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Evaluation of Hypoglycemic and Antioxidant potential of Different parts of *Cassia Fistula* l. in STZ-Induced Diabetic Rat

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ABSTRACT

The present study was undertaken with the objective to evaluate and compare the hypoglycemic and antioxidant potential of crude ethanol extract of the two different plant parts (Barks and Seeds) of Cassia fistula L. in vivo in order to select plant part with better efficacy to continue further studies with semi purified fractions. Albino wistar rats were made diabetic by single i.p injection of streptozotocin at a dose level of 60mg/Kg BW. Diabetic rats were treated with both the extracts (1/5th, 1/10th and 1/20th of respective LD_{50}) for twenty eight days orally once daily. FBS levels were estimated on 0,14th and 28th day. On 28th day animals were sacrificed under ether anesthesia and blood and tissues were collected and analysed for related biochemical parameters. Significant decrease in fasting blood glucose level on 14th and 28th day, and significant increase in hemoglobin content with reduction in glycosylated hemoglobin in blood, and increased catalase level in packed RBC, reduced lipid peroxidation and increased cellular glutathione level (GSH/NPSH) in packed RBC and liver were observed at the end of study with both the crude extracts. The results validate the in vivo antioxidant and hypoglycemic efficacy of both the extracts under our study. The efficacy of bark extracts came out better than the seeds extract on the basis of data accrued.

Keywords: *Cassia fistula; Barks and seeds; hypoglycemic and antioxidant activity; diabetic rats, Lipid peroxidation, GSH.*

INTRODUCTION

Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes *de novo* free radicals generation (Baynes & Thorpe, 1997). Oxidative stress has significant effect in the glucose transport protein (GLUT) and in insulin receptor activity (Mukherjee, 1989). Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of b-cells and protect pancreatic islets against the cytotoxic effects of streptozotocin (Coskun *et al,* 2005). In diabetes mellitus, protein glycation and glucose auto-oxidation may generate free radicals, which in turn catalyse lipid peroxidation (Baynes, 1991). Disturbances of antioxidant defense systems in diabetes mellitus such as alteration in antioxidant enzymes (Maritim *et al,* 2003), lowered vitamin levels (West, 2000), decreased ceruloplasmin levels (Anwar & Meki, 2003) have been reported. Decreased lipid peroxidation and improved antioxidant status may be one mechanism by which dietary treatment contributes to the prevention of diabetic complications (Armstrong *et al,* 1996).

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Therefore, a potent broad spectrum scavenger of the ROS may serve as a possible preventive intervention for free radical mediated cellular damage and diseases (Ahmad *et al*, 1998). From recent studies, it has been found that a number of plant products including polyphenols, terpenes, and various plant extracts exerted an antioxidant action (Zhou and Zheng, 1991; Quinn and Tang, 1996; Seymour *et al*, 1996; Prasad *et al*, 1996). Considerable amount of data have been generated on antioxidant properties of food plants around the globe (Kaur and Kapoor, 2002). However, traditionally used medicinal plants warrant such screening.

Cassia fistula Linn. (CF) commonly known as "golden shower" plant and belonging to *Leguminosae* family is one such plant reported to be effective as an antidiabetic agent in the various folk systems of medicine (Daisya et al, 2010).The plants are fast growing small tree or tall shrubs and are found growing throughout tropical countries of the world (Kritikar et al, 1991). Almost all parts of the plants are of medicinal use in the various folk system of medicine in Southeast Asia, and scientific studies have validated many of the ethnomedicinal observations. (Vasudevan et al, 2008; Perumal et al, 1998; Gupta et al, 2000; Yadav and Jain, 1999; Kuo et al, 2002). The preliminary antioxidant effect of the crude extracts of stem bark, seeds, flowers, and fruit pulp of *CF* has been assessed (Siddhuraju et al, 2002). The hydro alcoholic extracts of seeds have significant radical scavenging activity *in vitro* (Bhalodia et al, 2011; Jothy et al , 2011). The literature survey shows that there are a very few numbers of *in vivo* studies on the effect of these extracts on oxidative biomarkers in animal model. In milieu of these observations, the present study has been undertaken to evaluate the hypoglycemic and antioxidative property of this plant's bark and seeds crude ethanolic extracts *in vivo.*

MATERIALS AND METHODS

Collection of Plant Materials:

The barks and seeds of *Cassia fistula* (CF) were together collected at Shantiniketan campus of Visva-Bharati University, Birbhum district, India, in May 2013 and authenticated by botanist Professor DR Sudhendu Mandal, UGC Professor of Botany, Visva-Bharati Shantiniketan, India. The herbarium for future reference has been kept in Department of Veterinary Pharmacology and Toxicology, WBUAFS, Belgachia, Kolkata.

