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ANTIHYPERGLYCEMIC AND ANALGESIC ACTIVITIES OF ETHANOLIC EXTRACT OF *CASSIA FISTULA* (L.) STEM BARK

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ABSTRACT

The present study was designed to evaluate antihyperglycemic and analgesic effects of ethanolic extract of *Cassia fistula (*CF) stem barks in rats and mice, respectively. The analgesic effect of extract was evaluated by acetic acid induced writhing test method while antihyperglycemic effect was investigated by oral glucose tolerance test (OGTT) in normal and alloxan induced diabetic rats. Diclofenac (10 mg/kg, i. p.) and metformin (150 mg/kg, p. o.) were used as reference drugs for comparison. The extract significantly ($P<0.05$) reduced blood sugar level in alloxan induced diabetic (hyperglycaemic) and glucose induced hyperglycemic (normo-hyperglycaemic) rats orally at 250 mg/kg and 500 mg/kg body weight respectively. The glucose tolerance results showed significant ($p<0.05$) improved at the dose 250 mg/kg and 500 mg/kg body weight (b. wt.) of ethanolic extract respectively. On the Other hand, the analgesic activity of extract at 200 mg/kg and 400 mg/kg dose level were produced 45% and 62% writhing inhibitory response but diclofenac was observed 82% of that when compared to control group. The plant's extract produced dose-dependent, significant ($P<0.05$) analgesic effects against chemically induced nociceptive pain in mice. Preliminary phytochemical screening of the plant extract showed the presence of alkaloids, triterpenoids, flavonoids, saponins and tannins etc. were present in the plant which has antihyperglycemic and analgesic properties. However a glucose tolerance hypoglycemic test is comparable to diabetic control group and effect is a dose dependent. The findings of this experimental animal study indicate that *Cassia fistula* stem-bark ethanolic extract possesses analgesic and antihyperglycemic properties; and thus lend pharmacological credence to the folkloric, ethnomedical uses of the plant in the treatment and/or management of painful, inflammatory conditions, as well as in the management and/or control of type 2 diabetes mellitus.

INTRODUCTION: According to World Health Organization projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 [1]. Diabetes mellitus is a chronic metabolic disorder in the endocrine system with multiple etiology, is characterized by chronic hyperglycemia together with disturbances in carbohydrate, protein and fat metabolism results from a decrease in circulating concentration of insulin (insulin

deficiency), a decrease in the response of peripheral tissues to insulin (insulin resistance) or both [2]. It is becoming the third "killer" of mankind after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality [3]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs [4].

The American Diabetes Association reports that about 50% of people with diabetes have some form of nerve damage known as diabetic neuropathy. Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Diabetes can destroy small blood vessels, which in turn can damage the nervous system, and these damaged nerves can cause pain. When a person has pain that is caused by nerve damage from diabetes, it is called diabetic nerve pain. Day by day, the number of people suffering from diabetic nerve pain has also increased. Diabetic nerve pain is a common diabetes complication, as are kidney and eye (retinopathy) conditions. The most common type of diabetic neuropathy is peripheral neuropathy such as burning, throbbing, or painful tingling in your hands or feet [5, 6].

Recently, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas, d-phenylalanine and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side effects in the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes [7, 8]. Hence plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabetic drugs. Many traditional plants treatment for diabetes are used throughout the world.

Analgesics relieve pain as a symptom, without affecting its cause [9]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. For instances, common pain medicines like aspirin may not work for nerve pain In this respect, new compounds with improved pain management capacity and fewer side effects are being searched every nook and corner of the world. Though considerable progress has been achieved in medical science during the last decades, management of chronic pain still remains a challenge for medical community [10]. As a result, more and more people are turning to herbal medicines as the alternative treatment of diabetes and along with pain.

According to the WHO, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. In developing countries 80% population are using traditional medicine in primary medical problems [11, 12]. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one [13]. The anti-hyperglycemic effect of these plants are for their ability to restore the function of pancreatic tissues by increasing insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.

