IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 February, 2012; received in revised form 16 May, 2012; accepted 25 May, 2012

ANTIDIABETIC ACTIVITY AND PHYTOCHEMICAL INVESTIGATIONS OF *CASSIA FISTULA* LINN. BARK

Shrikant N. Malpani*[1] and K. P. Manjunath [2]

Leben Laboratories Pvt. Ltd., L 4, Phase-III, M.I.D.C [1], Akola-444104, Maharashtra, India
Department of Pharmacognosy, K.L.E.S' College of Pharmacy [2], Hubli-580031, Karnataka, India

Keywords:
Cassia fistula,
Antidiabetic,
Alloxan-induced diabetic

Correspondence to Author:

Shrikant N. Malpani

Research Associates (F & D Dept.), Leben Laboratories Pvt. Ltd., L-4, Phase-III, M.I.D.C., Akola – 444 104, Maharashtra, India

ABSTRACT

Daily oral administration of the total alcoholic extract and its ethyl acetate fraction of the bark of *Cassia fistula* in alloxan induced diabetic rats exhibited significant reduction in blood glucose levels and also found effective in restoring the blood lipids to normal levels. The activity was found comparable with standard drug glibenclamide. The ethyl acetate fraction showed more significant antidiabetic effect. The chromatographic isolation of ethyl acetate fraction summarizes the presence of flavonoidal moiety.

INTRODUCTION: Diabetes is one of the most prevalence chronic diseases in the world. This is a chronic incurable condition due to insulin deficiency that affects 10% of the population. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025. For a long time, diabetes has been treated with several medicinal plants or their extract based on the folklore medicine.

Nowadays herbal medicines are highly recommended for the treatment of diabetes inspite of other therapeutic option, which can produce serious side effects and in addition they are not safe during pregnancy.

Therefore, the search for the more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plant has become more important [1-2].

Cassia fistula Linn. also known as golden shower, Indian laburnum, belongs to the family Leguminoceae. In traditional medicine, it is used in the treatement of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, and as antipyretic, analgesic & laxative [3-4].

Cassia fistula is a moderate sized deciduous tree, distributed throughout India. It is 8-15m to 24m in height, with greenish grey smooth bark when young & rough, dark brown when mature. Leaflets 8–12 pair, flowers yellow, long drooping racemes. Pod cylindrical & pulpy. Seeds light brown, hard & shiny [5-7].

MATERIALS AND METHODS:

Plant: The bark of *Cassia fistula* L. were collected from the local areas of Hubli, Karnataka, and authenticated by Dr. B.D. Huddar, Head, Department of Botany, H.S.K. Science Institute, Hubli (Ref. No: SKA & H.S.K./Auth-2008-09, dated: 28/07/2008). A voucher specimen (No.07PG353, Shrikant Malpani) has been deposited in the PG Pharmacognosy laboratory of the college for future reference.

Extraction: 200 g of shade dried and powdered stem bark was extracted with 95% ethanol at temperature 40-60^{0}C, in a Soxhlet extractor. The solution was evaporated giving a dark brownish residue (27.39%) stored in dessicator.

20 g of total alcoholic extract was dissolved in water and water soluble portion was fractionated with pet ether (yield 0.076%), benzene (yield 0.076%) and ethyl acetate (yield 69.72%). The suspension of alcoholic extract and its ethyl acetate fraction were prepared by using 1% Tween in saline. The phytochemical screening of the extracts revealed the presence of tannins, flavonoids, glycosides, carbohydrates, steroids and triterpenoids [8].

Animals: Wistar albino rats (200-300 g) of both sex used for the antidiabetic activity. The rats were purchased from Sri Venkateshwara Traders, Bangalore-21. They were housed in polypropylene cages and fed with laboratory diet and water ad *libitum*. All the protocols were performed in accordance with Institutional Animal Ethical Committee.

Toxicity studies in mice: In house bred albino mice of either sex weighing between 20-30gm were used during investigation. The animals were fasted over night. As per following the OECD guideline no. 420 fixed dose method procedure, the effective dose for both extract was found to be 200mg/kg body weight [9].

Antidiabetic activity:

- **Induction of diabetes:** The experimental diabetes in overnight fasted rats was induced by single intraperitoneal administration of 120 mg/kg alloxan monohydrate. After one hour of alloxanisation the animals were given feed *ad libitum* & 5% dextrose solution for a day to avoid early hypoglycemic phase. The blood glucose was monitored after every 24 hr of alloxanisation. Rats with blood glucose level more than 200 mg/dl were included in study [10].

- **Standard drug used:** Glibenclamide tablet (Daonil Tab.) manufactured by Aventis Pharma was used as standard drug. It was purchased from Nandi Pharma, Vidyanagar, Hubli-31. The tablets were suspended in distilled water using tween-80 as suspending agent & used for study.

