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Antidiabetic activity of methanolic extract of stem bark of *Elaeodendron glaucum* Pers. in Alloxanized rat model

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ABSTRACT

Phytotherapy is considered to be minimum toxic or no side effects in comparison to modern allopathic medicines. Report of ethnobotany suggested that about 800 medicinal plants possess antidiabetic potential. *Elaeodendron glaucum* Pers. (Family/ Genus: Celastraceae; Hindi- Jamrassi, bakra; ED) is a medium sized tree which is distributed throughout India (the hotter part), Australia America, South Africa & Tropical Asia. Therefore, in this context since no extensive work has been performed for possible hypoglycemic properties of this plant, in the present experiment we have designed to standardize to total phenolic content of methanolic extract of ED (MED) and to evaluate the antidiabetic potential in normal and alloxan induced diabetic rats. Inbred adult male Charles-Foster (CF) albino rats were used in the experiment for hypoglycemic activity in oral glucose tolerance test (OGTT) and normoglycemic rats, and antidiabetic activity in alloxan induced rats. Preliminary phytochemical screening revealed that MED showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the MED showed total phenolic content (285.2 mg/g). Results showed that the continuous post-treatment for 21 days with the MED showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxan induced rat models. Further, isolation and establishment of exact mechanism of action of specific compound from MED is to be carried out in the future.

Keywords: *Elaeodendron glaucum*, diabetes, hyperglycemia, dyslipidemia and oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is an endocrinal disorder associated with depleted insulin secretions, damaged pancreatic β -cells with altered carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases etc. It has been estimated that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 millions and would go up to 40 millions by the end of 2010 which further will reach to maximum of 74 millions by 2025 [1]. It has been suggested that diabetes is the third leading cause of death due to high incidence of morbidity and mortality after cancer and cardiovascular disorders. Complications such as renal failure, coronary artery disorder, cerebro-vascular disease, neurological complications, blindness, limb amputation, long term damage, dysfunctions and failure of various organs and eventually premature death are associated with chronic hyperglycemia [2]. Therapy for DM includes long term use of oral hypoglycemic agents or insulin therapy and lifestyle modifications, dietary control and regular physical exercises [3]. Recently, it has been reported that phytotherapy is considered to be less toxic and minimal or no side effects in comparison to modern allopathic medicines [4]. Report of ethnobotany suggested that about 800 medicinal plants possess antidiabetic potential [5] and the bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and flavonoids are effective drugs both in preclinical and clinical studies [6,7]. About 80% of population belonging to developing countries using traditional medicines of plant origin and continue to be an important for their primary health care needs.

Elaeodendron glaucum Pers. (Family/ Genus: Celastraceae; Hindi-Jamrassi, bakra; ED) is a medium sized tree which is distributed throughout India (the hotter part), Australia America, South Africa & Tropical Asia. ED leaves were 3-7 cm x 5-12 cm, elliptic, acuminate, glabrous and dark green in color with grey or bluish bark is generally grown for its medicinal importance [8-11]. In Madhya Pradesh state (India) the ED is found in dry deciduous forest region [12]. Report based on literature survey revealed that the leaves are used as stimulant, fumigant, analgesic [13] and antidote for snake bite [14]. Seed were reported to possess *in vitro* cytotoxic activity against human carcinoma cells [15, 16]. Traditionally, fresh extracts of stem bark and leaves of ED are used in cuts and wounds for healing in Santhal & Paharia tribes of Bihar, India [17]. Further, the Indian tribals of Bundelkhand region of the Madhya Pradesh state used the stem bark for treatment of "Madhumeha" disease [18]. Chemical investigations suggested that the plant leaves consisting of numerous biologically active compounds such as elaeodendrosides B, C, F, G, K and L [19], elaeodendrosides A, D, E, H, I, J and elaeodendrogenin [20]. The bark containing n-octacosanol, friedelin, β -sitosterol, betulonic acid, 23-hydroxy betulin and β -sitosterol- β -D-glucoside [21], elaeodendrosides T and U [22], nor-triterpenes [23].

Therefore, in this context since no extensive work has been performed for possible hypoglycemic properties of this plant, in the present experiment we have planned to standardize to total phenolic content of methanolic extract of ED (MED) and to evaluate the antidiabetic potential in normal and alloxan induced diabetic rats. Further, an attempt has been undertaken to establish a possible mechanism of action.

MATERIALS AND METHODS

Chemicals and reagents

The ED stem bark were harvested from the Bilaspur region which was identified and authenticated by one of our co-author and was deposited in herbarium (Voucher Specimen No. DOP/HB/459). Glibenclamide (GL) was supplied as gift sample by Hoechst Pharmaceuticals, Mumbai, India. Alloxan monohydrate was procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Folin-Ciocalteu reagents were from Sigma-Aldrich Inc. (St. Louis, MO, USA); One Touch Glucometer (Accu-chek Sensor) and Diagnostic-kits were purchased from Roche Diagnostics GmbH, Mannheim, Germany. All other reagents and chemicals used were of analytical/ pharmacopoeial grade purchased from E. Merck India Ltd and Ranbaxy respectively.

Plant material and extraction

The samples were washed thoroughly with fresh running water, dried under shade room temperature ($25\pm 1^\circ\text{C}$) for 10 days, pulverized in an hammer mill and screened through 40 # mesh sieve to coarse sized. Coarse powders (1 kg) were separately Soxhlet extracted with methanol solvent (2.0 L, 72 h) at the temperature between $45\text{--}65^\circ\text{C}$. The viscous semisolid masses were dried at 40°C in rotary evaporator frozen and lyophilized and thereafter stored in airtight containers at refrigerated conditions between $4\text{--}8^\circ\text{C}$ till subsequent uses [24]. Percent yield of the fractions was found to be 10.4% w/w.

