

# Comparative study of antidiabetic activity of *Cajanus cajan* and *Tamarindus indica* in alloxan-induced diabetic mice with a reference to *in vitro* antioxidant activity

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## ABSTRACT

**Background:** Oxidative stress not only develops complications in diabetic (type 1 and type 2) but also contributes to beta cell destruction in type 2 diabetes in insulin resistance hyperglycemia. Glucose control plays an important role in the pro-oxidant/antioxidant balance. Some antidiabetic agents may by themselves have antioxidant properties independently of their role on glucose control. **Objective:** The present investigation draws a comparison of the protective antioxidant activity, total phenol content and the antihyperglycemic activity of the methanolic extract of *Cajanus cajan* root (MCC) and *Tamarindus indica* seeds (MTI). **Materials and Methods:** Antidiabetic potentials of the plant extracts were evaluated in alloxan-induced diabetic Swiss albino mice. The plant extracts at the doses of 200 and 400 mg/kg body weight was orally administered for glucose tolerance test during 1-hour study and hypoglycemic effect during 5-day study period in comparison with reference drug Metformin HCl (50 mg/kg). *In vitro* antioxidant potential of MCC and MTI was investigated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity at 517 nm. Total phenolic content, total antioxidant capacity and reducing power activity was also assayed. **Results:** There was a significant decrease in fasting serum glucose level ( $P < 0.001$ ), reduction in blood glucose level ( $P < 0.001$ ) in 5-days study, observed in the alloxan-induced diabetic mice. The reduction efficacy of blood glucose level of both the extracts is proportional to their dose but MCC is more potent than MTI. Antioxidant study and quantification of phenolic compound of both the extracts revealed that they have high antioxidant capacity. **Conclusion:** These studies showed that MCC and MTI have both hypoglycemic and antioxidant potential but MCC is more potent than MTI. The present study suggests that both MCC and MTI could be used in managing oxidative stress.

**Key words:** Alloxan, antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl, hyperglycemia, metformin hydrochloride, oxidative stress

## INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. At low-to-moderate concentrations, they possess various physiological roles ranging from cellular signal transduction to defense against pathogens.<sup>[1]</sup> During oxidative stress, there is an overproduction of ROS and

RNS on one side and a deficiency of enzymatic and nonenzymatic antioxidant defense system on the other, resulting in degradation of cellular components, DNA, carbohydrates, proteins and lipids. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications,<sup>[2]</sup> a devastating illness with significant morbidity and mortality, which has increased steadily worldwide.<sup>[3]</sup> During diabetes or insulin resistance, glucose concentrations in blood remain high due to failure of insulin-stimulated glucose uptake by fat and muscle. Consequently, glucose uptake by insulin-independent tissues increases resulting in enhancement of oxidant production and impairs antioxidant defenses by multiple interacting

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nonenzymatic, enzymatic catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) and mitochondrial pathways.<sup>[4,5]</sup> The level of these antioxidant enzymes critically influences the susceptibility of various tissues like beta-islet (insulin-releasing cell), which is among those tissues that have the lowest levels of intrinsic antioxidant defenses and is associated with the development of complications in diabetes (kidney, eye, blood Vessel and nerve damage).<sup>[6,7]</sup> Diseases linked with hyperglycemia-induced oxidative stress mediated free radicals generation can be prevented by antidiabetic and antioxidant therapy.<sup>[8]</sup> Despite progress in the management of diabetes by synthetic drug (insulin) and oxidative stress using synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), rutin, tertiary butylated hydroquinone and gallic acid (GA) esters are less effective and suspected to cause health hazard.<sup>[9]</sup> So, current researches are directed towards improved, safe and natural antidiabetic and antioxidative plant products as widespread traditional medical treatment.<sup>[10]</sup> Even for populations which use herbs traditionally, encouraging the use of species with chemopreventive actions as costs are significantly low, herbs have usually little or no toxicity during long-term oral administration and are relatively available at large scale.<sup>[11]</sup>

