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EFFECT OF *TAMARINDUS INDICA* LINN. AND *CASSIA FISTULA* LINN. STEM BARK EXTRACTS ON OXIDATIVE STRESS AND DIABETIC CONDITIONS

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Abstract: *Tamarindus indica* and *Cassia fistula* are traditionally important medicinal plants. Stem barks of these plants have not been much explored for their potential hypoglycemic and oxidative stress conditions. The main aim of present study was to evaluate antidiabetic activity along with renal complications and antioxidant potential of alcoholic extracts of stem barks of these plants. Alcoholic extracts of stem barks of *Tamarindus indica* and *Cassia fistula* were evaluated for anti-hyperglycemic effect in alloxan-induced diabetic rats. Biochemical parameters including blood glucose, serum cholesterol, triglycerides, serum albumin, total protein and creatinine were studied. Antioxidant potential in DPPH, nitric oxide and hydroxyl radical induced *in vitro* assay methods were evaluated. Acute toxicity studies were carried out to establish the safety of the drugs according to OECD guidelines. There was a significant decrease in blood glucose level in diabetic rats treated with the alcoholic extracts of both plants. Serum cholesterol, serum triglyceride, serum creatinine, serum albumin, total proteins and body weight were recovered to normal levels at the end of the studies. Alcoholic extract of stem bark of both plants showed significant antioxidant activity in DPPH, nitric oxide and hydroxyl radical induced *in vitro* assay methods. Acute toxicity studies with the extracts of both plants showed no signs of toxicity up to a dose level of 2000 mg/*p.o*. It can be concluded from the study that *Tamarindus indica* and *Cassia fistula* stem barks possess blood glucose lowering effect along with antioxidant effect and protective effect on renal complications associated with hyperglycemia.

Keywords: antidiabetic, oxidative stress, renal complications, *Cassia fistula*, *Tamarindus indica*

Diabetes is a complex heterogeneous disorder affecting millions of people worldwide. The pathogenesis involves development of insulin resistance accompanied by defective insulin secretion from pancreatic β-cells resulting in an increased blood glucose level. Increased blood glucose level may further elevate various other diabetic complications. It has been found that the generation of reactive oxygen species (oxidative stress) may play an important role in the etiology of diabetic complications. Exposure of endothelial cells to high glucose can further leads to the production of superoxide anion, which may quench nitric oxide affecting general homeostasis of the vasculature (1). Such kind of biochemical changes may further lead to other diabetic complications including cardiovascular disorders, renal dysfunction and diabetic retinopathy. There are so many synthetic agents employed in the management of diabetic condition but generally having inadequate efficacy and serious side effects (2).

Hundreds of plants have been screened scientifically for their effect on diabetic conditions but search for the appropriate solution is still going on. There are wide variety of newer therapeutic agents/strategies being examined for the treatment of type 2 diabetes, most of all currently under preclinical and early clinical stages of drug development (3, 4). Looking into this aspect, the two important medicinal plants namely *Cassia fistula* (CF) and *Tamarindus indica* (TI) stem barks were selected to find out their antidiabetic potential along with the effect on oxidative stress and renal complications associated with the disease.

Fruit, seeds, leaves and flowers of these plants have been screened for various activities including diabetes and oxidative stress. Lesser reports have been found on the stem barks. Also not much literature was available on studies showing the effect on oxidative stress along with changes in the parameters indicating the recovery of renal damage. This study includes the evaluation of antidiabetic potential along with the effect on oxidative stress and

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other biochemical parameters indicating protective effect of the test drugs against renal damage.

Cassia fistula Linn. (Golden shower tree) is a flowering plant belonging to family Leguminosae. Leaves, flowers, fruit, stem bark and root have been used in traditional system of medicine for the treatment of various diseases. Leaves and pods have been widely used as strong purgatives and laxative (5-8).

Tamarindus indica Linn. (Tamarind) family Leguminosae is a large tropical tree with a short massive trunk, ferny pinnate leaves, small yellow flowers and flat reddish brown pods. It is widely used as a food and medicine (9-12).