Preliminary phytochemical screening and standardization to total phenolic content

Methanolic extract was subjected to identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols [25]. Assaying method of Velioglu *et al.*, 1998 [26] was followed for estimation of total phenolics content in methanolic extract using Folin-ciocalteu reagent with slight modification. Briefly, 100 μl concentration of plant extract (1 mg/ml) were prepared, to this added 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) and mixed thoroughly in a 100 ml volumetric flask. Then, mixture was allowed to stand for 5-8 min at $25\pm 1^\circ\text{C}$ (ambient temperature). Thereafter, Na_2CO_3 (2% w/v, 1 ml) was added and allowed it to incubate at room temperature ($25\pm 1^\circ\text{C}$) for 2 h intervals. The absorbance of reaction mixture against blank was determined at λ_{max} 725 nm using Double-Beam UV-Vis Spectrophotometer (Simadzu-1800) respectively. The calibration curve ($0.02\text{--}0.1\text{ mg ml}^{-1}$) was plotted against reference gallic acid. The total phenolic content was expressed as milligrams of gallic acid equivalent/ g of methanolic extract.

Animals

Charles foster (CF) male albino strain rats weighing (160–200 g), aged 8-14 weeks older were obtained from School of Pharmacy, Chouksey Engineering College, Bilaspur and were used in the study. Rats were acclimatized for a period of 7 days before experimentation, housed in groups of six in polypropylene cages, lined soft wood shavings as bedding (renewed every 24 h), 12/12 h light/dark cycles, relative humidity 50-60% RH and at temperature $22\pm 3^\circ\text{C}$, were fed with rat pellet diet (Gold Moher, Lipton India Ltd) and water *ad libitum* regularly. Ethical clearance was taken from institute animal ethics committee (Regd. No.1169/ac/08/CPCSEA) were in accordance with the strict guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Oral toxicity studies

The acute oral toxicity study was performed according to OECD guidelines on oral administration to animals of either sex (n=6; overnight fasted) with fixed extracts dose of 5, 50, 300 mg/kg and 2000 mg/kg respectively. The dose was found tolerable due to which three dose levels were selected for antidiabetic activity assessment: (i) Median dose: 200 mg kg⁻¹ b.w (One-tenth of the maximum lethal dose), (ii) Low dose: 100 mg kg⁻¹ b.w. (One-twentieth of the maximum lethal dose) and (iii) High dose: 400 mg kg⁻¹ b.w. (Twice that of one-tenth dose) were selected for further studies. Thereafter, rats were observed individually after dosing for first 30 min periodically and daily thereafter, till 14 days for any toxicity sign of gross changes in skin and fur, eyes and mucous membranes, circulatory, respiratory, autonomic and central nervous systems, and behavior pattern if any.

Experimental Design

Study on Oral Glucose Tolerance Test (OGTT)

OGTT of plant extracts was carried out in overnight fasted normal rats, which were equally divided into five groups of six rats each. Group of normal control received only vehicle (1 ml of 0.3% CMC; p.o.) and standard group received 1 ml of reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group from third to fifth were administered with 1 ml of MED (100, 200 and 400 mg/kg, p.o.) respectively. Thereafter, following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, GmbH, Germany).

Study on normoglycemic rats

Studies for normoglycemic were carried out in overnight fasted normal rats, which were equally divided into five groups of six rats each. The normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and 1 ml of standard group received reference drug GL suspended in the vehicle (0.25 mg/kg, p.o.), while group from third to fifth were administered with 1 ml of MED (100, 200 and 400 mg/kg, p.o.) respectively. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level.

Study on Alloxan-induced diabetic rats

Diabetes was induced by intraperitoneal injection (single dose) of alloxan monohydrate (120 mg kg⁻¹ b.w.) in 0.9% w/v NaCl solution (normal saline) to overnight fasted normal rats. Blood glucose level was checked by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats shown FBG > 250 mg/dl were considered to be diabetic and were selected for studies. Animals selected were fasted over night and then divided into five groups (n=6) as follows: Group-I: Normal control rats (non-alloxanized) that was administered with vehicle (1 ml of 2.5% v/v Tween-80 in distilled water; p.o.) only; Group-II: Diabetic control rats (Untreated, alloxanized); Group-III: Diabetic rats administered once with glibenclamide (0.25 mg kg⁻¹ b.w.) as reference standard drug while; Group-III to VI: Diabetic rats administered with MED (100, 200 and 400 mg/kg/day) respectively. Treatment was continued for a period of 21 days following oral

administration to the experimental animals by gastric intubation, using a force-feeding needle. Plasma glucose was estimated on withdrawing blood samples were from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively all groups of animals. The body weight, food and fluid intake of all groups of animals were monitored on a daily basis for 21 days at regular time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day. At the end of 21th day, all the rats were euthanized by pentobarbitone sodium (60 mg/kg) and sacrificed by cervical dislocation. Blood sample was withdrawn from abdominal aorta into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum and plasma. Serum samples were stored at -20°C until utilized for further biochemical estimation parameters.

Biochemical estimations

The centrifuged serum (15 min, 2500 rpm) was analyzed for lipid profiles viz. total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), urea, creatinine, protein and albumin were estimated in a Hitachi Auto analyzer using commercial kit (Ecoline, E-Merck, India; Roche Diagnostics, GmbH, Germany) following standard procedures. Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boehringer Mannheim, Germany). The level of hemoglobin (Hb) and glycosylated hemoglobin (HbA_{1c}) was estimated using Drabkin reagent.