Being a tropical country, Bangladesh is abundant in medicinal plants used in traditional medicine. In this connection two Bangladeshi medicinal plant of known hypoglycemic effect *Cajanus cajan* and *Tamarindus indica* were subjected for study in order to assess their antidiabetic and oxidative stress inhibitory effect. *C. cajan*, local name Arhar (family- Fabaceae), is a perennial shrub native to Asia, most common pulse eaten all over Asian subcontinent. The extracts or components of *C. cajan* are commonly used all over the world for the treatment of diabetes, dysentery, hepatitis and measles, as a febrifuge to stabilize the menstrual period.<sup>[12-14]</sup> As a traditional Chinese medicine, the leaves of *C. cajan* have been widely used to arrest blood, relieve pain and kill worms.<sup>[15]</sup> Nowadays, the leaves are used for the treatment of wounds, aphtha, bedsores and malaria, as well as diet-induced hypercholesterolemia, among others.<sup>[16-18]</sup> Protective effects of extracts from *C. cajan* leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported.<sup>[19]</sup> Chemical constituent investigations have indicated that *C. cajan* leaves are rich in flavonoids and stilbenes, which are considered responsible for the beneficial efficacies of *C. cajan* leaves on human health.<sup>[20-22]</sup>

*T. indica*, local name tetul, is a perennial herb belonging to the dicotyledonous family of Fabaceae. It grows naturally in tropical and subtropical regions and now is one of the most important plant resources as food materials and is accepted as herbal medicine in parts of the world.<sup>[23]</sup> It

was used as a traditional medicine for the management of diabetes mellitus in human and experimental animals.<sup>[24,25]</sup> The potential antioxidant activity of Tamarind seeds has already been reported.<sup>[26,27]</sup> *T. indica* seed coat may play an important role in chemical protection from oxidative damage by possessing endogenous antioxidants such as phenolic compounds. The potential antioxidant activity of Tamarind seeds have already been reported and the isolated antioxidant components are, 2-hydroxy-30, 40-dihydroxyacetophenone, methyl 3, 4-ihydroxybenzoate, 3, 4-dihydroxyphenylacetate and epicatechin in addition to oligomeric proanthocyanidins, phenolic compounds are procyanidin B2, epicatechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer, procyanidin hexamer, polymeric tannins, polymeric tannins.<sup>[28]</sup> It has been shown that aqueous extract of *T. indica* seeds have potent antidiabetic and antihyperlipidemic activities in STZ-induced diabetic male rat.<sup>[29]</sup> Here we studied the methanolic extract of *C. cajan* root (MCC) and methanolic extract of *T. indica* seeds (MTI) to compare their hypoglycemic activity. As antidiabetic activity is closely related with antioxidant activity, in this communication, we have also assessed the antioxidant activity and total phenol content of these two plants.

## MATERIALS AND METHODS

### Collection of plant parts

Roots of the plant *C. cajan* and seeds of *T. indica*, were collected during the month of May 2011 from the area of Konda Thana, Dhaka, Bangladesh. The plants were taxonomically identified and voucher specimen No- DACB 36424 and DACB 36425, have been maintained for *C. cajan* and *T. indica*, respectively, in the Bangladesh National Herbarium, Dhaka, Bangladesh.

### Preparation of methanolic extract of *C. cajan* root

About 387 g of dried powdered material was refluxed with 500 ml of 95% methanol for 3 h. The extracts were then filtered through filter paper (Double Rings filter paper 102, 11.0 cm). The filtrates (methanol extract) were concentrated at 50°C under reduce pressure using rotary evaporator (STUART RF3022C, UK) to afford an oily concentrate of greenish black color of extract. Finally, we got 16 g methanolic extract. The extract transferred to a closed container for further use and protection.

### Preparation of Methanolic extract of *T. indica* Seeds

The seeds of *T. indica* were dried in an oven for 2 days at 40°C, crushed in an electrical grinder and then powdered. Extraction was performed by taking 25-g powder in 250 ml of distilled water for 18 h in a soxhlet apparatus and a deep

brown aqueous extract was obtained. The extract was dried at reduced pressure and transferred to a closed container for further use and protection.

