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ANTIHYPERGLYCEMIC ACTIVITY OF *CASSIA FISTULA* FRUIT EXTRACTS IN STREPTOZOTOCIN INDUCED DIABETES

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ABSTRACT

Aim: This study was planned to evaluate the antihyperglycemic effect of *Cassia fistula* fruit extracts on diabetic rats which is induced by streptozotocin. **Methods:** Petroleum ether, ethanol and aqueous extracts of *Cassia fistula* fruits were tested (500 mg/ kgp.o.) for 21 days and on the last day, serum was subjected for various biochemical parameters viz. glucose, cholesterol, triglycerides etc. **Results:** Ethanol and aqueous extracts of *Cassia fistula* fruits have shown a significant ($P<0.001$) anti-diabetic activity in streptozotocin induced diabetic rats. Further, this is confirmed by significant restoring of biochemical parameters and improvement in body weight. **Conclusion:** The results are obtained in this study indicate the significant of antihyperglycemic effect of ethanol and aqueous extracts in streptozotocin induced diabetic rats by mediated through restoration of biochemical parameters level. This observed effect of title plant could be due to high phenolic constituents present in it.

KEYWORDS

Anti-diabetic, *Cassia fistula*, Serum cholesterol and Streptozotocin.

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INTRODUCTION

Diabetes mellitus is a chronic disease. It is characterized by hyperglycaemia resulting from inherited defects in insulin secretion and/or acquired deficiency in insulin production or action or both[1]. This will affect metabolic processes of glucose, protein and lipid leads to acute and chronic complications. The major complication includes atherosclerosis involving renal, cardiovascular and peripheral vessels and is more likely to increase risk

for coronary heart disease because of alteration in serum lipid profile.

Diabetes is a large public health problem in the world. To treat diabetes modern medicine does not offer safety. Hence, searching for herbal origin offers safe and potent remedy for the treatment of diabetes. Literature review showed that, many Indian medicinal plants employed traditionally in the treatment of diabetes have been scientifically validated[2].

Cassia fistula Linn (Fabaceae) commonly known as Indian laburnum is a plant distributed thought India. Cassia fistula has many different uses and these were based on different parts of plant.

Cassia fistula Linn. Commonly called as Indian laburnum is been widely employed for many ailments since ancient times. The various parts of *C. fistula* are used traditionally as anti-pyretic, astringent, strong purgative and in the management of heart diseases, migraine, jaundice, piles, rheumatism, ulcers, diabetes, inflammation, chest and liver complaints[3].

Literature report said that no scientific validation done on antidiabetic activity of *Cassia fistula* fruits. Hence, we undertook present research to demonstrate antidiabetic activity of *Cassia fistula* fruits in streptozotocin induced diabetic rats.

MATERIAL AND METHODS

Plant materials collection and preparation of extracts

The *Cassia fistula* fruits were collected from the surrounding fields of Aurangabad (Maharashtra) in the month of July after authenticated by Dr. M. B. Mulimani, S.B. Arts and Science College, Bijapur. A voucher specimen has been deposited at the herbarium of our college.

The Fruits were dried in shade at room temperature (RT). The dried fruits were powdered by using grinder to coarse powder, packed into Soxhlet column and extracted by using Petroleum ether solvent for 24 hrs and procedure repeated for 70% alcohol and distilled water. The obtained crude extracts were stored in air tight containers in refrigerator below 10^0C for further studies. The stock solution of hydro-alcohol and aqueous extracts were prepared using distilled water and for petroleum ether extract using 2% tween 80.

Determination of Phytochemicals

Preliminary phytochemical screening

Preliminary phytochemical investigation was carried out on all three extracts of *Cassia fistula* fruits for detection of various phytochemicals as per standard procedures explained in Practical Pharmacognosy by C.K. Kokate[4] and R.K. Khandelwal[5]. For standardization, estimation of total phenol and flavonoid content as carried out by a standard procedure.