Preparation of Plant Extracts and phytochemical screening: *The* plant materials (barks and seeds) were shade dried and pulverized in mechanical mill. The appropriate dried powdered plant materials (200 gm) were kept in 2000 ml of 90% ethanol at room temperature for 10 days. The ethanol liquor was then filtered off using cotton plug. The filtrate obtained was subjected to repeated filtration for three times and was converted to a reduced mass on removal of ethanol under reduced pressure in a rotary vacuum evaporator. The semisolid mass is the crude ethanolic extract (CEA) of CF bark (CFBE) and seeds (CFSE). The average yield was 17.78 gm of CFBE and 13.29 gm of CFSE. Phytochemical screening of CFBE and CFSE were done according to standard natural product chemistry methods (Sarkar *et al*, 2006, Brahmachari G, 2009) before storing at – 20 deg C for further analysis. A known amount of each CEA was dissolved in ethanol and used for experiment in diabetic albino wistar rats.

Chemicals and Reagents:

Glucose estimation kits were obtained from Span Diagnostics Ltd. India. Glycosylated hemoglobin estimation kit was procured from Lab care Diagnostics (India) Pvt. Ltd. Streptozotocin (STZ) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Standard drug glibenclamide pure samples were procured from M/S Hindustan Chemicals & Pharmaceuticals, West Mumbai, India. All other chemicals (Analytical Grade) were procured from Hi-Media Laboratories Ltd, India,and Merck India Ltd. All organic solvents were of analytical grade purchased from local companies.

Animals:

The study was conducted on Albino Wistar Rats of either sex, five to six weeks of age weighing between 150–200 g, housed in polypropylene cages at an ambient temperature of 25 ± 2^{0}C with 12 h light and 12 h dark cycle. The rats were fed standard diets and water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 1 week. All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC), West Bengal University of Animal and Fishery Sciences, West Bengal, India.

Acute Toxicity Study:

The acute oral toxicity study was carried out in accordance to the guidelines set by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Twenty seven rats were fasted overnight and divided into 9 groups (N=3 in each group). Group 1 was administered vehicle only and served as control. Group 2-5 were administered range of concentration (1500, 2000, 2500 and 3000 mgkg^{-1}BW) of CFBE. Group 6-9 were administered range of concentration (1500, 2000, 2500 and 3000 mgkg^{-1} BW) of CFSE. The animals were observed continuously for the first two hours, followed by once every hour up to 6 h for any changes in behavioral, neurological, and autonomic profiles and then every 24 hours up to 14 days to identify lethality. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate, convulsion, water and food intake, state of stool, body weight and body temperature. On day 15th the animals were sacrificed under ether anesthesia. Hematological and serum biochemical parameters such as hemoglobin content, RBC and WBC total count, serum total protein, ALT, AST, urea nitrogen, cholesterol, creatinine and alkaline phosphatase were determined. Liver, spleen, and kidneys were dissected out and observed for gross morphological changes.

Induction of Type I diabetes:

The rats were fasted overnight and administered freshly prepared STZ solution (60 mg/kg body weight) dissolved in cold citrate buffer, (0.1M, pH 4.5) intraperitoneally. Diabetes was identified by polydipsia, polyuria, and by measuring non fasting plasma glucose levels. Animals with postprandial glycemia over 225 mg/dL, 3 days after STZ administration, were considered diabetic. Whole blood was collected from the tail vein of the rats for glucose estimation in serum. Control rats received only citrate buffer. (Mondal *et al*, 2012).