### Instruments

The molecular absorption spectra and absorbance at specific wavelengths were recorded with a HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of 1-cm light path.

### Chemicals and reagents

All chemicals and drugs were obtained commercially and were of analytical grade. DPPH (Sigma Chemical Co., USA.), Alloxan (Fluka, Germany), ammonium molybdate (Merck, Germany), sodium phosphate (BDH, England), potassium ferricyanide  $K_3[Fe(CN)_6]$ , trichloroacetic acid ( $CCl_3COOH$ ), Folin–Ciocalteu reagent, GA [ $C_6H_2(OH)_3COOH$ ], ascorbic acid (AA) and metformin hydrochloride (General Pharmaceutical Bangladesh Ltd) and glucose estimation kit (Human, Germany).

### *In vivo* study for assessment of antihyperglycemic activity

#### Experimental animal

Experiment was conducted on adult albino mice of mix sex with the weights of 50–55 g procured from International Centre for Diarrhoeal Disease Research Bangladesh (ICDDRDB). All mice were fed normal laboratory chow food containing 16% protein, 66% carbohydrate, 8% fats and water. All mice were housed at a (12:12) h light and dark cycle at 25°C and relative humidity (60–70%).

**Ethical Approval:** Animal experiment was done by following guidelines followed by ICDDRDB which were approved by the institutional animal ethical committee.<sup>[30]</sup>

#### Acute toxicity test

Acute toxicity study was performed for the extract according to the acute toxic classic method as per the method of Lorke.<sup>[31]</sup> The animals were divided into six groups containing 10 animals each. The MCC and MTI suspension were administered orally in increasing dose up to 1500 mg/kg, b.w as we were investigating in low doses. These animals were observed for mortality and toxicity for 14 days.

#### Preparation of the drug solution

MCC and MTI were dissolved separately in normal saline to prepare dose level of 200 and 400 mg/kg/day, body weight for administration into mice.

#### Oral glucose tolerance test

A glucose tolerance test is the administration of glucose to determine how quickly it is cleared from the blood. This

study includes measurement of the blood sugar levels of fasted mice and after 30 min, 1 and 2 h after taking glucose drink in same mice. For this, the normal nondiabetic mice, who had fasted overnight, were randomly divided into six experimental groups. Each group contained six mice.

Group 1: Treated with distilled water (Negative control normal group).

Group 2: Treated with Metformin HCl 50 mg/kg body weight (positive control normal group).

Groups 3 and 4: Treated with 200 mg/kg body weight of MCC and MTI separately (test groups).

Groups 5 and 6: Treated with 400 mg/kg body weight of MCC and MTI separately (test groups).

The non-diabetic mice in all groups were orally feed glucose (3 g/kg body weight) after 30 min of the above pretreatment.

### Experimental design

Animals were divided into seven groups and for each group four animals were taken. The investigation was carried out for 4 weeks. The first 2 weeks were for the induction of diabetic condition in mice and the following 2 weeks were experimental period with crude MCC and MTI on fasted mice.

Group-1: Normal saline-treated mice.

Group-2: Normal saline-treated alloxan-induced diabetic mice.

Group-3 and 4: Each MCC and MTI of 200 mg/kg body weight treated alloxan-induced diabetic mice, respectively.

Group-5 and 6: Each MCC and MTI of 400 mg/kg body weight treated alloxan-induced diabetic mice, respectively.

Group-7: Metformin hydrochloride 50 mg/kg body weight treated alloxan-induced diabetic mice.

### Induction of experimental diabetes

Diabetes was induced in Swiss albino mice of mix sex by intravenous injection of aqueous alloxan monohydrate (55 mg/kg, i.v.).<sup>[32]</sup> Two week after alloxan injection, mice with plasma glucose level (blood collected from tail vein) of greater than 10 mmol/L were included in the study.