EXPERIMENTAL

Plant materials

Stem barks of both the plants were collected around the area near Greater Noida U.P. India in the month of May. Herbariums of the collected herbs were submitted at National Bureau of Plant and Genomic Research (NBPGR), Pusa Campus, New

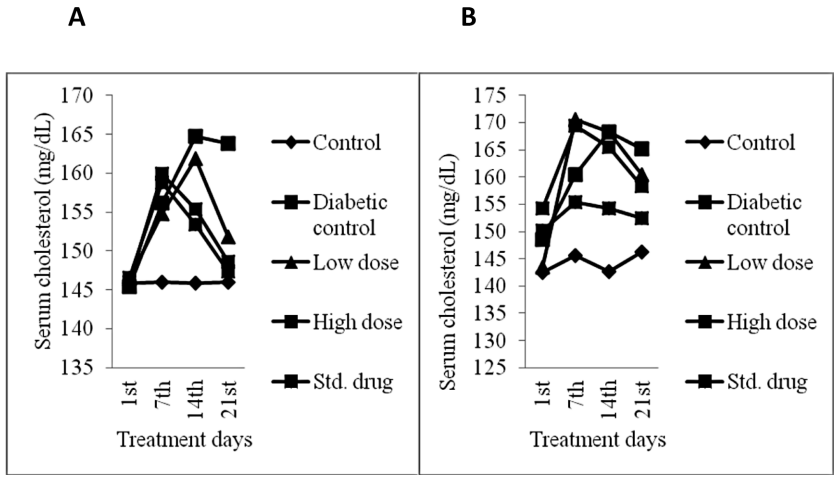

Figure 1. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (**B**) bark on serum cholesterol level (SCL) on alloxan-induced diabetic rats. The serum cholesterol level in mg/dL is presented as the mean ± SEM

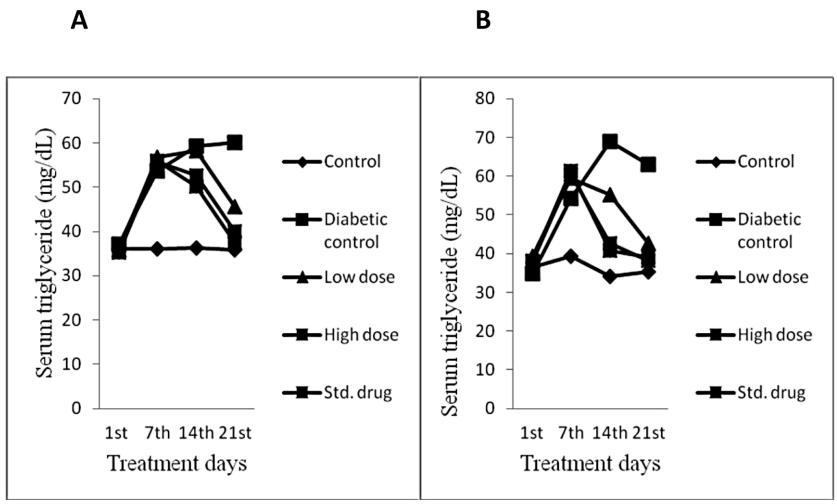

Figure 2. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (**B**) bark on serum triglyceride level (STL) on alloxan-induced diabetic rats. STL in mg/dL is presented as the mean ± SEM

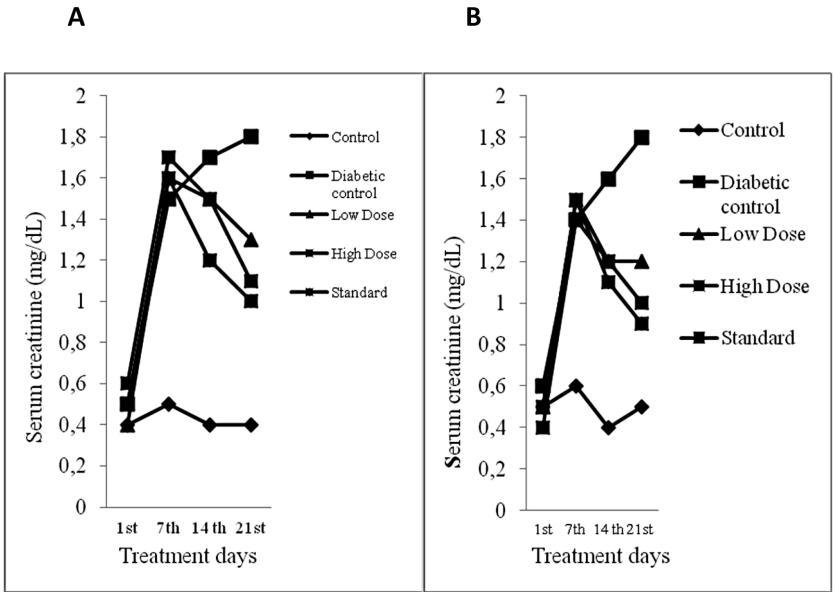

Figure 3. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (**B**) bark on serum creatinine level on alloxan-induced diabetic rats. The serum creatnine level in mg/dL is presented as the mean ± SEM