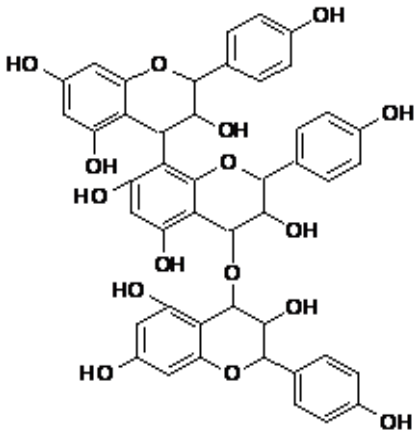
- **Experimental design:** The rats were divided in five groups of six animals each as follows: Group-I served as Normal Control, Group-II as Negative control (Untreated), Group-III as Standard control (5mg/kg), Group-IV received Ethyl acetate extract; Group-V received Total alcoholic extract. All the doses were given orally for 14 days.

- **Bioassay:** On 15th day of treatment, blood samples were collected by retro-orbital plexus puncture method under mild ether anesthesia and serum was separated by centrifugation. Serum glucose, cholesterol (CHL) and total triglyceride (TGL) levels were evaluated using a commercial kit [11]. Body weights of rats were taken before and after treatment [12].

- **Statistical Analysis:** The data were subjected to the analysis of variance (one way ANOVA) to determine the significance of changes, Dunnett's multiple comparison were made to analyze the significance of difference within the experimental groups. *P* values of 0.05 or less were taken as significant.

Chromatographic isolation: The ethyl acetate fraction was found to contain three compounds chromatographically. The compounds were separated by silica gel column using different organic solvents of increasing polarity [13]. Compound-I was eluted with benzene: ethyl acetate (3:7 v/v), compound-II with benzene: ethyl acetate (1:9 v/v).

Compound-I: Rf 0.730, solvent- benzene: ethyl acetate (1:9 v/v); UV (CH_3OH): 288 nm; FT-IR 3367.65 cm^{-1}(OH Stretching) 1610.88 cm^{-1}(C=C Stretching, Aromaticity) 1160.81 cm^{-1} (– C-O-C Stretching); ^{1}HNMR δ Value 2.1 – 2.8 (– CH, 9H, Alkyl-H) 6.8 – 7.8 (Ar-H, 17H, Ar-H), 8.4 – 10.4 (OH, 8H, Phenolic-H); ^{13}C-NMR δ Value 21.15 – 60.15 (-C – H, Alkyl carbon), 40.13 – 79.44 (CH – OH), 144.46 – 170.61(CH = CH, Aromatic); Mass spectra shows base peak at m/z 381 and M^+ peak at m/z 986.



COMPOUND- I

Compound-II: Rf 0.840, solvent- benzene: ethyl acetate (1:9 v/v); UV (CH_3OH): 282 nm; FT-IR 3365.72 cm^{-1}(OH Stretching),1704.68 cm^{-1} (C=O Stretching), 1610.29 cm^{-1} (C=C Stretching, Aromaticity), 1369.38 cm^{-1} (CH_3 Bending), 1163.22 cm^{-1} (C-O-C Ether linkage): ^{1}HNMR δ Value 8.8 (OH,1H, Phenolic), 6.5 – 7.5 (Ar-H, 4H), 4.8 – 5.3(OH, 4H, Alcoholic), 3.8 – 4.8 (OCH_3,6H), 1.0 - 2.8 (CH,5H, Alkyl, CH_2,2H, Alcoholic); ^{13}C-NMR δ Value 29.53 – 40.47(C – H, Carbon), 78.37 – 83.13 (C – O, Carbon), 114.77 – 157.24 (C = C, Aromatic); Mass spectra shows base peak at m/z 353 and M^+ peak at m/z 463.

RESULT AND DISCUSSION: It is well known fact that alloxan monohydrate induces diabetes mellitus in rats by selective necrotic action on the beta cells of pancreas leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals like increased blood glucose level, increased levels of cholesterol and triglyceride and decreased protein content [14].

As expected in alloxan treated rats, there was significant increase in blood glucose, cholesterol (CHL) and triglyceride (TGL) levels. The diabetic animals showed significant decrease in blood glucose level after 14 days treatment. Moreover it also decreased the levels of cholesterol (CHL) and triglyceride (TGL) increased by alloxan treatment (**Table 1, Figure 1 and 2**). Alloxan treatment of the rats has showed the loss in body weight as compared to normal rats (**Table 2, Figure 3**).