Estimation of lipid peroxidase

Lipid peroxidation was estimated by assessing the plasma malondialdehyde (MDA) formation using the method described by Uchiyama and Mihara, 1978 [27] with minor modification by Sunderman et al., 1985 [28] based on the thiobarbituric acid reactive substances (TBARS) test. Plasma samples were added (1/10, v/v) to ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.75 ml of 20% acetic acid, and 0.75 ml of 0.8% TBA solution. Then mixture was made up to 2.0 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of *n*-butanol/pyridine mixture (15:1, v/v) were added and the mixture shaken vigorously. Thereafter, mixture was centrifuged at 4000×*g* for 10 min and the absorbance of the supernatant (pink-colored) was measured at 532 nm using UV/VIS Spectrophotometer against the blank reagent. Standardization of TBARS reaction was carried out by analysis of tetraethoxypropane standard solutions, which yield MDA, mole for mole, under the described reaction conditions.

Estimation of superoxide dismutase (SOD)

Estimation of superoxide dismutase (SOD) activity a free radical scavenging enzyme, was performed using the method of Ukeda et al., 1997 [29]. Briefly, into 2.6 ml of 50 mmol/L sodium citrate buffer (pH 9.4) were added 0.1 ml each of 30 mmol/l xanthine (dissolved in 1 mol/L NaOH), 3 mmol/l EDTA, 0.8 mmol/L XTT (3-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)-benzenesulfonic acid hydrate) dissolved in buffer at 50°C and 0.05 ml of sample solution containing SOD or water. The reaction consists of the addition of 0.02 ml of xanthine oxidase solution (500 mU/ml) with the formation of a red formazan dye. The SOD activity was monitored for 30 s at 470 nm (25°C) by the degree of inhibition of this reaction.

Estimation of catalase (CAT)

Catalase (CAT) was assayed according to the method of Aebi, 1984 [30]. The homogenate was prepared with 50 mmol/l phosphate buffer, pH 7.0, with a drop of TritonX100 and centrifuged at 15,000×g for 15 min at 4°C. To 3.0 ml of phosphate buffer, 0.05 ml of 90 mmol/l hydrogen peroxide solutions and 0.02 ml of extract or water were added. The absorbance was read at 240 nm for 30 s respectively.

Statistical Analysis

The results are expressed as mean±S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by *Post-hoc* Student Newman Keuls test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of MED (100, 200 and 400 mg/kg) on oral glucose tolerance test (OGTT) and plasma glucose level in normoglycemic and diabetic rats:

Table-1: Hypoglycemic effect of MED (100, 200 and 400 mg/kg) in oral glucose tolerance test (OGTT)

Groups	0 min	30 min	60 min	90 min	120 min
CON	87.1±1.22	141.9±3.06	133.2±0.82	117.1±2.19	112.3±4.37
GL	84.1±1.32	102.8±3.74 ^a	96.7±3.21 ^a	79.1±1.21 ^a	66.1±3.22 ^a
MED-100	85.7±1.26	132.7±3.19	121.7±2.45	95.4±4.26	82.6±1.75
MED-200	86.6±2.11	117.6±2.15 ^a	107.7±3.25 ^a	85.3±4.72 ^a	74.6±2.10 ^a
MED-400	87.3±1.44	121.4±3.15 ^a	119.1±2.14 ^{a,c}	99.4±3.45 ^{a,c}	97.3±1.76 ^{a,c}

All values are Mean±SEM. ^a $P < 0.05$ compared to control, ^c $P < 0.05$ compared to GL (One-way ANOVA followed by Student Newmann keuls test)

Table-1 illustrates the effect of MED (100, 200 and 400 mg/kg) on OGTT at different time points. Statistical analysis by One-way ANOVA showed that there was no significant difference among the groups at 0 min [$F(4, 25) = 0.14$, $P > 0.05$]. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups [$F(4, 25) = 3.18$, $P < 0.05$]. Post-hoc test revealed that GL (0.25 mg/kg) and MED (200 and 400 mg/kg) showed significant attenuation in the plasma sugar level compared to control. Further, statistical analysis at 60 min showed that there was significant difference among the groups [$F(4, 25) = 87.54$, $P < 0.05$]. Post-hoc by Student Newmann Keuls test revealed that GL and MED (200 and 400 mg/kg) showed significant decrease in the plasma sugar level compared to control. Furthermore, MED (400 mg/kg) showed significant increase in the sugar level ($P < 0.05$) compared to GL and this trend was similar at 90 min [$F(4, 25) = 21.19$, $P < 0.05$] and 120 min [$F(4, 25) = 33.19$, $P < 0.05$].

Time dependant effect of MED (100, 200 and 400 mg/kg) on the level of plasma glucose level in fasted normoglycemic rats is depicted in Table-2. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [$F(4, 25) = 0.09$, $P > 0.05$]. Similarly, statistical analysis at 7 day showed that there was significant difference among the groups [$F(4, 25) = 5.89$, $P < 0.05$]. Post-hoc test revealed that GL and MED (400 mg/kg) showed significant decrease in the plasma sugar level compared to control. Further, MED (400 mg/kg) showed significant reduction in blood sugar levels compared to GL, MED

(100 and 200 mg/kg). Furthermore, the similar trend like at 7 day was observed at 14 day [F (4, 25) = 18.86, $P < 0.05$] and 21 day [F (4, 25) = 15.52, $P < 0.05$].

Table-2: Hypoglycemic effect of MED (100, 200 and 400 mg/kg) in fasted normoglycemic rats

Groups	Day 0	Day 7	Day 14	Day 21
CON	75.8±3.71	77.6±2.75	74.2±4.72	72.5±2.12
GL	72.7±1.65	55.8±3.48 ^a	49.6±3.47 ^a	51.4±1.42 ^a
MED-100	75.6±3.45	66.4±3.21	62.2±5.31	66.1±1.44
MED-200	73.5±1.78	64.1±2.46	63.4±2.36	58.9±1.35
MED-400	73.2±2.71	59.7±5.39 ^{a,c,d,e}	51.7±3.15 ^{a,c,d,e}	51.6±3.05 ^{a,c,d,e}

All values are Mean±SEM. ^a $P < 0.05$ compared to control, ^b $P < 0.05$ compared to diabetes, ^c $P < 0.05$ compared to GL, ^d $P < 0.05$ compared to MED-100 and ^e $P < 0.05$ compared to MED-200 (One-way ANOVA followed by Student Newmann keuls test).