### Statistical analysis

The data obtained in the animal experiments was subjected to statistical analysis. All values are expressed as Mean  $\pm$  S.E.M (Standard Error of Mean). The data were assessed by the analysis of variance (ANOVA) and the group means were evaluated by the *post-hoc* Dunnet test using SPSS program (SPSS 15.0, USA). Mean values were considered significantly different if  $P < 0.05, 0.001$ .

### *In vitro* study for antioxidative activity evaluation DPPH radical scavenging effect

DPPH radical scavenging effects of MCC and MTI were performed according to the method of Braca.<sup>[33]</sup>

The 0.1 mmol/L solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 mL of extract's solution at different concentrations (5, 10, 25 and 50 µg/mL). After 30 min, absorbance was measured at 517 nm. Vitamin C (AA) was used as a reference drug. The percentage inhibition was evaluated by comparing the absorbance values for the control and experimental samples<sup>[34]</sup> following the equation:

$$\text{Percentage of inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where  $A_0$  is the Absorbance of the control,  $A_1$  is the Absorbance of the plant extract/standard.

$IC_{50}$  value was calculated from the equation of line obtained by plotting a graph of concentration (µg/mL) versus % inhibition.

### Total antioxidant activity capacity test

The antioxidant activity of MCC and MTI were evaluated by the phosphomolybdenum method according to the procedure describe by Prieto.<sup>[35]</sup> The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The antioxidant activity is expressed as the number of equivalents of AA using the following equation:

$$C = (c \times V)/m$$

Where, C= Total antioxidant activity of extract, in AA (mg/mL), c =The concentration of AA established from the calibration curve (mg/ml), V= The volume of extract (mL), m = the weight of pure plant methanolic extract (g).

### Determination of total phenolic content

The concentration of total phenolic was based on the method described by Velioglu<sup>[36]</sup> with some modification. Phenols of extracts react with phosphomolybdic acid in Folin–Ciocalteu reagent in alkaline media and produce a blue-colored complex (molybdenum blue). That can be anticipated colorimetrically at 760 nm after 2 h against a blank. GA was used to construct a standard curve (0–50 mg/L). The amount of total phenols were expressed as milligram gallic acid equivalents (GAE)/dried weight, calculated from the calibration curve.

### Ferric reducing/antioxidant power assay

The reducing power of MCC and MTI was determined according to the method described by Oyaizu.<sup>[37]</sup> For the measurement of the reductive ability, transformation of Ferric ion ( $Fe^{3+}$ ) to Ferrus ion ( $Fe^{2+}$ ) was investigated in the presence of extracts. Increased absorbance of the reaction mixture indicated increased reducing power. AA was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final

reaction mixture of two parallel experiments was taken and is expressed as mean  $\pm$  standard deviation.

## RESULTS

### Acute toxicity

During the acute toxicity study of the extracts, either mortality or any considerable symptoms of toxicity was not found after the oral administration of MCC and MTI upto a dose of 1 g/kg body weight in mice. Even no significant changes in general behavior was observed in mice up to 14-days study.

### Antidiabetic investigations

#### Oral glucose tolerance test

The result of glucose tolerance test are summarized in Table 1. The results of OGTT showed that after a single dose load of 200 mg/kg and 400 mg/kg of both the extracts in mice, there was a significant reduction ( $P < 0.001$ ) of fasting blood glucose level during the 2-h study period. Within 30 min of administration of glucose load, there was a progressive increase in the postprandial blood glucose level (BGL) of all the rats which peaked at 30 min. At 30 min, the MCC treated groups (200 and 400 mg/kg) had 52.04% and 18.23% increase in BGL compared to control (189.42%). Hence treatment with MCC suppressed the rise in BGL at 180 min by 17.66% (200 mg/kg) and 59.67% (400 mg/kg). The MCC evoked a progressive, significant and nondose-related decrease in BGL up to 180 min, at which the BGL were close to basal levels [Table 1]. At 30 min, the MTI-treated groups (200 and 400 mg/kg) had 70.55% and 17.86% increase in BGL compared to control (189.42%). Hence treatment with MTI suppressed the rise in BGL at 180 min by 15.74% (200 mg/kg) and 11.72% (400 mg/kg). Thus MTI also evoked a progressive, significant and nondose-related decrease in BGL up to 180 min, at which the BGL were close to basal levels.