Total Phenolic Content

The total phenolics content of plant was determined UV spectrophotometrically using folin-ciocalteu method. Aliquots (0.1 ml) of the extracts taken and were mixed with 0.5 ml of folin-ciocalteu reagent and made up to 3 ml with distilled water. After 3 min, 2 ml of sodium carbonate (20%) was added to reaction mixture and mixed it thoroughly. The sample was then incubated for 5 min at 50°C and cooled. The absorbance of solution was measured (at 650 nm) against the reagent blank. The total phenolic content were expressed in terms of mg gallic acid equivalent per gm of plant extract. The coefficient of determination was r^2 =0.9968.

Total Flavonoid Content

The determination of total flavonoid content in *Cassia fistula* was based on aluminium chlroride method. A volume of 0.5 ml of $AlCl_3$ ethanol solution (2 %) was added to 0.5 ml of sample solution (final concentration of 0.1 mg/ml). After 1 h incubation at room temperature, the absorbance was measured at 420 nm against blank. The total flavonoid content were calculated in terms of mg quercetin equivalent per gm of sample. The coefficient of determination was r^2 = 0.9965[6].

Experimental Animals

Male albino rats (150-200 g) and Male albino mice (20-25 g) are used throughout the experiments. The animals were obtained from Sri. Venkateshwara Enterprises, Bangalore (237/CPCSEA). Before initiation of experiment, all animals were made

acclimatized for10 days under standard environmental condition such as temperature (26 ± 2^0C), relative humidity (45-55%) and 12 hlight/dark cycles. All the animals were fed with rodent pellet diet (VRK Nutritional industries, Pune, India) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) (Reg. No. 1076/c/07 CPCSEA) for performing this research on animals.

Determination of Acute toxicity (LD_{50})

The acute toxicity for all three extracts of *Cassia fistula* was determined in albino mice. The animals were fasted overnight prior to the experiment and fixed dose method was adopted as per OECD Guideline No. 423; (Annexure-2d) of CPCSEA. Group of three mice were taken for each test dose[7].

Study of anti-diabetic activity

The following treatment was done as per below,

Group I - Served as normal control and did not received any treatment.

Group II - Streptozotocin (50 mg/kg, i.p) and served as diabetic control.

Group III - Streptozotocin + Glibenclamide (0.6 mg/kg, p.o.) and served as Standard.

Group IV - Streptozotocin + Petroleum ether extract (500 mg/kg, p.o.).

Group V - Streptozotocin +Hydro alcohol extract (500 mg/kg, p.o.).

Group VI - Streptozotocin + Aqueous extract (500 mg/kg, p.o.).

Fasting blood glucose of animal was determined after depriving food for 16 hrs with water *ad libitum*. Diabetes was induced by a streptozotocin monohydrate injection of 50 mg/kg i.p. After 3 days of streptozotocin injection, the hyperglycaemic rats (glucose level between 250-300 mg/dl) were selected for the study and were divided into different groups comprising of 6 rats each for the anti-diabetic study. The extracts were administered from the same day except normal control animals and diabetic control groups for a period of 21 days. During this period, the all animals had free access to standard diet and water. Body weight and blood glucose levels were estimated on 1st, 7th and 21st day of the treatment. On the lastday, biochemical estimations were completed with blood samples collected from overnight fasted rats by carotid bleeding under mild ether anaesthesia.

The parameters considered were fasting blood glucose, serum urea, serum total cholesterol, serum triglycerides and other parameter viz. body weight[8].

Measurement of serum biochemical parameters

The level of biochemical parameters like fasting blood glucose, serum urea, serum total cholesterol, serum triglycerides were determined using the Hitachi 912 clinical chemistry auto-analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

Statistical analysis

The data obtained from above findings subjected to the statistical analysis using one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results. The $p<0.05$ implies significance.

RESULTS

Determination of Phytochemicals

Preliminary phytochemical analyses revealed the presence of fats and oils in petroleum ether extract, whereas phenolics, flavonoids, tannins, Saponins are present in aqueous and ethanol extracts.

Total Phenolic and Flavonoid Contents

Table No.1 shows the amount of phenolics and flavonoids present in each extract. The concentration of phenolics in ethanol and aqueous extracts were higher when compared to petroleum extract. Flavonoids were below the detection limit in case of petroleum ether extract.