Hence, treatment with herbal drugs has an effect on protecting *β*-cells and smoothing out fluctuation in glucose levels [14]. There is a need, therefore for new compounds that may effectively reduce insulin resistance or potentiate insulin action in genetically diabetic or obese individuals. The search for such drugs with a potential to reduce long-term complications of diabetes is, therefore of current interest.

Cassia fistula Linn also known as the golden shower tree (Bengali name, sonalu or bandor lathi), belongs to the family Leguminosae family, is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin and a tonic [15] and has been reported to treat many other intestinal disorders like healing ulcers [16].

In traditional medicine, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of haematemesis, pruritus, leucoderm and diabetes has been suggested [17]. The plant has documented to possess analgesic [18], anti-inflammatory [19], antioxidant [20], antidiabetic [21], as well as hepatoprotective activity [22]. However, a detailed pharmacological screenings of the *Cassia fistula* barks extract have not been reported. In the present study, we investigated the anti-hyperglycemic and analgesic activity of the stem barks of ethanolic extract of *Cassia fistula*.

MATERIALS AND METHODS:

Collection of Plant Materials: The fresh stem barks of the plant *Cassia fistula* Linn. were collected from Sirajganj district, Bangladesh in April 2010 and the plant authenticity were confirmed from the Bangladesh National Herbarium, Dhaka.

Preparation of Plant Extract: The collected stem barks were washed and sun dried under shadow for several days. The dried stem barks were powdered in an electrical grinder after overnight drying in an oven below 50°C. The powdered plant barks were extracted with 96% ethanol at room temperature. The bottle were kept at room temperature and allowed to stand for several 7-10 days with occasional shaking and stirring. The extracts thus obtained were filtered through cotton and then through filter paper (Whatman Fitter Paper No. 1). The filtrate was defatted with petroleum ether for several times. Then, the defatted liquor was allowed to evaporate using rotary evaporator at temperature 40-45°C. Finally, a highly concentrated ethanol extract were obtained and kept in desiccators to dry to give a solid mass (Yield 10g of extract from 500 g of plant powder material).

Drugs and Chemicals: The standard drug, Metformin hydrochloride was the generous gift samples from Chemico Laboratories Ltd. Alloxan monohydrate was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Blood samples analyzed for blood glucose content by using BioLand G-423 glucose test meter (BioLand, Germany). All chemicals and solvents were of reagent grade.

Experimental Animals: Eight weeks Long Evans rats (160-180g) and Six weeks Swiss albino mice (20-30g) of either sex were purchased from ICDDR, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 h light: 12 h dark cycle). The rats were fed with standard pellet diet obtained from ICDDR, Dhaka and water ad libitum. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute. The study protocol was approved by the animal Ethics Committee of the Institution.

Induction of Diabetes: After fasting 16h, diabetes was induced into rats by in intra-peritoneal injection (i. p.) of alloxan monohydrade (120 mg/kg), dissolved in saline (100 µl/rat, ip.). After 72h, plasma glucose levels were measured by glucometer (Bioland, Germany) using a blood sample from tail-vein of rat. Rats with blood sugar level higher than 11.5-12.5 mmol/l were considered as moderate diabetic.

Experimental Design: In the experiment, a total of 45 rats were used. The animals were divided into five groups and each group comprises of five rats. Group I received vehicle 0.5% methyl cellulose stands for normal control. Group II received vehicle 0.5% methyl cellulose serves as diabetic control. Group III selected for diabetic standard drug group which received metformin orally at a dose of 150 mg/kg. Group IV and Group V were received 250 and 500 mg/kg body weight rats CF extract orally after chemical diabetes.