#### Result of long-term antidiabetic study

The results after chronic administration of MCC and MTI summarized in Table 2, showed significant difference ( $P < 0.001$ ) which was observed between experimental and diabetic control mice in lowering fasting blood glucose level. At a dose of 200 mg/kg body weight, MCC significantly lowered blood glucose level and showed reduction of 37.17% and MTI lowered 47.47% blood glucose level of on day 5. The extracts at 400 mg/kg body weight dose, produced maximum reduction of 54.51% and 51.76% of blood glucose level for MCC and MTI, respectively, on day 5 whereas inhibition of 63.43% was found for metformin HCl on day 5 as a peak. So both the extracts have potent hypoglycemic effect.

### In vitro antioxidative potentiality

#### DPPH radical scavenging effect

DPPH radical scavenging activity is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant compound due to the formation of a nonradical form (DPPH-H). In this study, DPPH was effectively scavenged by MCC and MTI and the percentage (%) of scavenging were found to be concentration dependant, i.e., scavenging capacity increases with the increase of concentration of both the extracts. The IC<sub>50</sub> value was found to be 17.44 µg/mL and 30 µg/mL for MCC and MTI, respectively. The standard antioxidant, AA exhibited 50% inhibition at a concentration of 12 µg/mL.

#### Total antioxidant capacity

Total antioxidant capacities of the extracts evaluated by Prieto procedure and are expressed as the number of equivalents of AA. It was found to be 826.87 ± 10.27 mg/g equivalent of AA for MCC and 298.38 ± 9.86 mg/g equivalent of AA for MTI.

#### Total phenolic content

The total phenolic content in the extracts of MCC and MTI were determined according to the colorimetric Folin–Ciocalteu method with GA as a standard compound (data not shown). A wide range of total phenolic content was found in the plant materials to be 297.42 ± 8.4 mg/g plant extract (in GAE) for MCC and 248.97 ± 7.51 mg/g plant extract (in GAE) for MTI.

#### Ferric reducing/Antioxidant power (FRAP) Assay

The FRAP assay measures the antioxidant effect of any substance in the reaction medium in term of its reducing ability and it reflects total antioxidant power involving the single electron transfer reaction. Table 3 shows the reductive capabilities of the plant extracts compared to AA at different concentration. The reducing power of the extracts, MCC and MTI founded remarkable and the reducing power of the extract observed to rise as the concentration of the extract gradually increased.

## DISCUSSION

The results of entire experiment indicated that both MCC and MTI established their potency against diabetes and oxidative stress by lowering postprandial hyperglycemia, serum blood glucose level in diabetic mice and greatest antioxidant capacity with huge amount of phenolic compounds. In comparative evaluation of *C. cajan* root (MCC) and *T. indica* seeds (MTI), first one was found to be more efficacious to later one.

#### In vivo antidiabetic effect

Insulin-dependent diabetes mellitus study model has been developed here to explore the antihyperglycemic activity of MCC and MTI. Diabetes was induced using alloxan, a beta-cytotoxin, which causes selective destruction of

**Table 1: Glucose tolerance test after administration of 200 and 400 mg/kg body weight of methanolic extract of *Cajanus cajan* root (MCC) and methanolic extract of *Tamarindus indica* seeds (MTI) in mice**

Group	Blood glucose level (mmol/L)			
	Initial 0 min	30 min	60 min	120 min
Control	5.01 ± 0.22	14.5 ± 0.37	15.3 ± 0.39	16.2 ± 0.28
Met. Hcl 50 mg	5.11 ± 0.20	7.9 ± 0.11	7.4 ± 0.15	7.5 ± 0.19
MCC. 200 mg	5.40 ± 0.21	8.21 ± 0.56**	7.31 ± 0.41**	6.76 ± 0.39**
MTI. 200 mg	5.40 ± 0.21	9.21 ± 0.56**	8.31 ± 0.41**	7.76 ± 0.39**
MCC. 400 mg	5.43 ± 0.19	6.42 ± 0.28**	6.12 ± 0.30**	5.8 ± 0.22**
MTI. 400 mg	5.43 ± 0.19	6.4 ± 0.26**	6.12 ± 0.30**	5.65 ± 0.28**