Determination of acute toxicity LD_{50}

The Petroleum ether, 70% ethanolic and aqueous extracts was studied for acute toxicity at a dose of 2000 mg/kg orally in albino mice. The extracts were found no mortality of the animals. So, 2500 mg/kg was considered as LD_{50} cut off value.

Hence, the doses selected for the investigation of anti-hyperglycemic activity as per OECD guidelines No. 423 (Annexure - 2D) fixed dose method are mentioned below.

1. 500 mg/kg petroleum ether extract (1/5th of 2500 mg/kg).

2. 500 mg/kg 70% ethanolic extract (1/5th of 2500 mg/kg).
3. 500 mg/kg aqueous extract (1/5th of 2500 mg/kg).

Anti-diabetic study

Effect of *Cassia fistula* fruits extracts on fasting blood glucose level in diabetic rats

A marked rise in fasting blood glucose level was observed in diabetic control rats compared to normal control. Ethanolic and water extracts of *Cassia fistula* (500 mg/kg) exhibited significant anti-hyperglycaemic activity on 21th day post treatment. Whereas the petroleum ether extract (500 mg/kg) was found to be statistically non-significant. Glibenclamide, standard drug had shown a significant reduction in blood glucose compared to diabetic control. The results are shown in the Table No.2.

Effect of *Cassia fistula* fruit extracts on body weight in diabetic rat

Normal control animals were shown no change in their body weight but diabetic rats showed significant reduction in body weight during 21 days. Rats treated with ethanolic and water extracts have demonstrated significant restoration of streptozotocin mediated body weight reduction. However, petroleum ether extract was found statistically not significant on body weight of the animals. Results are shown in Table No.3.

Effect of *Cassia fistula* fruit extracts on biochemical parameters

Pre-treatment of animals with Ethanolic and water extracts of *Cassia fistula* fruits have demonstrated significant decreased in serum cholesterol, serum triglycerides and serum urea levels when compared to diabetic rats. But, the petroleum ether extract decreases the serum cholesterol, serum triglycerides and urea levels but less significant. Results are shown in Table No.4.

DISCUSSION

Diabetes mellitus is a complex chronic disorder and it is characterized by chronic hyperglycaemia and abnormality of lipid profile like cholesterol, low density lipoproteins (LDL) and high density lipoprotein (HDL) and triglyceride leading to series of secondary complications. These complications include polyuria, polyphagia, polydypsia, ketosis, retinopathy and cardiovascular diseases[9].

The most widely used animal model for the investigation of anti-diabetic drugs is STZ induced diabetes model. This model induces diabetes mellitus that mimics the human diabetes[10]. STZ leads to diabetes by causing selective necrosis of pancreatic β-cells and thus reducing the insulin secretion[11]. STZ breaks the DNA strands of β-cells by methylation and also generates potential free radicals such as nitric oxide by intracellular STZ metabolism which leads to necrosis of β-cells[12]. In STZ induced diabetes, the body weight of the animal is decreased due to metabolic changes caused by deficiency of insulin because of destruction of β-cells[13].

In the present study the ethanolic and aqueous extracts of *Cassia fistula* fruits have shown a significant antidiabetic activity in STZ induced diabetic model. These results are comparable with control. Petroleum ether extract showed less significant antidiabetic activity than the ethanolic and aqueous extracts. The observed potential antidiabetic activity of ethanolic and aqueous extracts of *Cassia fistula* fruits possibly by potentiating the insulin effect either by increasing the secretion of insulin[14] or by regeneration of damaged β- cells in STZ induced diabetes[15]. The body weight usually decreases as diabetes progresses[16], but ethanolic and aqueous extracts significantly inhibited the decrease in body weight. This effect is also an indication of the anti-diabetic activity of both extracts.

Diabetes mellitus is associated with altered levels of serum lipids and is considered as one of the risk factor for coronary artery diseases[17]. Hyperlipidemia is the main complication of diabetes mellitus characterised by raised cholesterol and triglycerides levels[18]. In STZ induced diabetes the levels of serum cholesterol and triglycerides are elevated[19] mainly due to the uninhibited actions of lipolytic hormones on the fat depots, due to the impairment of insulin secretion[20,21]. Ethanolic and

aqueous extracts supplementation lowered significantly these serum values to a normal range.