Determination of the Oral Glucose Tolerance (OGTT) in Normal Rats:

OGTT experiment was performed as explained by Gokee *et al* (2008). The normal rats were divided into 4 groups (n = 3) and were fasted overnight (18 h). The next day the rats were administered either drinking water (NC) or glibenclamide (PC) (0.5 mgkg^{-1} used as the standard drug) or 1/5th of LD_{50} of the two extracts. Glucose (2 gkg^{-1}) was administered 30 min after feeding the extracts. Blood was withdrawn from the tail vein at 30, 60, 120 and 180 min of glucose administration and blood glucose is estimated using biochemical kits in a spectrophotometer (Systronic 125).

Experimental Design:

The *in vivo* hypoglycemic and antioxidative effect of the extracts was studied in STZ- induced diabetic rats. The rats (n=3 per group) were divided into 9 groups: group 1(NC): normal rats treated with vehicle alone; group 2(DC): diabetic control treated with STZ solution (60 mgkg^{-1} body weight) dissolved in cold citrate buffer, 0.1M, pH 4.5, intraperitoneally; group 3(PC): diabetic rats treated orally with glibenclamide @0.5mg/Kg BW; group 4,5 and 6 diabetic rats were administered CFBE@1/5th, 1/10th and1/20th of its LD50 respectively(CFBE110, CFBE220, CFBE440); groups 7, 8 and 9 diabetic rats were administered CFSE@1/5th, 1/10th and1/20th of its LD50 respectively (CFSE110, CFSE220, CFSE440); the vehicle, standard drug, and extracts were administered orally to respective groups once daily for 28 days. FBS was estimated on 0, 14th and 28th day in blood collected from tail vein (using Reagent Kit). On day 28, the fasted animals were euthanized under ether inhalation. The fasted blood was collected by cardiac puncture with and without EDTA and processed for estimation of hemoglobin, glycosylated Hb(in whole blood using Reagent Kit), catalase (Bergmeyer, 1974), reduced glutathione (GSH) (Prins and Loos,1969), malondialdehyde (MDA) in packed RBC(Jain,1988) . Liver tissue was dissected out, washed with ice cold NSS and a 10% homogenate (w/v) was prepared in 0.1M, pH 7.4 PBS, centrifuged at 4^{0}C at 5000g for 30 mins and the supernatant was stored at -20^{0}C before analysis for MDA (Placer *et al*, 1966) and cellular glutathione (NPSH) (Sedlak and Lindsay, 1969).

Statistical Analysis:

Statistical analysis was performed using Graph Pad Prism software package Version 6.0. The values were analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, Tukey's multiple comparison test or Sidak's multiple comparison test as applicable for the data.

RESULTS AND DISCUSSION

The detailed study of plant constituents for two crude ethanolic extracts have been done by standard natural products chemistry methods (Brahmachari, 2009) and is depicted in Table 1. Bark extracts show the presence of alkaloids, terpenoids, flavonoids, polyphenols, anthraquinones and tannins, whereas seed extracts show the presence of anthraquinones, terpenoids, saponins, tannins,glycosides, phenolics and flavonoids. Tannins, fatty acids, glycosides, phenolic compounds and flavonoids in higher amounts had been reported in hydroalcoholic seed extract of CF by Bhalodia *et al*, (2011). Oxyanthraquinone, dihydroxyanthraquinone, catechin, lupeol, ß-sitosterol and hexacosanol, phenolics, flavonoids had been reported to be present in CF bark extracts with different solvents. (Bahorun et al, 2005)

The acute toxicity studies revealed that the oral administration of the extracts caused death in the higher doses of 2.5 and 3gm kg^{-1} BW. So the extracts are toxic to some extent. All the rats receiving CFBE and CFSE at dose levels below those survived throughout the experimental period without exhibiting any abnormalities. The rats did not show any symptoms of toxicity such as fatigue, loss of appetite, change in fur color, weight loss, etc. Comparative analysis of various hematological and biochemical parameters in the CFBE and CFSE treated and control animals, clearly showed that there was no significant alteration except marginal variations in certain parameters. The pathological examination confirmed that the extracts treated organs (lung, spleen, liver and kidney) did not show any significant morphological

changes in comparison to control. The observed probits were plotted against log dose and LD_{50} values were obtained from the point of intersection. The LD_{50} values obtained for both CFBE and CFSE were 2200mg /kg B Wt.