ANOVA done in SPSS Version 15.0 followed by Dunnet's T Test where \*\* indicates  $P < 0.001$

**Table 2: The effect of 5-day treatment of MCC and MTI on blood sugar of alloxan-induced diabetic mice**

Groups	Blood glucose level				
	1st. Day	2nd. Day	3rd Day	4th. Day	5th. Day
Control (non diabetic)	5.10 ± 0.17	5.67 ± 0.47	4.98 ± 0.13	5.50 ± 0.35	5.0 ± 0.26
Control (Diabetic)	11.65 ± 0.42	14.23 ± 0.31	17.16 ± 0.49	17.54 ± 0.32	17.93 ± 0.51
MCC. 200 mg/kg/day	12.20 ± 0.30	11.31 ± 0.48**	9.63 ± 0.37**	8.76 ± 0.39**	7.32 ± 0.21**
MTI. 200 mg/kg/day	10.20 ± 0.30	9.31 ± 0.48**	8.63 ± 0.37**	7.76 ± 0.39**	6.12 ± 0.21**
MCC. 400 mg/kg/day	11.07 ± 1.03	9.47 ± 1.29**	6.47 ± 0.72 **	5.75 ± 0.41**	5.30 ± 0.43**
MTI. 400 mg/kg/day	9.8 ± 0.52	8.62 ± 0.5**	6.82 ± 0.62 **	6.25 ± 0.35**	5.62 ± 0.27**
Metformin HCl 50 mg/kg/day	12.46 ± 0.67	8.75 ± 0.31**	5.53 ± 0.27**	4.46 ± 0.14**	4.26 ± 0.32**

ANOVA done in SPSS Version 15.0 followed by Dunnet's T Test where \*\* indicates  $P < 0.001$  Values are mean ± SEM, (n = 5); \*:  $P < 0.05$ , Dunnet test as compared to control. Experimental groups (Group-3 and 4 and Group-5 and 6) at the dose of 200 mg/kg and 400 mg/kg, respectively, were compared with diabetic control (Group-2) on corresponding day using Dunnet's test

**Table 3: Reducing power of MCC, MTI and standard AA**

Concentration (microg/ml)	% Reducing power		
	AA	MCC	MTI
25	100 ± 9.27	42 ± 5.02	48 ± 10.012
50	157 ± 11.88	83 ± 8.27	61 ± 7.28
100	220 ± 6.77	110 ± 6.11	70 ± 6.49
200	270 ± 12.57	225 ± 9.56	193 ± 9.43

insulin-secreting pancreatic  $\beta$ -cells through reactive oxygen species-dependent oxidative damage<sup>[38]</sup> supported here by diminished serum insulin level.<sup>[39]</sup> The insulin-dependent alteration in the carbohydrate metabolism results elevation in levels of fasting blood glucose,<sup>[40]</sup> and leading to various metabolic aberrations the animals namely decreased protein content.<sup>[41]</sup> In this study the experimental groups were treated with standard (Metformin HCl, 50 mg/kg/day) and test samples MCC and MTI to evaluate and compare hypoglycemic activity.