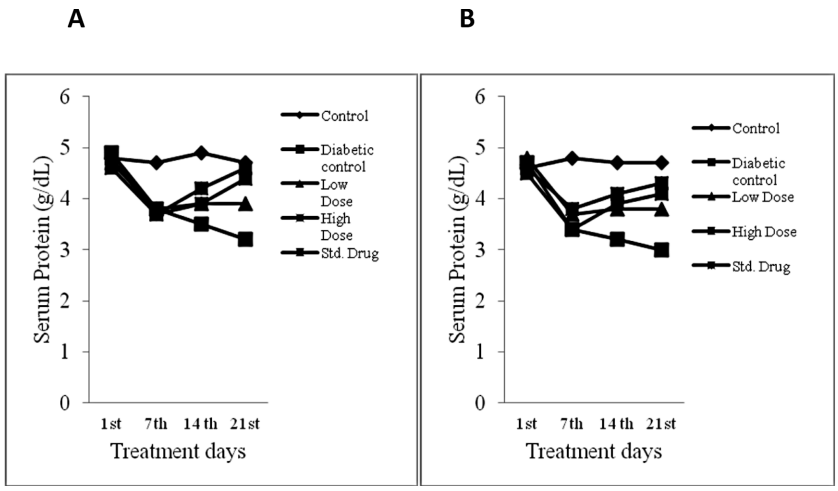

Figure 4. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (**B**) bark on serum protein level on alloxan-induced diabetic rats. The serum protein level in g/dL is presented as the mean ± SEM

Delhi, India for authentication and voucher no. NHCP/NBPGR/2009/1 was provided.

Preparation of extracts

The plant materials were washed thoroughly to remove adhered foreign matter, dried in shade and grinded into coarse powder with the help of a mechanical grinder. Coarse powdered drugs were extracted in Soxhlet extractor with ethanol. The extracts were dried under vacuum and lyophilized.

Acute toxicity

Acute oral toxicity study was performed acc. to OECD-423 guidelines (acute toxic class method) (16). Wistar rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals,

Table 1. Effect of alcoholic extracts of *Cassia fistula* and *Tamarindus indica* bark on blood glucose level on alloxan-induced diabetic rats.

Treatment groups	*Cassia fistula*				*Tamarindus indica*			
	1st day	7th day	14th day	21st day	1st day	7th day	14th day	21st day
Control	89.00 ± 0.03	89.85 ± 0.14	89.57 ± 0.02	89.43 ± 0.11	83.25 ± 2.55	84.50 ± 2.26	84.0 ± 2.17	85.37 ± 2.12
Diabetic control	232.29 ± 0.11	235.57 ± 0.21	239.57 ± 0.24	238.00 0.05	241.25 ± 2.08	246.42 ± 10.50*	295.00 ± 3.61	296.14 ± 4.45
Low dose	238.43 ± 0.03	226.00 ± 0.04**	202.29 ± 0.12**	132.86 ± 0.11	247.62 ± 2.32	250.42 ± 3.50*	194.5 ± 3.59*	165.14 ± 2.41*
High dose	240.00 ± 0.22*	230.00 ± 0.16*	182.00 ± 0.05	104.57 ± 0.18	244.12 ± 2.67	248.62 ± 6.71*	183.62 ± 2.47*	148.25 ± 2.61*
Standard drug	237.86 ± 0.31	233.00 ± 0.03*	172.29 ± 0.04*	90.86 ± 0.04	267.37 ± 1.19	280.0 ± 6.74*	147.87 ± 2.27*	88.12 ± 1.80*

The blood glucose level in mg/dL is represented as the mean ± SEM. * $p < 0.05$ *vs*. diabetic control group (n = 6-8 animals).