The diabetes mellitus is associated with renal function and the marker of renal function is urea. In diabetes mellitus due to insulin deficiency the protein catabolism is increased rather than a reduction in the synthesis of proteins[22]. This increased protein catabolism causes synthesis of end product urea and further enhanced breakdown of liver and plasma proteins in chemical induced diabetes leads to the accumulation of urea nitrogen[23]. Ethanolic and aqueous extracts significantly decreased this parameter which could be due to decreased disturbances in protein metabolism may be because of better glycaemia control.

A positive correlation between antidiabetic activity and flavonoid content was found suggesting that, the antidiabetic capacity of the plant extracts is due to a great extent to their flavonoid content[24].

Table No.1: Total phenolics content (TPC) and total flavonoid content (TFC) of *Cassia fistula* fruit extracts

S.No	Extract	TPC (mg of Gallic acid equivalents/g extract)	TFC (mg of Quercetin equivalents/g extract)
1	Petroleum ether	1.60 ± 0.04	---
2	Ethanol	143.38 ± 0.24	32.59 ± 1.06
3	Aqueous	136.47 ± 0.62	26.49 ± 0.28

Table No. 2: Effect of *Cassia fistula* fruit extracts on fasting blood glucose level in STZ induced diabetic rats

Groups	Fasting blood glucose level (mg/dl)			
	Initial day	7th day	14th day	21th day
I	112.02±3.38	118.44±3.52	108.79±2.55	112.54±3.28
II	337.24±5.36	352.29±4.08	376.03±4.99	360.33±6.75
III	349.28±3.53	124.06±3.43***	109.21±2.07***	115.39±2.78***
IV	320.60±3.22	308.42±4.82^{ns}	293.45±3.51^{ns}	257.50±7.97^{ns}
V	342.05±4.08	172.38±4.32**	145.42±3.37***	122.36±3.06***
VI	351.72±4.50	169.34±3.04**	151.68±5.43***	125.23±7.56***

Values are Mean ± S.E.M; n=6

^{Ns} non-significant, **P < 0.01and ***P < 0.001 vs, Diabetic control.

Table No.3: Effect of *Cassia fistula* fruit extracts on body weight of theanimal in streptozotocin induced diabetic rats

Groups	Body weight of the animal (g)			
	Initial day	7th day	14th day	21th day
I	212.52±3.25	214.5±4.39	221.2±3.66	226.2±4.19
II	185.39±2.43	178.09±3.25	171.80± 4.52	165.26±3.31
III	172.84±3.22	178.43±4.76*	183.32±4.98**	184.32±2.56**
IV	187.07±3.09	182.06±3.88ns	175.22±2.36ns	167.40±1.94ns
V	192.49±2.33	193.55±3.74*	196.78±5.24*	198.32±1.89**
VI	184.40±3.78	187.05±3.51*	189.73±4.25*	192.42±1.83**

Values are Mean ± S.E.M; n=6

Ns non-significant, *P < 0.05 and **P < 0.01 vs, Diabetic control.

Table No.4: Effect of *Cassia fistula* fruit extracts on biochemical parameters in STZ induced diabetic rats

Groups	Serum Triglycerides (mg/dl)	Serum Cholesterol (mg/dl)	Serum Urea (mg/dl)
I	84.26±1.52	93.50 ±0.60	24.05 ±0.98
II	163.25±2.52	164.4 ±2.34	73.63 ±0.42
III	94.38±1.94***	97.23±1.26***	33.60 ±2.69***
IV	159.29±2.81ns	158.29±1.88ns	68.90 ±0.92ns
V	98.80±2.13***	94.83±1.07***	33.17±1.74***
VI	95.37±1.39**	97.87±2.02**	42.83±1.35*

Values are Mean ± S.E.M; n=6

Ns non-significant, * P < 0.05, ** P < 0.01 and ***P < 0.001 vs, Diabetic control.

CONCLUSION

The results obtained in this study indicate the significant anti-hyperglycemic effect of ethanol and aqueous extracts in streptozotocin induced diabetic rats, which is mediated through restoration of biochemical parameters level. This observed effect of title plant could be due to high phenolic constituents present in it. Further study is needed to isolate the phytoconstituents responsible for the observed effect.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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