Antihyperglycemic Activity Tests: The animals of Group IV and Group V received oral administrations of bark extract of *C. fistula* at a dose 250 and 500 mg/kg/ml body weight using intrgastric tube. Group III received metformin (150 mg/kg body weight), while Group II serves as diabetic control (vehicle 0.5% MC). The blood samples were analyzed for blood glucose content by Glucometer.

Oral Glucose Tolerance Test (OGTT) in diabetic rats: After fasting for overnight, a baseline blood glucose level was estimated (0 minutes). Without delay, a glucose solution (2 gm/kg body weight) was administered by gavage. At the same time standard drug and plant extracts were administered orally to the respective animal groups. Four more blood samples were taken at 30, 60, 90, 120 minutes after glucose administration and blood glucose level was estimated in all the experiments by using glucometer (Bioland-423, Germany).

Oral Glucose Tolerance Test (OGTT) in glucose induced hyperglycemic rats: For oral glucose tolerance test (OGTT) animals were divided into four groups (each group comprises five rats). Group VI to Group IX was prepared for testing of hypoglycemic effect after glucose-induced hyperglycemia in rats. Group VI received vehicle 0.5% methyl cellulose stands for normal control.

Group VII received metformin orally at a dose of 150 mg/kg and Group VIII and Group IX were received 250 and 500 mg/kg body weight of rats CF extract orally. Four more blood samples were taken at 30, 60, 90, 120 minutes after glucose administration and blood glucose level was estimated in all the experiments by using glucometer.

Analgesic Activity Test: In the experiment, a total of 20 mice were divided into four groups (each group comprises five mice). Group I served as vehicle control mice received vehicles (1% Tween 80 in saline), Group II served as standard group and received diclofenac (10 mg/kg i.p) as standard drug, Group III and Group IV received 200 and 400 mg/kg orally of CF extract respectively. The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Writhing was induced in mice by intraperitoneal administration of 0.1 ml of 1% Acetic Acid. Extract and vehicle were administered orally 30 minutess before intraperitoneal administration of 1% acetic acid but diclofenac sodium was administered intraperitoneally 15 minutess before injection of acetic acid. After an interval of 5 minutes, the mice observed for specific contraction of body referred to as "writhing" for the next 10 minutes [23].

Phytochemical Screening Tests: Phytochemical tests have been performed according to the literature by Nayak and Pereira (2006) [24].

Test for Saponins: 300 mg of extract was boiled with 5 ml water for two minutes. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

Test for Tannins: To an aliquot of the extract, sodium chloride is adding ed to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

Test for Triterpenes: 300 mg of extract was mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

Test for Alkaloids: 300 mg of ex tract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour which indicated the presence of alkaloids.

Test for Flavonoids: The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnings and potassium hydroxide solution.

Salkowski's reaction for Steroids: A few crystals of the compounds are dissolved in chloroform and a few drops of concentrated sulfuric acid are added to the solution. Production of brown color indicates the presence of steroids.

Statistical Analysis: Data were expressed as mean ± Standard error of mean (SEM). Statistical comparison was performed by one-way ANOVA, followed by Dunnett's Multiple Comparison Test. Results considered as significant when p values were less than 0.05 ($p<0.05$). Statistical calculations and the graph were prepared using GraphPad Prism Software version 5 (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

RESULTS: The effect of the ethanolic extract of *C. fistula* on the fasting blood glucose tolerance was investigated in normal and alloxan-induced diabetic rats using metformin HCl as standard antidiabetic agent.

Antihyperglycemic activity of CF extract in alloxan induced diabetic rats: The mean blood glucose concentration of control and different doses of *C. fistula* extract treated animals were estimated on 0, 2, 6, 16, 24 hours respectively as shown in **Table 1**. In case of alloxan induced diabetic rats metformin reduced blood glucose level were significantly compared to diabetic control rats.

The blood sugar levels in rats of Group CF 250 and Group CF 500 were lowered after 2, 6, 16, 24 hours of treatment and the effects were dose-dependent. Group CF 250 and Group CF 500 rats showed significant glucose lowering efficacy between hours 2-16 and were comparable to group diabetic control animal.