Table 2. *In vitro* free radical scavenging effect of *C. fistula* and *T. indica* bark extracts by DPPH method.

Drug	% Scavenging (the mean ± SEM) of triplicates						
	4 µg/mL	8 µg/mL	15 µg/mL	30 µg/mL	60 µg/mL	125 µg/mL	250 µg/mL
C. fistula	22.02 ± 0.002*	24.21 ± 0.002*	27.91 ± 0.001	33.02 ± 0.001	41.12 ± 0.002	45.22 ± 0.002	55.21 ± 0.002
T. indica	20.15 ± 0.002*	22.54 ± 0.001*	23.34 ± 0.001*	30.77 ± 0.001*	37.4 ± 0.001*	45.22 ± 0.002*	50.13 ± 0.002*
Vitamin C	0.1 µg/mL	0.2 µg/mL	0.4 µg/mL	0.6 µg/mL	0.8 µg/mL	1.0 µg/mL	—
	6.03 ± 0.002	15.22 ± 0.001*	32.51 ± 0.001*	48.18 ± 0.003*	64.15 ± 0.001*	81.12 ± 0.001*	—

Data were analyzed using one way ANOVA followed by Dunnett's multiple comparison test, * $p < 0.01$.

then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 100, 150, 300 up to 2000 mg/kg body weight.

Selection of animals

Healthy Wistar rats of either sex (150-200 g) were selected for study. They were housed in plastic cages at temperature of 24 ± 1°C with 12-h light and dark cycle. They were provided with standard diet *ad libitum*. All the animals were acclimatized for one week prior to the study. The test extracts and the standard drugs were administered in the form of a suspension in water using 5% Tween 80 as suspending agent. All the pharmacological experimental protocols were approved by the institutional Animal Ethics Committee.

Experimental design

The diabetic and normal animals were divided into seven groups and each group contained eight animals.

Group I – Normal control rats received 1 mL 5% Tween-80 by oral administration.

Group II – Diabetic control rats received 1 mL 5% Tween-80 by oral administration.

Group III – Diabetic rats received 250 mg/kg of alcoholic extracts of *C. fistula*.

Group IV – Diabetic rats received 250 mg/kg of alcoholic extracts of *T. indica* bark.

Group V – Diabetic rats received 500 mg/kg of alcoholic extracts of *C. fistula*.

Group VI – Diabetic rats received 500 mg/kg of alcoholic extracts of *T. indica*.

Group VII – Diabetic rats received standard reference drug (gliclazide).

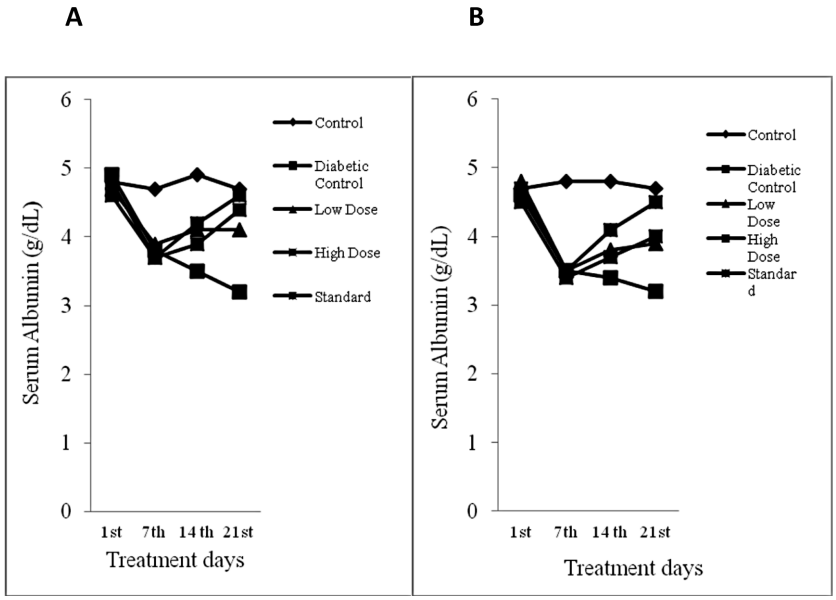

Figure 5. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (B) bark on serum albumin level on alloxan-induced diabetic rats. Serum albumin level in g/dL is presented as the mean ± SEM