Table-3 showed time dependant effect of MED (100, 200 and 400 mg/kg) on the level of plasma glucose level in alloxan treated rats. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (5, 30) = 0.96, $P > 0.05$]. Further, statistical analysis at 7 day showed that there was significant difference among the groups [F (5, 30) = 4.28, $P < 0.05$]. Post-hoc test revealed that DM, GL and MED (100, 200 and 400 mg/kg) showed significant increase in the plasma sugar level compared to control. Further, GL and MED (200 and 400 mg/kg) groups showed significant decrease in the blood glucose level compared to DM. The MED (100 and 200 mg/kg) groups showed significant increase in sugar level compared to GL. Further, MED (200 and 400 mg/kg) groups showed significant difference compared to MED (100 mg/kg). In addition, MED (400 mg/kg) group showed significant decrease in blood sugar level compared to MED (200 mg/kg). Furthermore, the similar trend like at 7 day was observed at 14 day [F (4, 25) = 17.89, $P < 0.05$] and 21 day [F (4, 25) = 34.04, $P < 0.05$].

Table 3: Hypoglycemic effect of MED (100, 200 and 400 mg/kg) in Alloxan induced animals

Groups	Day 0	Day 7	Day 14	Day 21
CON	75.1±2.43	76.7±1.87	75.1±3.33	72.1±3.11
DM	343.2±2.75	422.6±4.06 ^a	396.3±9.45 ^a	363.3±9.23 ^a
GL	306.3±1.25	253.2±8.77 ^{a,b}	195.6±11.52 ^{a,b}	96.6±12.56 ^{a,b}
MED-100	339.3±1.22	397.1±11.15 ^{a,c}	289.7±13.29 ^{a,c}	276.7±14.23 ^{a,c}
MED-200	319.7±5.31	273.5±12.24 ^{a,b,c,d}	253.5±12.21 ^{a,b,c,d}	161.5±11.51 ^{a,b,c,d}
MED-400	316.5±5.41	243.7±10.45 ^{a,b,d,e}	216.6±10.73 ^{a,b,d,e}	120.6±11.23 ^{a,b,d,e}

All values are Mean±SEM. ^a $P < 0.05$ compared to control, ^b $P < 0.05$ compared to diabetes, ^c $P < 0.05$ compared to GL, ^d $P < 0.05$ compared to MED-100 and ^e $P < 0.05$ compared to MED-200 (One-way ANOVA followed by Student Newmann keuls test).

Effect of MED (100, 200 and 400 mg/kg) on body weight, food and fluid intake of diabetic rats:

The effect of MED (100, 200 and 400 mg/kg) on initial and final body weight is illustrated in figure-1(A). Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups during initial body weight estimation [F (5, 30) = 0.78, $P > 0.05$]. Further, statistical analysis revealed that there was significant difference among the groups during final body weight estimation [F (5, 30) = 7.82, $P < 0.05$]. Post-hoc test revealed that DM

and MED (100, 200 and 400 mg/kg) showed significant decrease in body weight compared to control. GL and MED (400 mg/kg) groups showed significant increase in body weight compared to DM. MED (100 and 200 mg/kg) groups showed significant decrease in body weight compared to GL. The body weight was significantly increased in MED (400 mg/kg) compared to MED (100 and 200 mg/kg).