Table 2 demonstrates that the groups treated with CFBE at dose level of 440 $mgkg^{-1}$ showed initial rise in blood sugar up to 30 min, then the level decreased significantly (P<0.01) maintained up to 180 min. A similar pattern was also observed in case of CFSE at 440mg kg-1 starting from 60 min through 180min.So at these time points glucose tolerance of the animals has been significantly increased by both the extracts. As compared to control no significant difference (P>0.01) between two plant extracts was observed in this study through the entire duration of experiment.

Diabetes generates detrimental ROS inducing exhaustion of cellular anti oxidative systems that leads to liver and kidney damage and other diabetic complication. Hence drugs having hypoglycemic and anti oxidative effects are helpful under such situation. The oxidative stress biomarkers include superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia (Matrim et al, 2003). Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. Clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. These consequences of oxidative stress can promote the development of complications of diabetes mellitus (Matrim et al, 2003).

Significant hyperglycemia in STZ-treated diabetic rats was observed on 14^{th} and 28^{th} day of our experiment on comparison to control (P<0.01). The two crude extracts of CF administered orally once daily for 28 days reduced the fasting blood glucose level significantly (P<0.01) (Fig 1and 1a) at a dose dependent fashion as compared to diabetic control. Tukey's multiple comparison test for blood glucose mean values also reveals that there is significant difference (P<0.01) in hypoglycemic effects between CFBE and CFSE at their highest dose levels (440$mgKg^{-1}$BW), the former being more potent at the end of the study. Hypoglycemic effect exhibited by these two groups is not comparable to that of standard drug glibenclamide (P>0 .01). Glibenclamide, a standard antidiabetic drug, stimulates insulin secretion from beta cells of islets of Langerhans. Hence hypoglycemic effect of both the bark and seeds extracts of CF may be due to their insulin stimulating property. The methanol extract of the stem of CF and catechins, a flavonoid, isolated from the bark extract and seed extract from different organic solvent are shown to reduce the blood glucose levels in STZ-induced diabetic rats (Daisya et al, 2010; Einstein et al, 2012). The earlier researchers have suggested that flavonoids, having potent antioxidant activities present in plant extracts has protective effect in diabetes by decreasing oxidative stress and preservation of pancreatic beta-cell integrity (Coskun *et al*, 2005). They also inhibit cycloxygenases and promotes β-cell regeneration besides having insulin secretary property (Singh et al, 1976; Geeta et al, 1994; Gupta *et al*, 1994). Antihyperglycemic and antioxidant activity were reported in STZ-induced diabetic rats by rutin, a polyphenol flavonoid by Kamalakkannan and Prince (2006). Cassia fistula barks is a known source of tannins, flavonoids, phenolics and xanthine glycosides (Gupta *et al*, 1989). It has been reported that the stem bark of CF is a potential source of lupeol, β-sitosterol and hexacosanol (Bahorun et al., 2005). The bark extracts of CF have been shown to have the highest antioxidant potential (Sen and Shukla, 1968; Sidduraju et al, 2002) mostly due to presence of fistucasidin (flavan-3-diol) (Lal and Gupta, 1972). It contains a 4-ortho hydroxyl group which provides active hydrogen to take part in reaction to scavenge O_2^-.Some of the compounds isolated from the seeds of *C. fistula* are two oxyanthraquinones, chrysophanol, chrysophanein. (Kuo et al, 2002) and galactomannan constitutes of different type of sugar moieties (Lal *et al*, 1976). Yadava and Verma (2003) reported a new bioactive flavone glycoside from the acetone soluble fraction of defatted seeds of Cassia fistula Linn. It was characterized as 5, 3', 4'- tri- hydroxy- 6- methoxy- 7- O- alpha- L-rhamnopyranosyl- (1->2)- O- beta- D-galactopyranoside compound. Study showed the hydroalcoholic extract of CF seeds have significant radical scavenging activity and contained tannins, fatty acids, glycosides, phenolic compounds and flavonoids in higher amounts (Bhalodia et al, 2011). Previous studies have conclusively shown that the phytochemicals epicatechin, flavanoids, proanthocyanidins, and kaempferol possess hypoglycemic effects (Pinent et al, 2008). Flavonoids are antioxidants, anti-inflammatory, anticarcinogenic and protective against coronary disease and metabolic disorders. The great diversity of flavonoid structures makes it difficult to establish common effects in the pancreas. Published data suggest that there might be direct effects of flavonoids on insulin secretion, as well as on prevention of beta-cell apoptosis, and they could even act via modulation of proliferation. The mechanisms of action involve mainly their antioxidant properties, but other pathways might also take place. It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas beta cell regeneration ability (Pinent et al 2008). Present study shows that CF bark extracts contain alkaloids, terpenoids, flavonoids, polyphenols and tannins, whereas seed extracts show the presence of tannins , anthraquinones, saponins, glycosides and flavonoids. Thus the significant anti-diabetic effect of CFSE and CFBE may be due to the presence of more than one hypoglycemic principle and their synergic properties (Wolfram *et al* 2006, Shimada *et al* 2007) and it may be suggested that these crude extracts are rich in antioxidant principles that may be effective in controlling hyperglycemia and its complications.