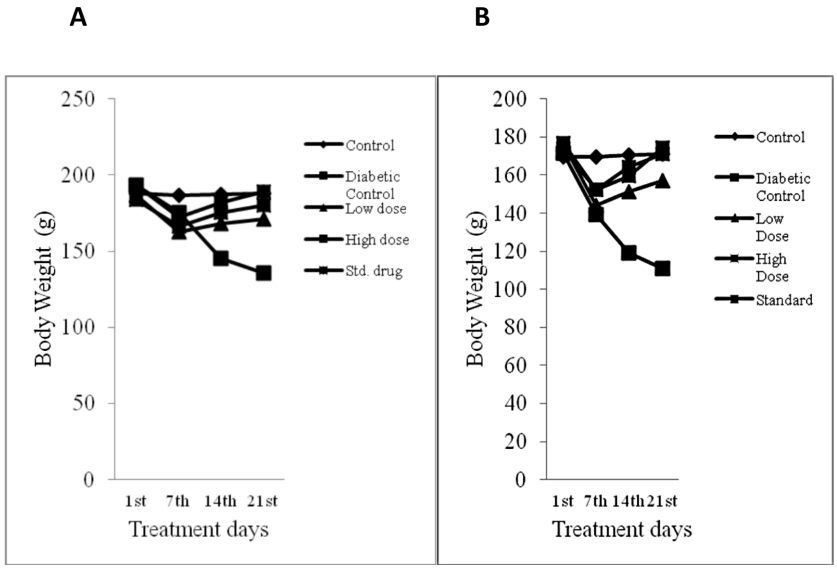

Figure 6. Effect of alcoholic extracts of *Cassia fistula* (**A**) and *Tamarindus indica* (**B**) bark on body weight on alloxan-induced diabetic rats. The body weight in grams is represented as the mean ± SEM

Anti-diabetic activity

The body weights of the animals were measured using a top loader weighing balance. Blood samples were obtained from the tail vein of the animals and their fasting blood glucose level was determined in mmol/L using a digital glucometer (Accu-chek® Active, Roche Diagnostic, Germany. Animals showing normal blood glucose level 70-100 mg/dL

were included in the study. Diabetes was induced by a single intraperitonial injection of freshly prepared alloxan (120 mg/kg) and it was confirmed by glucometer. Animals showing blood glucose level above 200 mg/dL were considered for the study after 24 h of alloxan administration. Standard drug – gliclazide (1.4 mg/kg body weight) tablet manufactured by Panacea Biotech Ltd. (B. No. 1048503) was used as standard drug. The dose was calculated on the basis of adult human effective dose.

Estimation of other biomarkers

Serum cholesterol

In vitro quantitative determination of the activity of cholesterol in serum was done using enzymatic kit (Nicholas India Pvt. Ltd., Ahmedabad, India). Cholesterol esterase (CHE) hydrolyses cholesterol ester. Free cholesterol is oxidized by the cholesterol oxidase (CHO) to cholest-4-ene-3-one and hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to produce pink colored quinoneimine dye. The intensity of color produced is proportional to cholesterol concentration.

Serum triglyceride

In vitro quantitative measurement of triglyceride concentration in serum was done using appropriate kit (Nicholas India Pvt. Ltd., Ahmedabad, India). Triglycerides in the sample are hydrolyzed by microbial lipase to glycerol and free fatty acid (FFA). Glycerol is phosphorylated by adenosine 5-triphosphate (ATP) to glycerol 3-phosphate (G-3-P) giving a colored complex with picrate in alkaline medium. The rate of formation of the complex is measured.

Serum proteins

In vitro quantitative measurement of total protein concentration in serum was done using appropriate kit (Span Diagnostics India Pvt. Ltd., Ahmedabad, India). Peptide bonds in protein react with cupric ion in alkaline solutions to form a colored chelate, the absorbance of which is measured at

Table 3. *In vitro* free radical scavenging effect of *C. fistula* and *T. indica* bark extracts by nitric oxide scavenging method.