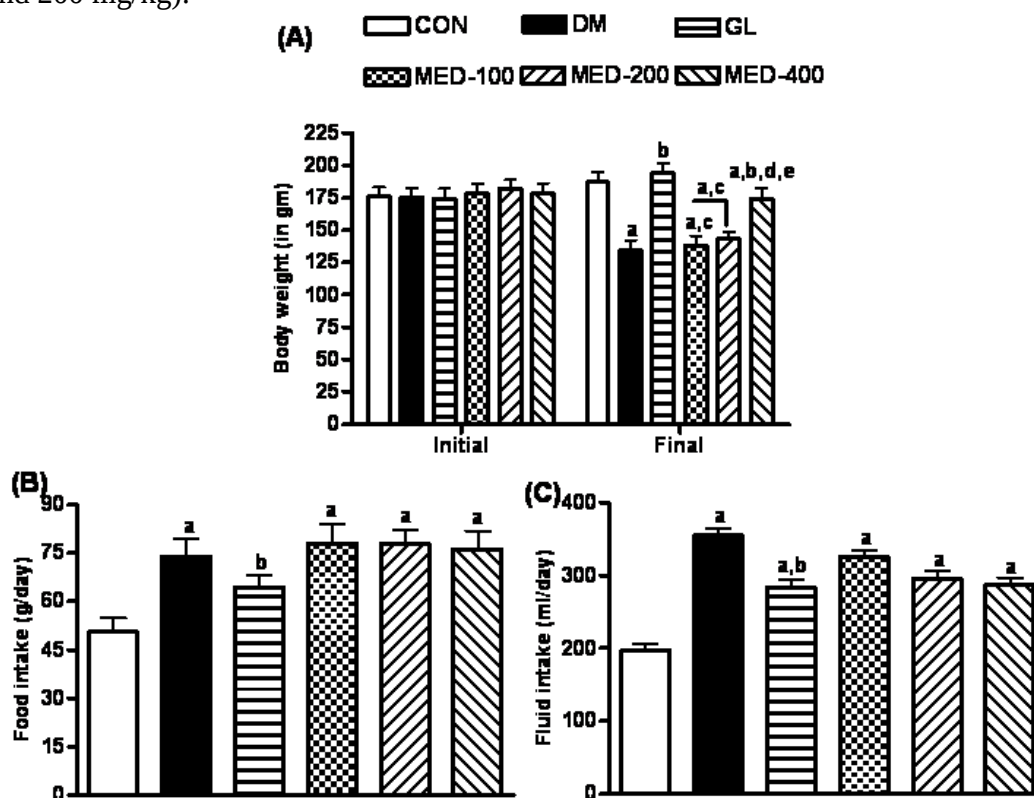


Fig-1. Effect of MED (100, 200 and 400 mg/kg) on the body weight (A), food (B) and fluid (C) intake
All values are Mean±SEM. ^a*P*<0.05 compared to control, ^b*P*<0.05 compared to DM, ^c*P*<0.05 compared to GL, ^d*P*<0.05 compared to MED-100 and ^e*P*<0.05 compared to MED-200 (One-way ANOVA followed by Student Newmann keuls test).

The effect of MED (100, 200 and 400 mg/kg) on food intake of diabetic rats are illustrated in figure-1 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [*F* (5, 30) = 3.85, *P*<0.05]. Post-hoc test revealed that all the groups except GL group showed significant increase in food intake compared to control. However, GL showed significant reduce in food intake compared to DM group. Figure-1 (C) depicts the effect of MED (100, 200 and 400 mg/kg) on fluid intake of diabetic animals. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [*F* (5, 30) = 92.78, *P*<0.05]. Post-hoc test revealed that all the groups showed significant increase in fluid intake compared to control. However, GL group showed significant reduction in fluid intake compared to DM.

Effect of MED (100, 200 and 400 mg/kg) on plasma lipid profile:

The effect of MED (100, 200 and 400 mg/kg) on TC, TG, LDL and HDL is depicted in figure-2. Statistical analysis by One-way ANOVA revealed that there was significant difference among

the groups [$F(5, 30) = 22.47$, $P < 0.05$]. Post-hoc test revealed that DM and MED (100 and 200 mg/kg) showed significant increase in TC level compared to control. The TC levels were decreased significantly in GL and MED (400 mg/kg) compared to DM. The groups MED (100 and 200 mg/kg) showed significant increase in TC levels compared to GL. Further, MED (400 mg/kg) group showed significant decrease in TC levels compared to MED (100 and 200 mg/kg). Furthermore, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [$F(5, 30) = 8.63$, $P < 0.05$]. Post-hoc test revealed that the TG levels were significantly elevated in all the groups compared to control. Further, MED (400 mg/kg) showed significant decrease in TG levels compared to DM, GL, and MED (100 and 200 mg/kg) groups.

Similarly, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [$F(5, 30) = 15.62$, $P < 0.05$]. Post-hoc test revealed that all the groups except GL showed significant increase in LDL levels compared to control. Further, GL and MED (400 mg/kg) showed significant decrease in LDL levels compared to DM. MED (400 mg/kg) showed significant decrease in LDL levels compared to MED (100 and 200 mg/kg) groups.

Fig. 2.

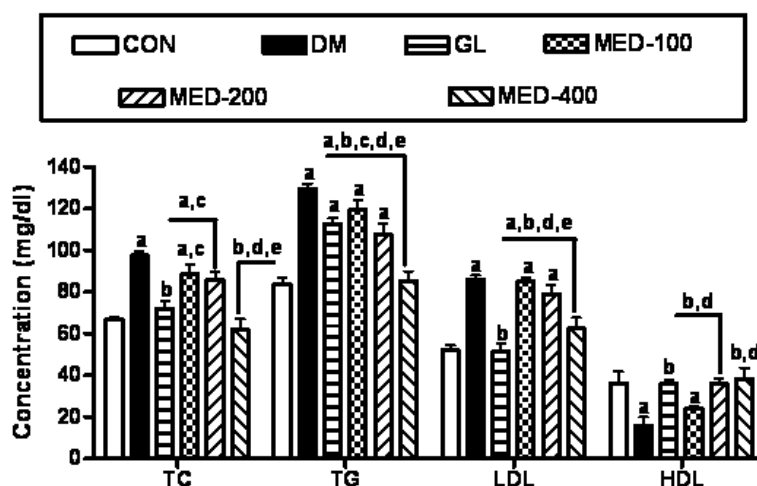


Fig-2. Effect of MED (100, 200 and 400 mg/kg) on plasma TC (A), TG (B), LDL (C) and HDL (D) levels
All values are Mean $\pm$ SEM. ^a $P < 0.05$ compared to control, ^b $P < 0.05$ compared to DM, ^c $P < 0.05$ compared to GL, ^d $P < 0.05$ compared to MED-100 and ^e $P < 0.05$ compared to MED-200 (One-way ANOVA followed by Student Newman keuls test).

Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [$F(5, 30) = 22.41$, $P < 0.05$]. Post-hoc test revealed that DM and MED (100 mg/kg) showed significant decrease in HDL levels, however there was no change in HDL levels of GL and MED (100 and 200 mg/kg) compared to control. Further, GL and MED (200 and 400 mg/kg) showed significant increase in HDL levels compared to DM. EP (200 and 400 mg/kg) showed significant increase in HDL levels compared to MED (100 mg/kg).

Effect of MED (100, 200 and 400 mg/kg) on liver function and plasma antioxidant profile:

The effect of MED (100, 200 and 400 mg/kg) on SGOT is depicted in figure-3 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups

[F (5, 30) = 22.63, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and MED (400 mg/kg) showed significant decrease in SGOT levels compared to DM. MED (100 mg/kg) showed significant increase in SGOT levels compared to GL. MED (400 mg/kg) showed significant decrease in SGOT levels compared to MED (100 mg/kg) group.