TABLE 1: EFFECT OF TOTAL ALCOHOLIC AND ITS ETHYL ACETATE FRACTION ON BLOOD GLUCOSE LEVEL, CHOLESTEROL AND TRIGLYCERIDE LEVELS OF THE RATS

Parameters → / Groups ↓	Blood glucose mg/dl	Triglycerides mg/dl	Cholesterol mg/dl
Normal	87.30 ± 4.648	73.82 ± 8.638	36.70 ± 3.365
Untreated	369.10 ± 7.974	150.5 ± 5.425	50.74 ± 2.055
Standard	124.70 ± 3.22***	81.15 ± 5.837***	40.51 ± 10.57
Ethyl acetate	183.00 ± 14.07***	85.79 ± 10.33***	37.00 ± 8.339*
Total alcoholic	293.00 ± 30.96*	81.79 ± 10.45***	45.76 ± 10.79

Values are Mean ± SEM, *N = 6*. *P < 0.05, **P < 0.01, *** P < 0.001, when compared to diabetic control.

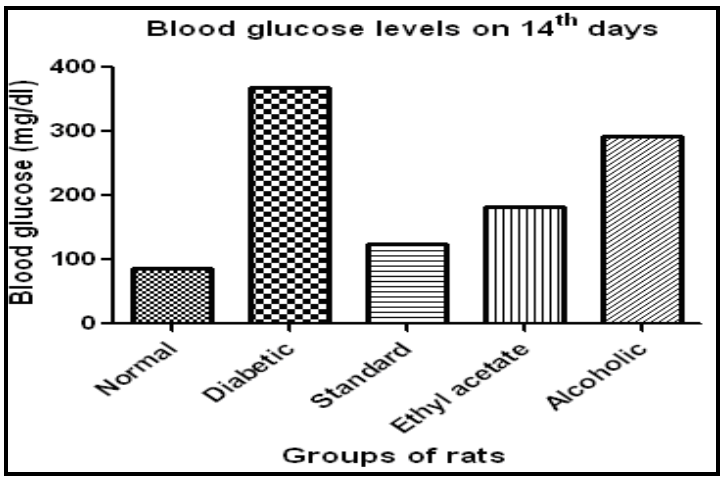


FIG. 1: EFFECT OF TOTAL ALCOHOLIC AND ITS ETHYL ACETATE FRACTION ON BLOOD GLUCOSE LEVELS

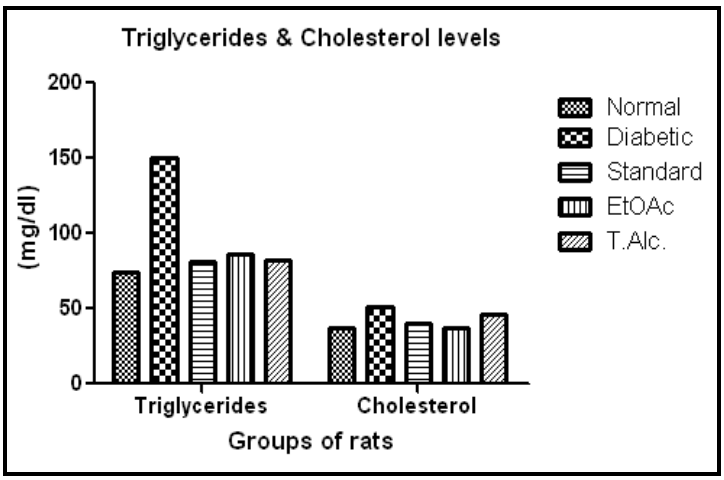


FIG. 2: EFFECT OF TOTAL ALCOHOLIC AND ITS ETHYL ACETATE FRACTION ON CHOLESTEROL AND TRIGLYCERIDE LEVELS

TABLE 2: PERCENTAGE INCREASE OR DECREASE IN BODY WEIGHTS (GMS) OF RATS

Parameters → / Groups ↓	Body weight before treatment	Body weight after treatment	% increase (+) or decrease (-)
Normal	163.7 ± 4.137	176.3 ± 2.261	+ 7.69
Untreated	283.3 ± 1.406	262.7 ± 4.333	- 7.27
Standard	210.3 ± 6.672***	198.00 ± 9.947***	- 5.84
Ethyl acetat	254.7 ± 2.616***	248.70 ± 3.211	- 2.36
Total alcoholic	216.3 ± 4.890***	208.70 ± 4.731***	- 3.51

Values are expressed as Mean ± SEM, *N = 6* ***P < 0.001, when compared to diabetic control.