Drug	% Scavenging (the mean ± SEM) of triplicates						
	4 µg/mL	8 µg/mL	15 µg/mL	30 µg/mL	60 µg/mL	125 µg/mL	250 µg/mL
C. fistula	36.12 ± 0.002	36.48 ± 0.001	37.22 ± 0.001	38.41 ± 0.002	39.02 ± 0.002	41.11 ± 0.001	41.67 ± 0.001
T. indica	35.13 ± 0.002	35.42 ± 0.001	35.86 ± 0.002	36.13 ± 0.001	36.48 ± 0.002	36.81 ± 0.002	37.21 ± 0.002
Vitamin C	0.1 µg/mL	0.2 µg/mL	0.4 µg/mL	0.6 µg/mL	0.8 µg/mL	1.0 µg/mL	—
	2.96 ± 0.001	12.11 ± 0.002*	32.28 ± 0.001*	39.45 ± 0.001*	63.24 ± 0.002*	73.36 ± 0.002*	—

Data were analyzed using one way ANOVA followed by Dunnett's multiple comparison test, * $p < 0.01$.

Table 4. *In vitro* free radical scavenging effect of *C. fistula* and *T. indica* bark extracts by hydroxyl scavenging method.

Drug	% Scavenging (the mean ± SEM) of triplicates				
	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
C. fistula	15.23 ± 0.001	26.43 ± 0.002	33.76 ± 0.001	44.34 ± 0.002	56.22 ± 0.002
T. indica	17.11 ± 0.002	31.43 ± 0.002	44.53 ± 0.001	57.41 ± 0.001	70.22 ± 0.002
	10 µg/mL	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL
Vitamin C	22.34 ± 0.001*	38.12 ± 0.002*	52.67 ± 0.001*	67.17 ± 0.001*	78.459,5 ± 0.004*

Data were analyzed using one way ANOVA followed by Dunnett's multiple comparison test, * $p < 0.01$.

578 nm. The biuret reagent contains sodium-potassium tartarate to complex cupric ions and maintains their solubility at alkaline pH. Absorbance data are proportional to protein concentration.

Serum albumin

In vitro quantitative measurement of albumin in serum was done using appropriate kit (Span Diagnostics India Pvt. Ltd., Ahmedabad, India). Albumin is the most abundant protein found in serum. The rat albumin test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). Developed yellow color was measured spectrophotometrically at 450 nm. The concentration of albumin is proportional to the optical density of the test sample.

Antioxidant activity

Scavenging of DPPH radical

This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity of the alcoholic extracts of CF and TI barks were examined *in vitro* using DPPH radical (9). The tested extracts were treated with different concentrations from a maximum of 250 µg/mL to minimum of 4 µg/mL. The reaction mixture consisted of 1 mL of 0.1 mM DPPH in ethanol, 0.95 mL of 0.05 M Tris-HCl buffer (pH 7.4), 1 mL of ethanol and 0.05 mL of the herbal extract. The absorbance of the mixture was measured at 517 nm exactly 30 s after adding the extract. The experiment was performed (in triplicate) and % of scavenging activity was calculated using the formula 100 – (100/blank absorbance × sample absorbance) (13).

Scavenging of nitric oxide

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentrations of the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL of Griess reagent (prepared by mixing equal volume of 1% sulfanilamide in 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula 100 – [100/blank absorbance × sample absorbance]. The activity was compared with ascorbic acid, which was used as a standard antioxidant (14).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} / ascorbate/ EDTA/ H_2O_2 system. The reaction mixture contained deoxyribose (2-8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (l mM), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4) and various concentrations (25-400 µg of extracts and standard 10 to 80 µg/mL) of standard drug in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C, deoxyribose degradation was measured at 532 nm (15).

Statistical analysis

The results obtained were expressed as the mean ± standard error of the mean (SEM) of three determinations. Where applicable, the data were subjected to a one-way analysis of variance (ANOVA test) followed by Dunnett's test.

RESULTS

Acute toxicity study

Alcoholic extracts of CF and TI stem barks did not cause any mortality up to 2000 mg/kg and so considered as safe (X-unclassified) (17).

Body weight and antidiabetic activity

Significant weight loss was observed in diabetic rats compared to normal rats. The administration of extracts of *C. fistula* and *T. indica* and a standard drug (gliclazide) improved the body weight as compared to the control diabetic rats. In diabetic control group there was significant increase in plasma glucose level from 88.29 mg/dL on 1st day to 238 mg/dL on 14th day of the study. In case of drug treated groups, the level of plasma glucose differed from 90 mg/dL to 104.57 mg/dL and 87.14 mg/dL to 99.14 mg/dL for *C. fistula* and *T. indica*, respectively.