TABLE 1: ANTIHYPERGLYCEMIC ACTIVITY OF CF EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

	Blood glucose level (mmol/l)				
Group→ Time in hours↓	Normal Control	Diabetic Control	Diabetic standard (Metformin)	CF extract (250mg /k g)	CF extract (500mg /kg)
0	6.04±0.27	18.07±0.54	19.07±0.59	18.31±0.48	17.04±0.63
2	5.88±0.21	17.27±0.67	13.21±0.73	14.68±0.44	13.90±0.61
6	6.10±0.30	17.14±0.65	7.58±0.32*	10.49±0.52	7.86±0.51*
12	6.94±0.44	17.35±0.44	6.31±0.34*	9.80±0.39*	6.51±0.41*
16	5.92±0.30	16.86±0.44	7.70±0.46*	11.11±0.68	7.98±0.39*
24	6.61±0.42	16.36±0.79	9.15±0.42	13.40±0.42	9.74±0.57

*p<0.05 indicates significant activity comparing with diabetic control group. Each group contains 5 animals. Control group received 0.5% methyl cellulose and standard group received 150 mg/kg Metformin. The results are expressed in Mean ± SEM.

Oral Glucose Tolerance Test (OGTT) in alloxan induced diabetic rats: For oral glucose tolerance test, the blood samples were analyzed for glucose content at 0, 30 60, 90, 120 minutes, respectively. The blood sugar levels of Group CF 250 and Group CF 500 were lowered significantly (p<0.05) were comparable to diabetic control and the effects were dose-dependent. Group CF 250 and Group CF 500 rats showed significant glucose lowering efficacy between 60-90 min and were comparable to diabetic standard shown in **figure 1**.

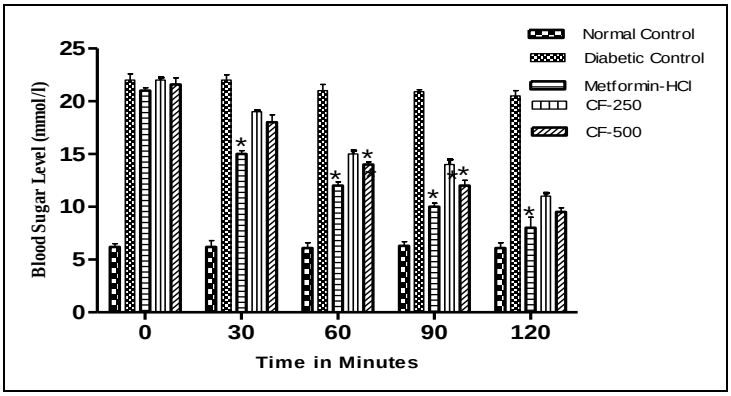

FIG. 1: EFFECT OF *C. FISTULA* EXTRACT ON ORAL GLUCOSE TOLERANCE TEST (OGTT) IN ALLOXAN DIABETIC RATS

The results are expressed in Mean ± SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150 mg/kg Metformin. *p<0.05 compared with diabetic control.

Oral Glucose Tolerance Test (OGTT) in glucose induced hyperglycemic rats: The effect of *C. fistula* extract in glucose induced hyperglycemia in normal rats shown in **figure 2**. The blood samples were analyzed for glucose content at 0, 30 60, 90, 120 minutes, respectively. The blood sugar were reduced in Group CF 250 and Group CF 500 significantly (p<0.05) and were comparable to normal rats and the effects were dose-dependent. It was found that extract have also hypoglycemic effect in glucose induced hyperglycemic rats.

