single disease, but numerous disease and symptoms were associated with it. There has been considerable recent debate regarding the extent to which increased oxidative stress contributes towards the development of diabetic complications. The fact that the role of antioxidant compounds in both protection and therapy of diabetes mellitus were also emphasized in previous scientific researches. It has been suggested that compounds or extracts having both hypoglycemic and antioxidative properties would be useful antidiabetic agents[18]. Hyperglycemia results in the generation of free radicals, which can exhaust antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid peroxidation.

In the present study, we have also measured the potential of *Cassia fistula* extract to inhibit lipid peroxidation in rat liver homogenate, induced by the $FeCl_2$–H_2O_2 system. Decrease in lipid peroxidation by *Cassia fistula* may be a result of it scavenging OH produced by $FeCl_2$–H_2O_2 and H_2O_2 in the reaction system; this is also confirmed by DPPH and NBT scavenging activity. GSH is mainly involved in the synthesis of important macromolecules and in the protection against reactive oxygen compounds GSH was observed in diabetic rats. The decreased GSH content contributes to the pathogenesis of complications associated with chronic diabetic state. The study showed an elevation in GSH level in tissue after extract treatment. So our extract has the ability to counteract the free radicals produced by the alloxan.

The exact mechanism of action needs further studies, but the present investigation gives some preliminary idea that the extract from leaves of *Cassia fistula* acts at more than one site, namely pancreas (release of hormone insulin), muscle and intestine (uptake of glucose through specific receptor). Also our extract was found to be a potent antioxidant agent. The toxic level of the extract was high (no death even with 10 times the effective dose), indicating the high margin of safety. These results indicate that it is worth undertaking further studies on possible usefulness of the hydroalcholic extract of the leaves of *Cassia fistula* in diabetes mellitus.

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