Other biomarkers

Serum cholesterol levels were found to be increased in diabetic controlled group from 145.90 mg/dL to 163.76 mg/dL. As compared to diabetic control, drug treated groups showed lower values i.e., 148.67 mg/dL and 99.14 mg/dL for CF and TI, respectively.

Animal groups treated with standard drug and CF showed significant decrease ($p < 0.01$) in the lev-

els of serum triglyceride when compared with diabetic control.

In case of renal parameters, diabetic animals treated with CF and TI extracts showed significant decrease in serum creatinine ($p < 0.05$) and an increase in serum albumin and total protein ($p < 0.01$) when compared with diabetic control.

Antioxidant activity

Alcoholic extracts of CF and TI barks showed promising free radical scavenging effect of DPPH in a concentration dependent manner up to a concentration of 250 µg/mL. CF showed more scavenging activity than TI. The reference standard ascorbic acid also demonstrated a significant radical scavenging potential in the concentration of 1 µg/mL. The DPPH radical inhibition (%) was 55.21, 50.13 and 81.12 for CF, TI and ascorbic acid, respectively. CF and TI bark extracts showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentration 250 µg/mL, showing 41.67 and 37.21% of NO inhibition, respectively. Ascorbic acid was used as reference standard. The CF and TI extracts (25-400 µg/mL) significantly scavenged the hydroxyl radicals generated by the EDTA/H_2O_2 system, when compared with that of ascorbic acid. The percentage scavenging of OH radicals by CF and TI was increased in a dose dependent manner. The standard vitamin C (10-80 µg/mL) also showed scavenging effect.

DISCUSSION

The vascular complications arising as a result of hyperglycemia are the major cause of morbidity and mortality in diabetic patients. The biochemical pathways associated with diabetes can increase the generation of free radicals. As a result, the diabetic patients become susceptible to various microvascular and macrovascular complications. In such circumstances it is important to control the generation or neutralization of free radicals produced during the diabetic conditions. Alcoholic extracts of both plants lowered the blood glucose level and were also found effective to fight against oxidative stress. Recovery in other biochemical parameters including blood cholesterol level, triglyceride, serum albumin, serum creatinine, serum protein concentration at the end of the study by the drug treated animals also indicates the preventive effect on cardiovascular system and nephroprotective effect (18).

Decrease in the serum albumin level indicates the leakage of the serum albumin in urine (albumin urea) due to thickening of the glomerulus in the early stages of diabetes (19) An increase in the serum albumin level at the 21st day of the study of *C. fistula* and *T. indica* extracts indicates the neproprotective effect of the extracts

Also an increase in the serum creatinine level in diabetic controlled animals indicates the hindrance in the filtration of the creatinine as a result of renal complications developed due to diabetic conditions. A decrease in the creatinine level in *C. fistula* and *T. indica* extracts treated animals at the end of the study again indicates the nephroprotective effect of the herbs.

Oxidative stress has significant effect in the causation of diabetes as well as diabetic related complications in human beings (20). In diabetes, oxidative stress coexists with a reduction in antioxidant status. Oxidative stress in diabetes increases glycation of proteins, inactivation of enzymes, alteration of structural functions of collagen basement etc. (21). Oxidative stress has significant effect in the glucose transport protein (GLUT) and in insulin receptor activity (22). It is known that scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and may alleviate the diabetes and reduce its secondary complications.

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons, DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired off in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the tested drugs to scavenge free radicals independently. Hydroxyl radical is the principal contributor for tissue injury. The formation of hydroxyl radical from Fenton reaction was quantified using 2, deoxy-D-ribose degradation. The extracts of *C. fistula* and *T. indica* inhibited hydroxyl scavenging activity.

Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine is used as the marker for NO scavenging activity (23). The chromophore formation was not complete in the presence of *C. fistula* and *T. indica* bark extracts, which scavenge the NO thus

formed from the sodium nitroprusside and hence, the absorbance decreases as the concentration of the extracts increases in a dose dependent manner.

CONCLUSION

It can be concluded from the study that *Cassia fistula* and *Tamarindus indica* stem barks have the potential as hypoglycemic agents along with oxidative stress and renal complications associated with diabetes. However, further research will be necessary to find out the active principals and the mechanism of action.

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