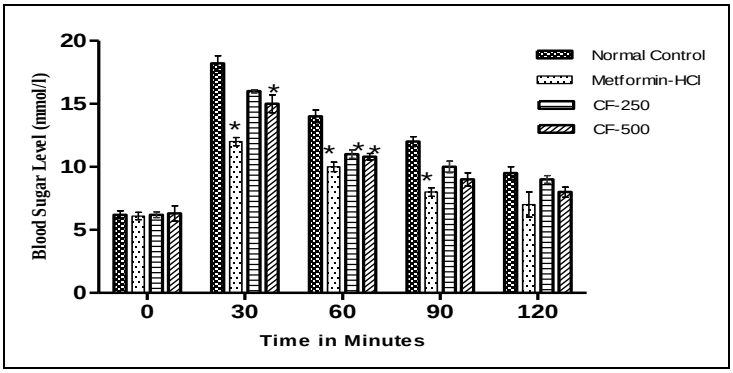

FIG. 2: EFFECT OF C. *FISTULA* EXTRACT ON THE GLUCOSE INDUCED HYPERGLYCEMIA IN NORMAL RATS

The results are expressed in Mean ± SEM. Each group comprised of 5 animals. Control group received 0.5% methyl cellulose and standard group received 150 mg/kg Metformin. *p<0.05 compared with normal control.

Analgesic activity of CF extract on Swiss Albino mice: The effect of the ethanolic extract of *C. fistula* was investigated on acetic acid-induced writhing response in mice using Diclofenac as reference standard drug. In writhing test the initial writhing of control mice, The analgesic activity of extract at 200 mg/kg and 400 mg/kg dose level were reduced 45% and 62% writhing inhibitory response but diclofenac was observed 82% of that in Group II. So, CF 200 and CF 400 mg/kg was found significantly (p<0.05) inhibitory response to pain induced by acetic acid when compared to control group I. Analgesic effect of the extract showed dose dependent antinociception against chemical induced pain in mice (**Fig. 3**). However, the reference drug Diclofenac was more potent than the plant extract at all dose level.

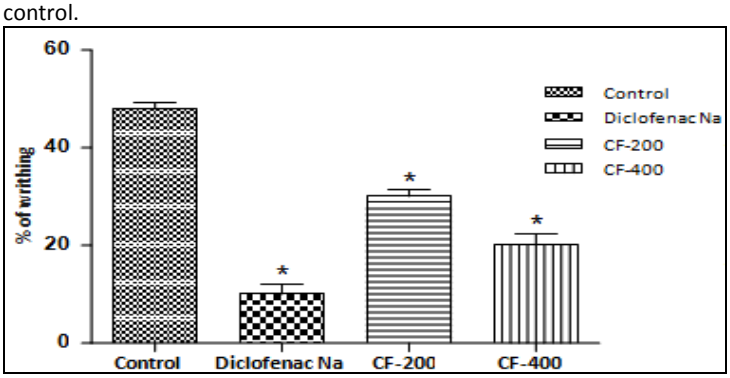

FIG. 3: EFFECT OF *C. FISTULA* EXTRACT ON WRITHING RESPONSE INDUCED ACETIC ACID IN MICE

The results are expressed in Mean ± SEM. Each group comprised of 5 animals. * indicate significant when compared with control ($p<0.05$).

Phytochemical Screening: The phytochemical analyses of crude extract revealed the presence of flavonoid, saponin, alkaloids, tannins and triterpenoid and negative result obtained for steroids. The results are summarized in **Table 2**.

TABLE 2: RESULTS OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

Plant Extract	Alkaloids	Saponins	Flavonoids	Tannins	Triterpenoids	Steroids
Ethanol Extract of *C. fistula*	+	+	+	+	+	-

+ = Presence; - = Absence

DISCUSSION: Diabetes mellitus is the world's largest growing metabolic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels [26]. Glycemic control is one of the targets for managing diabetes mellitus. Several studies have confirmed that effective control of blood glucose levels in type 2 diabetes substantially decrease the risk of developing diabetic complications [27]. Diabetes is a global disease with a huge adverse impact on the health and mortality. Traditional plant medicines are used throughout the world for the treatment of diabetic mellitus. The study of such medicine might offer a natural key to unlock for the future.