The effect of MED (100, 200 and 400 mg/kg) on SGPT is depicted in figure-3 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 11.41, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant increase in SGPT levels compared to control. GL and MED (200 and 400 mg/kg) showed significant decrease in SGPT levels compared to DM. MED (100 mg/kg) showed significant increase in SGPT levels compared to GL. MED (400 mg/kg) showed significant decrease in SGOT levels compared to MED (100 mg/kg) group.

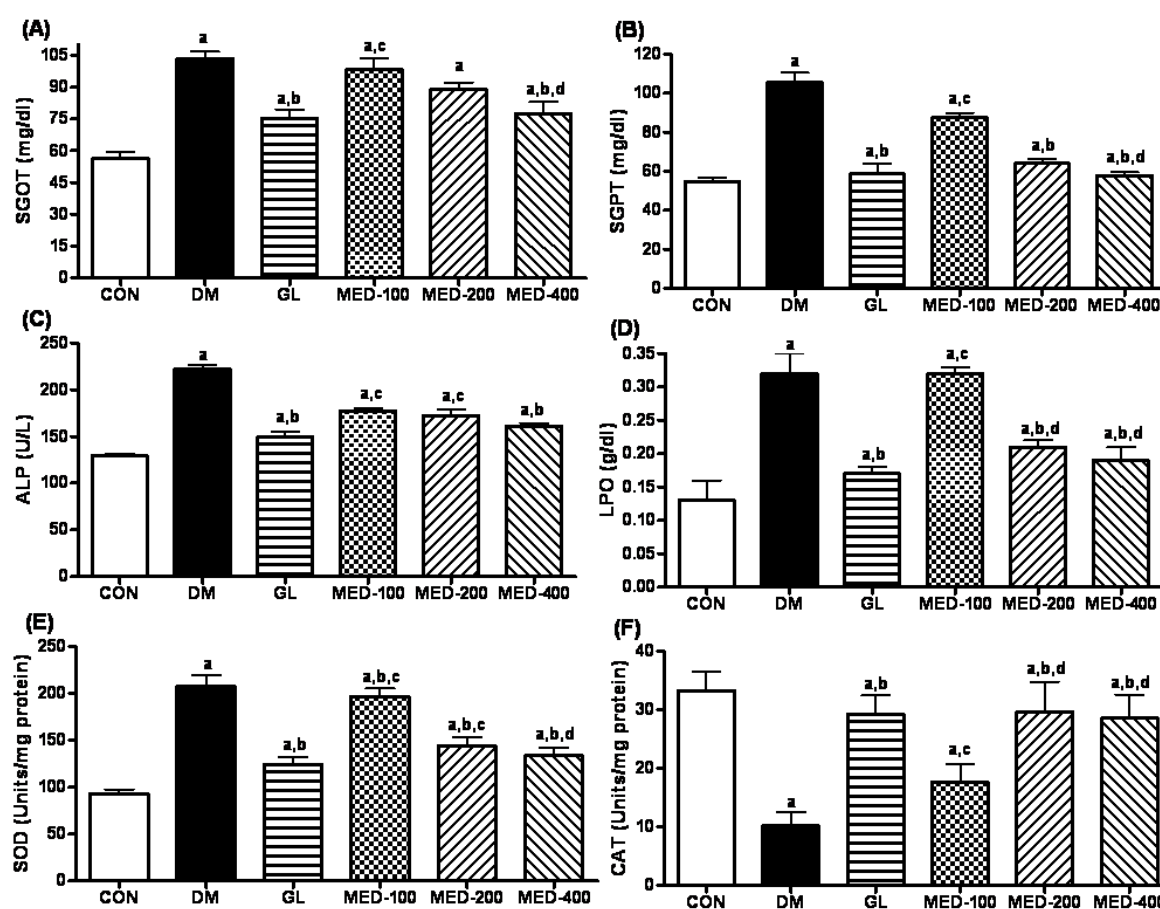


Fig-3. Effect of MED (100, 200 and 400 mg/kg) on the levels of SGOT (A), SGPT (B), ALP (C), LPO (D), SOD (E) and CAT (F)

All values are Mean $\pm$ SEM. ^a $P < 0.05$ compared to control, ^b $P < 0.05$ compared to DM, ^c $P < 0.05$ compared to GL, ^d $P < 0.05$ compared to MED-100 and ^e $P < 0.05$ compared to MED-200 (One-way ANOVA followed by Student Newman keuls test).

The effect of MED (100, 200 and 400 mg/kg) on ALP is depicted in figure-3 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups

[F (5, 30) = 29.48, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant elevation in ALP levels compared to control. GL and MED (400 mg/kg) showed significant decrease in ALP levels compared to DM. MED (100 and 200 mg/kg) showed significant increase in SGOT levels compared to GL.

The effect on LPO of MED (100, 200 and 400 mg/kg) is depicted in figure-3 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 22.79, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant elevation in LPO levels compared to control. GL and MED (200 and 400 mg/kg) showed significant decrease in LPO levels compared to DM. MED (100 mg/kg) showed significant increase in LPO levels compared to GL. MED (200 and 400 mg/kg) showed significant decrease in LPO levels compared to MED (100 mg/kg) group.

The effect of MED (100, 200 and 400 mg/kg) on SOD is depicted in figure-3 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 37.18, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and MED (100, 200 and 400 mg/kg) showed significant decrease in SOD levels compared to DM. MED (100 and 200 mg/kg) showed significant increase in SOD levels compared to GL. MED (400 mg/kg) showed significant decrease in SOD levels compared to MED (100 mg/kg).