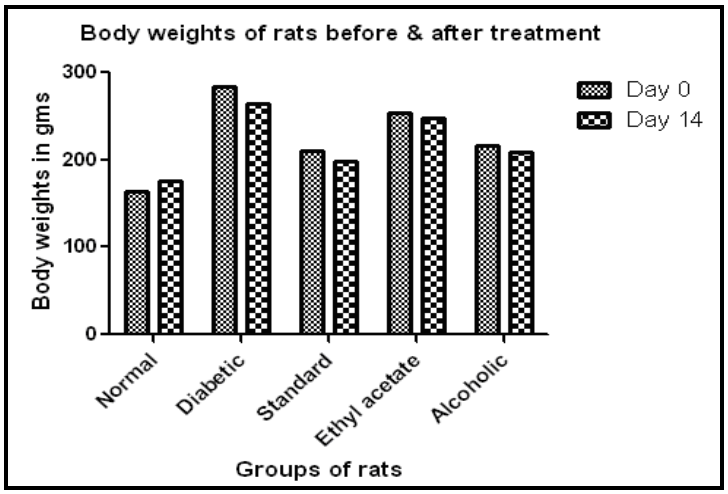


FIG. 3: EFFECT OF ALLOXAN TREATMENT ON BODY WEIGHT OF THE RATS

However, the ethyl acetate extract was more effective and results are comparable with that of reference drug, gliblenclamide. From the collective spectral data, the isolated compound-I was found similar to flavonoidal moiety (Compound-I). Hence, we can say that presence of flavonoid in the ethyl acetate fraction may be responsible for antidiabetic activity.

CONCLUSION: From the above results, it was concluded that the present study seems to support the claims by traditional medicine practitioners about the usefulness of *Cassia fistula* L. bark for the treatment of diabetes. From the phytochemical investigations and spectral data studies the correlation of antidiabetic activity can be made with the flavonoidal compounds.

ACKNOLEDGEMENT: The authors are thankful to the management KLE society, Belgaum & principal KLES College of pharmacy, Hubli for providing the necessary facility to carry out this study.

REFERENCES:

1. Kumar S, Kumar D, Deshmukh RR, Lokhande PD, More SN, Rangari VD: Antidiabetic potential of *Phyllanthus reticulates* in alloxan-induced diabetic mice. Fititerapia 2008; 79: 21-23.
2. Venkatesh S, Thilagavathi J, Shyam sundar D: Anti-diabetic activity of flowers of *Hibiscus rosasinensis*. Fitoterapia 2008; 79: 79-81.
3. Bhakta T, Banerjee S, Mandal SC, Maity TK, Saha BP & Pal M: Hepatoprotective activity of *Cassia fistula* leaf extract. Phytomedicine 2001; 8(3): 220-224.
4. Kannampalli P, Chandrashekaran VRM, Kuppannan G, Sivanesan K: Effect of *Cassia fistula* Linn. leaf extract on diethylnitrisamine induced hepatic injury in rats. Chemico-Biological Interactions 2007; 167 :12-18.
5. Orient Longman: Indian Medicinal Plants. A compendium of 500 species, Vol.2, (Orient Longman Ltd., Madras, 1994): 11-12.
6. Yoganarashimhan SN: Medicinal Plants of India. Vol.I, Bangalore, Interline Publishing Pvt. Ltd; 1994: 101-102.
7. Anonymous: A dictionary of Indian Raw Materials and Industrial Products: The Wealth of India. Vol.VII, New Delhi : Publication & Information Directorate, CSIR, 2003: 337-342.
8. Mukharjee PK. Quality Control of Herbal Drugs. 1st Edn. Business Horizon Pharmaceutical; 2001:183-219.
9. OECD [Organosation for Economic Co-opration and Development] 1992. Guideline 420: Acute oral toxicity – Fixed dose procedure, Paris: OECD.
10. Shankar MB, Parikh JR, Geeta M, Mehta RS & Saluja AK. Anti-diabetic activity of novel andrustane derivatives from *Syzygium cuminii* Linn. J of Natural Remedies 2007; 7/2: 214-219.
11. Adeneye AA, Ajagbonna OP, Ayodele OW. Hypoglycemic & antidiabetic activities on the stem bark aqueous & ethanol extracts of *Musanga cecropioides* in normal & alloxan induced diabetic rats. Fitoterapia 2007; 78:502-505.
12. Roy S, Sehgal R, Padhy BM, Kumar VL. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan induced diabetic rats. J of Ethnopharmacology 2005; 102: 470-3.
13. Gupta V, Agrawal Amulya , Singh J & Tiwari H. P.. Isolation & characterization of two flavonol & a xanthone glycoside from the stem bark of *Cassia fistula* Linn. Indian J of Chemistry March 1989; 28B: 282-4.
14. Dhanabal SP, Mohan Maruga Raja MK, Ramanathan M, Suresh B. Hypoglycemic activity of *Nymphaea stellata* leaves ethanolic extract in alloxan induced diabetic rats. Fitoterapia 2007; 78 : 288-291.

****