Measurement of glycosylated haemoglobin has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein 1995). The glycated haemoglobin levels increased in diabetic rats with a subsequent decrease in the levels of hemoglobin (Fig 2 and 3). The levels of glycated Hb decreased significantly (P<0.01) following treatment with both extracts for 28 days. However they do not exert action comparable to positive control (P>0.01).Significant difference (P<0.01) in action was observed between two extracts at all dose levels at the end of study. Hemoglobin level increased significantly with treatments by both the extracts. Agents with antioxidant or free radical scavenging power may inhibit oxidative reactions associated with glycation (Elgawish *et al,* 1996). Study conducted with polyphenolic flavonoids with its free radical scavenging capability effectively reduced the formation of glycated haemoglobin and increased the haemoglobin levels in diabetic rats. (Kamalakkannan and Prince, 2006).Hence flavonoids glycosides and polyphenols present in the crude extracts under present study may be suggested to constitute such antioxidative principles capable of free radical scavenging activities and decreasing in Hb glycation.

Lipid peroxidation measured as MDA (Malondialdehyde) was seen increased (P<0.01) in packed RBC and liver of streptozotocin-diabetic rats in this study. Diabetic rats when treated with the plant extracts at various dose levels, both drugs exhibited a significant (P<0.01) effect on MDA production in these cells and tissues and decreased their levels as compared with diabetic control rats (Fig 4 and 5). Significant difference (P<0.01) in reducing lipid peroxidation was found at all dose levels of the bark and seeds extracts in RBC system. In liver tissue, effect of CF bark extract at all dose levels was significantly higher than CF seeds extract (P<0.01). However they do not exert action comparable to positive control (P>0.01). MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress (Shodehinde and Oboh, 2013). Reactive oxygen species are increased by hyperglycemia. Hyperglycemia, which occurs during diabetes (both type 1 and type 2) and, to a lesser extent, during insulin resistance, causes oxidative stress. Oxidative stress may be important in diabetes because of its role in the development of diabetic complications. Lipid peroxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants (Korkina & Afanas'ev 1997). Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of reactive oxygen species (Bergendi *et al,* 1999). Elevated levels of MDA in these cells and tissue in our study is a clear manifestation of excessive formation of free radicals resulting tissue damage. Karpan *et al* (1982) observed an elevated level of lipid peroxides in plasma of STZ-induced diabetic rats. Yang *et al.* (2009) observed greater serum lipid peroxidation evaluated in terms of MDA in hyperglycemic mice and proposed that the increase in lipid peroxidation exacerbated the occurrence of myocardial infraction through NADPH oxidase activation (Yang et al, 2009). Significant decline in the concentration of MDA in RBC and liver of CF bark and seeds extract treated diabetic animals indicates the potential use of these extracts *in vivo* to counteract the oxidative stress induced changes in diabetes. Recently, a clinical study performed by Bandeira and coworkers (2012) aimed at characterizing blood oxidative stress in diabetic patients reported a significant higher lipid peroxidation which showed a close

relationship with high glucose levels as observed by the fasting glucose and glycosylated hemoglobin levels (Bandeira et al, 2012).