The effect of MED (100, 200 and 400 mg/kg) on CAT is depicted in figure-3 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 31.73, $P < 0.05$]. Post-hoc test revealed that all the groups showed significant reduction in CAT levels compared to control. GL and MED (200 and 400 mg/kg) showed significant increase in CAT levels compared to DM. MED (100 mg/kg) showed significant decrease in CAT levels compared to GL. MED (200 and 400 mg/kg) showed significant decrease in CAT levels compared to MED (100 mg/kg).

Effect of MED (100, 200 and 400 mg/kg) on Total Hb, HbA1c, plasma insulin, urea, creatinine and albumin:

The effect of MED (100, 200 and 400 mg/kg) on total Hb is depicted in figure-4 (A). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 0.07, $P < 0.05$]. Post-hoc test revealed that DM showed significant reduction and no other groups showed any change in total Hb levels compared to control. The GL and MED (100, 200 and 400 mg/kg) groups showed significant increase in total Hb levels compared to DM.

The effect of MED (100, 200 and 400 mg/kg) on HbA1c is depicted in figure-4 (B). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 15.58, $P < 0.05$]. Post-hoc test revealed that DM, GL and MED (100, 200 and 400 mg/kg) showed significant elevation in HbA1c levels compared to control. GL and MED (100, 200 and 400 mg/kg) showed significant decrease in HbA1c levels compared to DM. MED (100 and 200 mg/kg) showed significant increase in HbA1c levels compared to GL.

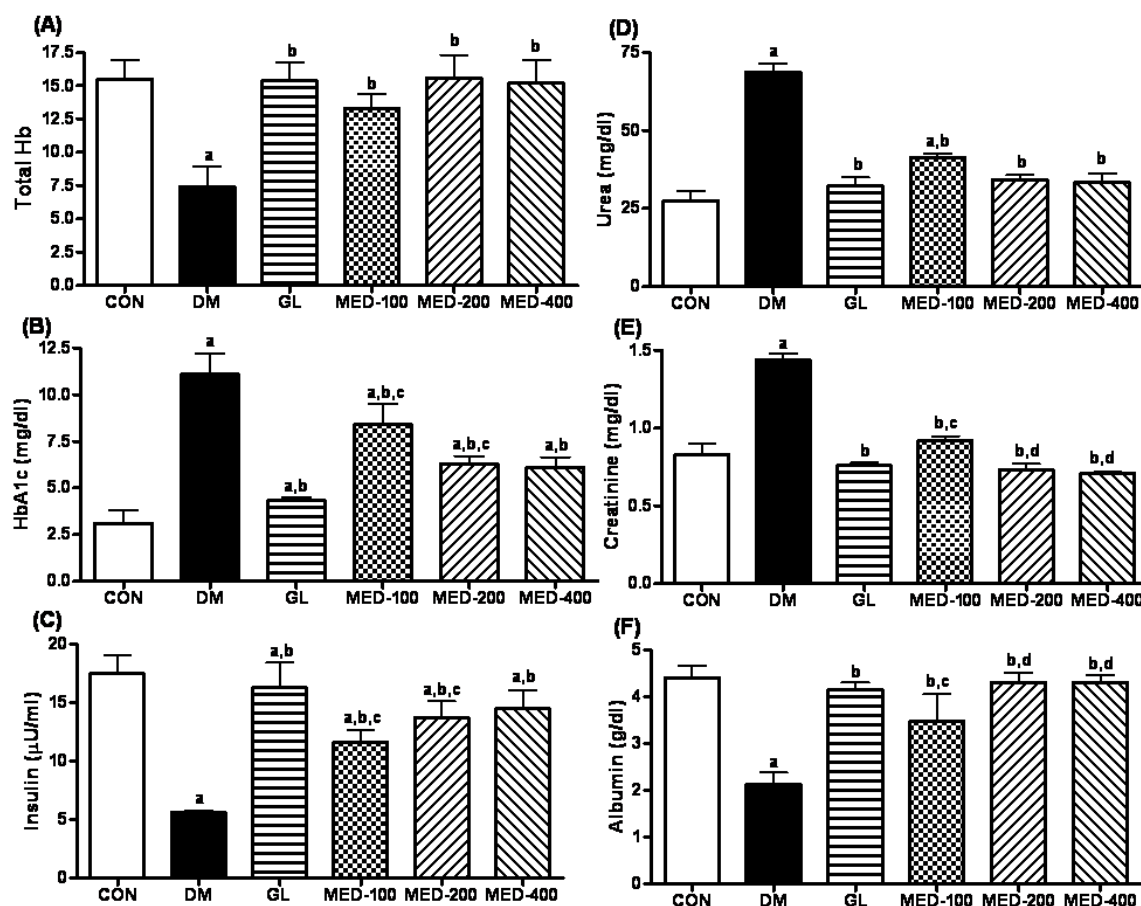


Fig-4. Effect of MED (100, 200 and 400 mg/kg) on the levels of Total Hb (A), HbA1c (B), Insulin (C), Urea (D), Creatinine (E) and Albumin (F)

All values are Mean $\pm$ SEM. ^aP<0.05 compared to control, ^bP<0.05 compared to DM, ^cP<0.05 compared to GL, ^dP<0.05 compared to MED-100 and ^eP<0.05 compared to MED-200 (One-way ANOVA followed by Student Newmann keuls test).

The effect of MED (100, 200 and 400 mg/kg) on plasma insulin is depicted in figure-4 (C). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 66.39, P<0.05]. Post-hoc test revealed that DM, GL and MED (100, 200 and 400 mg/kg) showed significant reduction in plasma insulin levels compared to control. GL and MED (100, 200 and 400 mg/kg) showed significant increase in plasma insulin levels compared to DM. MED (100 and 200 mg/kg) showed significant decrease in insulin levels compared to GL.