In the light of the literature on *Cassia fistula,* we made an attempt for the first time to study the antidiabetic and analgesic effect of ethanolic extract of bark in rats. Our present study revealed that ethanolic extract of *Cassia fistula* has significant effect in lowering fasting blood glucose level in alloxan and glucose induced diabetic rats. Metformin showed maximum reduction of blood glucose level at 12 hours and at the same time maximum reduction was obtained for extract in alloxan induced rats (Table 1). Blood sugar levels were then raised slightly for both extract and metformin treated rats group till observation probably due to loss of their duration of action. In glucose induced diabetic mice, a gradual declination of blood sugar level were observed in all treatment groups throughout the reading period. The extract showed dose dependent antihyperglycemic activity that is better antidiabetic effect was obtained at higher doses (500 mg/kg).

The experiment showed that Glucose Tolerance Test (OGTT) measures the body ability to use glucose, the body's main source of energy [28]. This test can be used to diagnose pre-diabetes and diabetes. Glucose lowering effects were found after oral administration of ethanolic extracts in rats (Figure 1 and figure 2). This may be due to the presence of hypoglycemic flavonoids, triterpenes or saponin glycosides that also requiring further investigation. The extracts have the properties to stimulate or regenerate the ß-cell for the secretion of insulin and are most effective for controlling diabetes by various mechanisms which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level. Induction of diabetes with alloxan was associated with decrease in hepatic glycogen, which could be attributed to decrease in the availability of the active form of enzyme glycogen synthetase probably because of low levels of insulin [29, 30].

In the present study, *C. fistula* restored the depressed hepatic glycogen levels possibly by increasing the level of insulin. Decreased activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animal resulting in depletion of liver and muscle glycogen content [31]. Treatment with plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx. The exact mechanism of action needs further investigation.

Cassia fistula has not been subjected to pharmacological investigations so far analgesic screening to provide scientific justification to its traditional claim in various pains. Therefore the present study has shown to establish remarkable analgesic potential of *Cassia fistula* (figure 3). Acetic acid-induced writhing model represent pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from phospholipids by the action of phospholipase A2 and other acyl hydrolase's [32].

The Prostaglandins mainly prostacyclin and prostaglandin-E have been reported to be responsible for pain sensation by exciting the A-fibres. Activities in the A-fibres cause a sensation of sharp well localization pain. Any agent that lowers the number of writhing will demonstrate analgesia preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [33]. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway [34, 35].

Flavonoids being powerful antioxidants [32] are reported to play a role in analgesic activity by targeting prostaglandins [36]. Since, stem bark of this plant contain flavonoids and glycosides [37]. Overall the analgesic action of *Cassia fistula* is assumed to be due to inhibition of prostaglandin synthesis.

The phytochemical screening of the plant *C. fistula* stem bark showed the presence of flavonoids, alkaloid, triterpenoids, saponins and tannins shown in Table 2. The ethanol extract of the plant *C. fistula* bark exhibited significant antihyperglycemic activity in alloxan-induced diabetic rats when compared with normal control. The activity also was comparable to that of the effect produced by metformin.

The present investigation established that the stem bark of the plant *C. fistula* have bioactive principles with anti-diabetic and analgesic potentials. However, further investigations were warranted to isolate bioactive compounds, to observe their effects on diabetic model and to find out the possible mechanism action for their beneficial effects both in normal and alloxan-induced diabetic rats.

CONCLUSION: The present investigation established that the stem barks of the plant *C. fistula* have antihyperglycemic and analgesic activities. This may be due to the presence of hypoglycemic saponins, triterpines, flavonoids and tannins. However, further investigations were warranted to isolate bioactive compounds responsible for these pharmacological activities.

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