The non protein thiols measured in RBC and liver (Fig 6, 7) decreased significantly (P <0.01) in diabetic rats as compared to normal control. The crude extracts of CF barks and seeds increased the cellular glutathione level in the present study as compared to diabetic control significantly (P<0.01). Significant difference (P>0.01) was found at 440mgkg^{-1} dose level of the bark and seeds extracts in increasing cellular glutathione content in RBC and liver tissue. However they do not exert action comparable to positive control (P>0.01).GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage (Tsai et al, 2012).Cellular reduced glutathione plays a major role in coordinating the body's antioxidant defense processes. It is present in all mammalian tissues as the most abundant nonprotein thiol that defends against oxidative stress (Lu, 2013). It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level (Rizvi and Chakravarty, 2011). There are several reports that claim reduced level of GSH in diabetes (Calabrese et al, 2012).Perturbation of GSH/NPSH status of a biological system can lead to serious consequences. Hyperlipidemia, inflammation, and altered antioxidant profiles are the usual complications in diabetes mellitus as results decreased GSH/GSSG ratio (Das et al, 2012).Glutathione depletion is a sign of cellular toxicity. Glutathione serves as a substrate for the enzyme glutathione peroxidase which catalyses the reaction of endogenously formed toxic hydroperoxides with reduced glutathione to produce oxidized glutathione (GSSG) and hydroxyderivatives (Christopherson, 1969). In the present study significant decrease in level of GSH in diabetic animals and attainment of significant improvements, particularly at highest dose levels of both the extracts, indicate that oxidative stress elicited by STZ was significantly reduced by these extracts.

The results of the present study show that catalase activity (Fig 8) decreased significantly in diabetic rats as compared to control. The CF bark extracts at dose levels of 220 and 440mgKg^{-1} BW and CFSE at 440mgKg^{-1} BW dose levels increased the catalase activity (P<0.05) as compared to diabetic control rats. The effect of CFBE at 440mgKg^{-1} BW is comparable (P>0.05) with positive control. The other doses of the extracts had no significant effect (P>0.05). Catalase protects pancreatic β-cells from damage by hydrogen peroxide (Tiedge *et al*, 1998).Catalase is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases (Chelikani et al, 2004). Catalase acts as main regulator of hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive small molecule formed as natural by-product of energy metabolism. Excessive concentration of hydrogen peroxide may cause significant damages to proteins, DNA, RNA, and lipids (Takemoto *et al* 2009). Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. The deficiency of this enzyme leads, in the pancreatic β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS (Góth and Eaton,2000).

Table No. 1: Phytochemical constituents present in crude ethanolic extracts of *Cassia fistula* barks and seeds.

Extract	Phytoconstituents							
	Tannins	Alkaloids	Saponin	Glycosides	Terpenoids	Flavonoids	Phenolics	Anthraquinones
Bark Extract	Yes	Yes	No	yes	Yes	Yes	yes	Yes
Seed extract	Yes	No	Yes	yes	Yes	Yes	Yes	Yes

Table No. 2: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) on blood glucose level over time in glucose induced hyperglycemic Albino Wister Rats.

Treatment groups	Blood glucose level (mg%)				
	0min	30min	60min	120min	180min
NC	101 ±1.52	152.00± 2.30	131.67± 2.03	111.7± 0.88	108.7± 0.67
PC	97.33± 0.66	119.3± 1.76*	111.3± 0.67*	104.0± 1.15*	102.00± 2.31*
CFBE@440mgKg^{-1}	97.33± 0.67	140.7± 0.67*	122± 1.15*	103.33 ±0.67*	98.67± 0.67*
CFSE@440 mgKg^{-1}	99.67± 1.20	143.3 ±2.4*	120.7± 0.67*	104.33 ±0.67*	90.00 ±5.292*

All values are Mean ± SEM (n=3) * implies significant as compared to normal control (P<0.01) Two way ANOVA followed by Dunnett's multiple comparison test.