The effect of MED (100, 200 and 400 mg/kg) on plasma urea levels is depicted in figure-4 (D). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 38.28, P<0.05]. Post-hoc test revealed that DM and MED (100 mg/kg) showed significant increase in plasma urea levels compared to control. The GL and MED (100, 200 and 400 mg/kg) groups showed significant decrease in plasma urea levels compared to DM.

The effect of MED (100, 200 and 400 mg/kg) on plasma creatinine is depicted in figure-4 (E). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [$F(5, 30) = 58.82, P < 0.05$]. Post-hoc test revealed that DM showed significant increase in plasma creatinine levels compared to control. GL and MED (100, 200 and 400 mg/kg) showed significant decrease in plasma creatinine levels compared to DM. MED (100 mg/kg) showed significant increase in creatinine levels compared to GL. MED (200 and 400 mg/kg) showed significant decrease in creatinine levels compared to MED (100 mg/kg).

The effect of MED (100, 200 and 400 mg/kg) on plasma albumin is depicted in figure-4 (F). Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [$F(5, 30) = 19.03, P < 0.05$]. Post-hoc test revealed that DM showed significant decrease in plasma albumin levels compared to control. GL and MED (100, 200 and 400 mg/kg) showed significant increase in plasma albumin levels compared to DM. MED (100 mg/kg) showed significant decrease in albumin levels compared to GL. MED (200 and 400 mg/kg) showed significant increase in albumin levels compared to MED (100 mg/kg).

DISCUSSION

In the present experiment, the continuous post-treatment for 21 days with the MED showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxan induced rat models.

Preliminary phytochemical screening revealed that MED showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the MED showed total phenolic content (285.2 mg/g). The increased level of glycosylated hemoglobin (HbA1c) is directly proportional to the decreased level of total hemoglobin in diabetic control experimental rats. Glycosylated hemoglobin (HbA1c) is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus [31]. Protein glycation is a non-enzymatic reaction between excess glucose present in the blood and free amino groups on the globin component of hemoglobin. Measurement of HbA1c level provides information of long-term glycemic status and to correlate with various complications related to DM. On oral administration of MED significantly decreased the HbA1c level possibly due to normoglycemic control mechanisms in experimental rats which also reflect the decreased protein glycation condensation reactions and the report obtained is concordant with the previous results [32].

A marked increase in serum concentration of TC, TG, LDL and decreased HDL was observed with diabetic rats than normal control group which is often linked with hyperlipidaemia. Hyperlipidemia certainly contributes to major risk factor for cardio vascular diseases [33,34]. During diabetic state, insulin deficiency contributes to derangements of various metabolic and regulatory mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids [35,36]. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood which resulted into elevated serum phospholipid level [37,38]. Our result

showed significantly ($P < 0.05$) fall in TC, TG, and LDL levels as well at the same time raised HDL level near to control on oral administration of MED (200 mg/kg) after 21 days repeatedly. This implies that plant may possess insulin-like activity which would be helpful to reduce the incidence of lipid born complications. The significant control on serum lipids may prevent from simultaneous coexistence of hypercholesterolemia and hypertriglyceridemia and also reduce the cardiovascular risk factors [39]. These findings are in agreement with previous studies carried out by Chakrabarti et al (2005) [40] whom used aqueous and methanolic seed extract dose (250 mg/kg) of *Caesalpinia bonducella* on alloxan induced diabetic rat models.

The enzymes SOD and CAT are major antioxidant defense systems of the body which protect the cell membrane and other cellular constituents against oxidative damage by free radical species (ROS) [41]. Decreased serum concentration of total antioxidant enzymes in alloxan treated diabetic rats were observed due to their utilization during inhibition or destruction of free radical species which also indicates an imbalanced ROS production and antioxidant scavenging systems. SOD is a metallo-protein enzyme primarily involved to catalyze the superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2) and finally decomposition into H_2O and O_2 during detoxification reactions by CAT [42,43]. Increased serum concentration of qualitative diagnostic enzymes such as SGPT, SGOT and ALP were observed in diabetic rats indicating an altered liver function and/ or liver mitochondrial injury in comparison to normal control rats. Insulin deficiency contributes to increased serum level of transaminase enzymes due to easily availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes during diabetes. On treatment with MED significantly reversed the elevated marker enzymes i.e. SGOT, SGPT, ALP and restored to normal values indicates a revival of insulin secretion into circulations and also its hepatoprotective effect. Increased free radical induces LPO refers to the oxidative degradation of lipids that impairs cell membrane functions resulting into cell damage and leading to several pathologies and cytotoxicity [44,45]. MDA ($CH_2(CHO)_2$), a reactive electrophile species which is one of the LPO end product analogy to advanced glycation end product is used as redox marker and measurement for elevated oxidative stress. Our present study showed a significant ($p < 0.05$) elevation of MDA level in serum of diabetic rats suggesting the tissue peroxidative damages. Oral administration of MED (200 and 400 mg/kg) significantly lowers the elevated level of LPO which suggests that MED (200 and 400 mg/kg) might prevent from oxidative stress and provides protection to vital tissues of liver, kidney, heart etc. indicating its antioxidant activities. Reports of earlier studies suggested that various plants was proved to possessing wide variety of natural antioxidant constituents such as tannins, saponosides, alkaloids, flavonoids, phenolic acids and poly phenols etc. which enhances free radical scavenging activities and responsible to ameliorate change in antioxidant enzymes which may be helpful for treatment of diabetic related complications.

The MED found to be potential antidiabetic extract in alloxan-induced diabetic model through reducing oxidative damage and modulating antioxidant enzymes by dose dependant manner. Further, isolation and establishment of exact mechanism of action of specific compound from MED is to be carried out in the future.

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