The glucose tolerance test was done to assess the ability of MCC and MTI to reduce and control postprandial hyperglycemia. Postprandial elevation of blood glucose constantly fuels chronic hyperglycemia, which is a risk factor for the development of life threatening and other complications of DM.<sup>[42]</sup> The results of glucose tolerance test indicated that both the extracts suppressed the rise in blood glucose level after a heavy glucose meal with maximum suppression at 60 min, the time of peak postprandial hyperglycemia, so were able to reduce and control postprandial hyperglycemia. The underlying mechanism may be due to enhancement of gluconeogenesis, which is characteristically activated at fasting state in diabetic animals or enhanced disposal of glucose by increased insulin sensitivity.<sup>[43]</sup> Similarly antihyperglycemic effect just after a glucose load may be due to delayed absorption of glucose by inhibitory activities on  $\alpha$ -glucosidase and  $\alpha$ -amylase in the gut responsible for digestion of carbohydrates. Inhibitory activities on  $\alpha$ -glucosidase and  $\alpha$ -amylase of *C. cajan* linn root constituents genistein<sup>[44]</sup> and *T. indica* seeds were evaluated.<sup>[45]</sup>

Chronic administration of MCC and MTI in diabetic mice resulted into efficient lowering of fasting blood glucose level suggesting that both the extracts might possess insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of  $\beta$ -cells. So in addition to hypoglycemic effect, the extracts also suppress postprandial rise in blood glucose level both of which are indices of effective glycaemic control.

In respect to maximum nonfatal doses studied revealed the nontoxic nature of MCC and MTI. There was no lethality or any toxic reactions found at any of these doses selected until the end of the study period. According to toxicity classification<sup>[41]</sup> both the extracts are nontoxic.

### **In vitro antioxidant activity**

*In vitro* findings indicated that both MCC and MTI attributed their antioxidative abilities in terms of total phenol content, total antioxidant capacity, DPPH radical scavenging activity and reducing power.

### **Total phenol content**

Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties.<sup>[46]</sup> Founded high yield of total free phenolics in both the extracts, might be due to the reflux of MCC and MTI using methanol. Because recovery of phenolic compounds from food samples is mainly depend upon the type of solvent used and the duration of extraction. In addition, the quantity of phenolic compounds in roots and seed samples is influenced by soil, environmental conditions, genotype (cultivar/variety), agronomic practices (irrigation, fertilization and pest management), maturity level at harvest and postharvest storage conditions. For instance, low temperature during the onset and duration of seed fill were shown to increase the isoflavone content by several folds in soybean.<sup>[47]</sup> Since, both the plant grows wildly in adverse environmental conditions such as drought, poor soil. A high phenolic content in the seed materials may contribute to the resistant function.

### **Total antioxidant activity**

The observed total antioxidant activity of MCC and MTI might be the contribution of investigated high phenolic compounds in both the extracts, which may be responsible for its anti-inflammatory and chemoprotective mechanism as well as justify the basis of using this plant's extract as folkloric remedies.

### **DPPH radical scavenging activity**

Potential DPPH radical scavenging activity revealed by MCC and MTI might confirm its hydrogen donating capacity and also its proposed ability to protect the consumers' health from various free radical-related diseases.

### **Reducing power assay**

Dose-dependent high yield of reducing ability of both the extracts exert antioxidant action by breaking the free radical chain by donating hydrogen atom.<sup>[48]</sup> So these antioxidant potentiality of MCC and MTI are an important approach for the management of oxidative stress ailment.

In the entire experiment, both MCC and MTI established their potency against diabetes and oxidative stress by lowering postprandial hyperglycemia, serum blood glucose level in diabetic mice and greatest antioxidant capacity with huge amount of phenolic compounds. These is may be due to its phytoingredients viz., GA compounds present in it as they have major antioxidative activity with redox properties, adsorption and neutralization capacity to free radicals, potency to extinguish singlet and triplet oxygen and scavenging of peroxides.<sup>[49]</sup> From previous study higher positive antioxidative efficacy of this phytochemical has been established.<sup>[50]</sup>

## CONCLUSIONS

Methanolic extract of MCC and MTI were found to contain appreciable levels total free phenolics with promising antioxidant and antidiabetic properties. This scientific information can serve as an important platform for the development of further safe and effective natural medicine. Elucidation of the molecular mechanisms involved and isolation of the bioactive molecules implicated may help the development of plant-derived potent drugs which can replace the clinically toxic. Therefore, further investigations need to be undertaken for full identification and characterization of the molecules responsible for antioxidant and antidiabetic activities of MCC and MTI.

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