Abbreviations: NC: normal control; PC: Positive control; CFBE: *Cassia fistula* bark extract CFSE: *Cassia fistula* seed extract

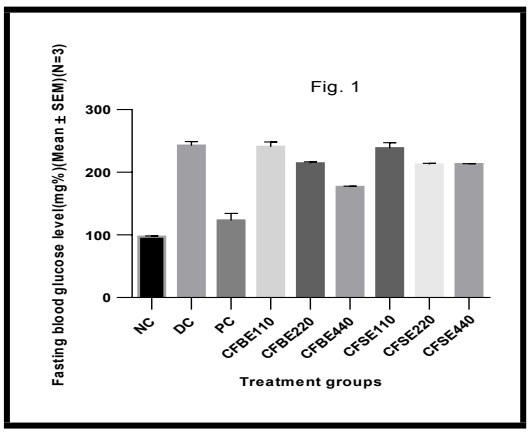

Fig. 1: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on Fasting Blood glucose levels (14th day) in diabetic Albino Wister Rats.

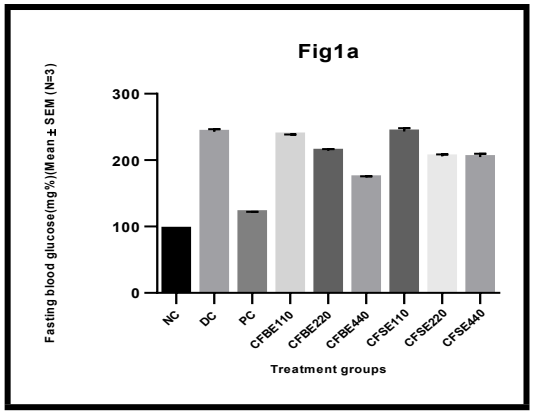

Fig. 1a: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on Fasting Blood glucose levels (28th day) in diabetic Albino Wister Rats.

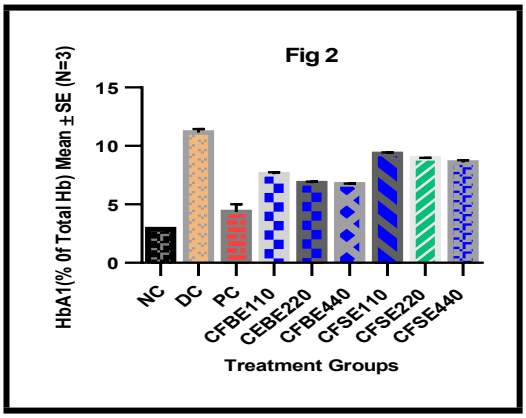

Fig. 2: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on Glycosylated Hb levels in diabetic Albino Wister Rats.

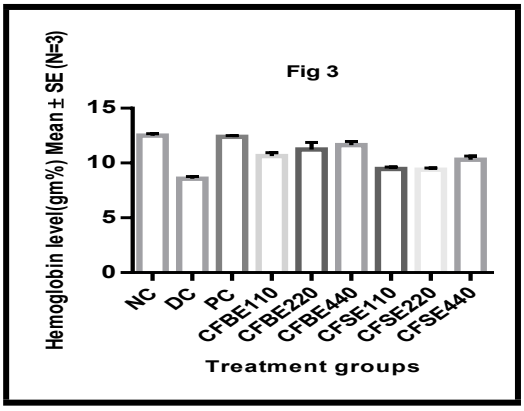

Fig. 3: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on Hb levels in diabetic Albino Wister Rats.

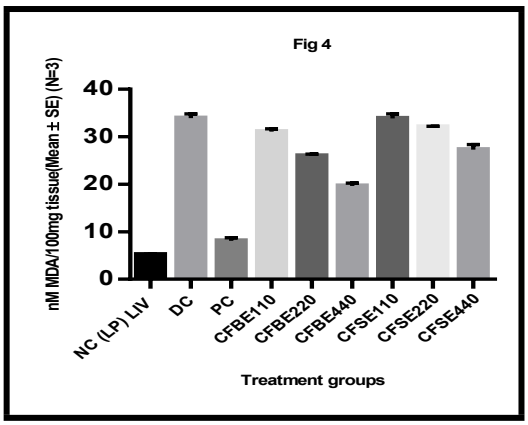

Fig. 4: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on MDA levels in liver of diabetic Albino Wister Rats.

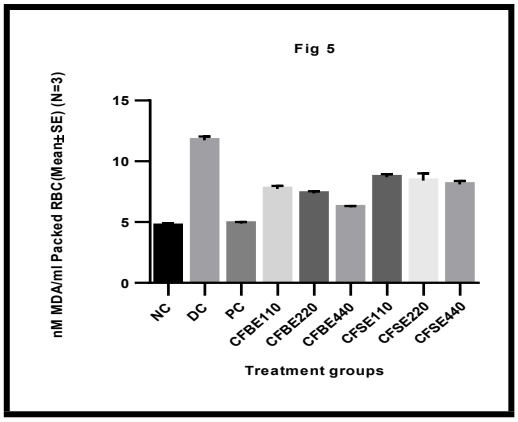

Fig. 5: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on MDA levels in packed RBC of diabetic Albino Wister Rats.

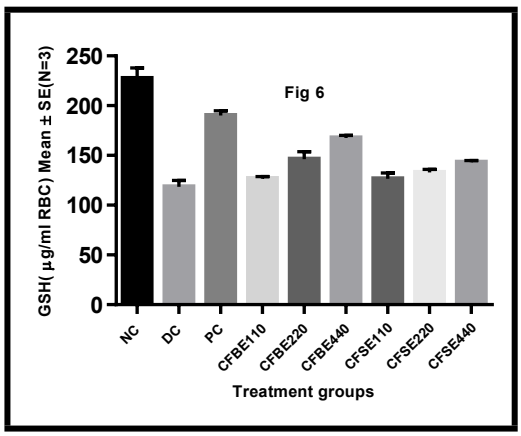

Fig. 6: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on GSH levels in packed RBC of diabetic Albino Wister Rats.

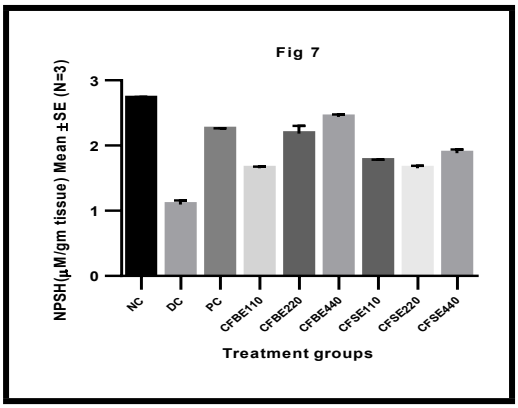

Fig. 7: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on NPSH levels in liver of diabetic Albino Wister Rats.

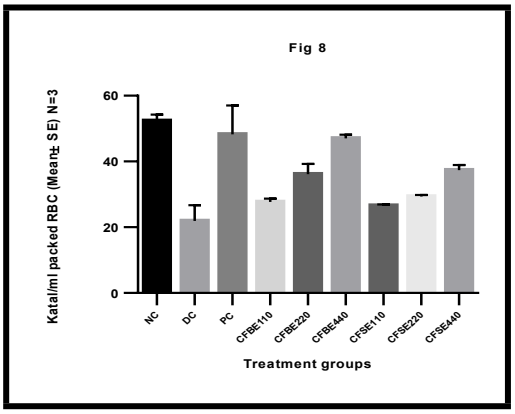

Fig. 8: Comparative effect of ethanolic extracts of *Cassia fistula* bark (CFBE) and seed (CFSE) at different doses on catalase activity in packed RBC of diabetic Albino Wister Rats.

CONCLUSION

On the basis of experimental observations and data accrued it may be primarily suggested that CF bark and seeds CEE possess hypoglycemic and antioxidative potential *in vivo* and CFBE being superior to CFSE. The actual mechanism of action of the antioxidative actions exerted by these two crude extracts at different dose levels is not clear, as it is a preliminary study. However, activities such as reduction of lipid peroxidation in tissues, enhancement of cellular glutathione and catalase activity in cells and reduction of hyperglycemia and hemoglobin glycation make them potential candidates for antioxidant activities which may partly be attributed to their phenolic, flavonoids, glycosides and terpenoids content. Further studies with semipurified and purified fractions are necessary to explore the underlying mechanism.

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