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Antidiabetic activity of methanolic extract of root bark of *Aegel marmelos* in Alloxan-induced animal model

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ABSTRACT

Aegel marmelos (L.) Corr., (Rutaceae; AG) is popularly known as bael tree, widely distributed throughout the Indian peninsula, Pakistan, Thailand, Bangladesh, Burma, and Sri Lanka. In the present study we have carried out the hypoglycemic activity of the standardized to total phenolic content of methanolic extract of AG (MAG) in experimental diabetic model. Inbreed 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. The results showed that the preliminary phytochemical screening revealed that MAG showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the MAG showed total phenolic content (198.8 mg/g). Moreover, the routine post-treatment for 21 days with the MAG showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxan induced rat model in terms of significant hypolipidemic and antioxidant activity. In future, the isolation of specific compound from MAG and establishment of proper mechanism of action should to be carried out.

Keywords: *Aegel marmelos*, diabetes, hypoglycemic, dyslipidemia and oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, is associated with absolute or relative deficiency in insulin secretion or insulin action with altered carbohydrate, lipid and protein metabolism.

Various complications such as neurological complications, coronary artery disorder, renal failure, cerebro-vascular disease, limb amputation, blindness, long term damage, dysfunctions and failure of various organs and eventually premature death [1] occurs. It has been predicted that Indian people are more genetically susceptible to diabetes and will reach to maximum of 74 millions by 2025 [2]. After cancer and cardiovascular diseases diabetes is becoming the third leading cause of death worldwide due high prevalence of morbidity and mortality. Management for DM includes modification in lifestyle, balanced diet, exercise, and long term use of oral hypoglycemic agents or insulin injection [3]. Recently, the marketed allopathic medicines have significant toxic and adverse side effects compared to plant based drugs [4]. It has been suggested that about 800 medicinal plants possess antidiabetic potential [5] and the bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and floavonoids 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. In the continuation of above mentioned reports, World Health Organization (WHO) has also recommended the traditionally used plant drugs for the treatment of various diseases [8].

Aegel marmelos (L.) Corr., (Rutaceae; AG) is popularly known as bael tree, widely distributed throughout the Indian peninsula, Pakistan, Thailand, Bangladesh, Burma, and Sri Lanka. It has been reported that it is used as ancient folk herbal medicines in the Ayurveda, Unani and Siddha systems for treatment of wide variety of ailments [9]. It has been reported from the preclinical studies that the leaves of AG possess several potential pharmacological activities such as anti-inflammatory, antipyretic [10], analgesic [11], hepatoprotective [12] and antifungal [13]. Traditionally, it is used in treatment of cancer, diarrhoea, dysentery, asthmatic complications [14], hypoglycemic and antioxidant activity [15-18]. The fruit (unripe) is used for diarrhea, dysentery, ulcers [19], hypoglycemic [16,20,21]. The fresh flowers are useful in allay thirst and vomiting, dysentery whereas seeds were reported to posses antimicrobial, anthelmintic [22], hypoglycemic activities [23]. Stem and root bark are used to treat ulcers [24], dyspepsia, and stomachalgia [19]. Phytochemical studies on various parts revealed that the plant consists of numerous biologically active compounds such as aegelin, citronellal, cineole, citral, marmelosin, aurapten, cuminaldehyde (4-isopropylbenzaldehyde), eugenol, lupeol, skimmianine, marmesinin, luvangetin, fagarine, marmelide, tanin, marmin and psoralen [9,14].

Therefore, in the present study we have carried out the hypoglycemic activity of the standardized methanolic extract of AG (MAG) in experimental diabetic model.

MATERIALS AND METHODS

1.1. Chemicals and reagents

AG root barks were harvested from dense forest of Achanakmar foothills located at Chhattisgarh State (India) which was further identified and authenticated by Dr N Shiddamallayya of Regional Research Institute (Ay.), Bangalore (India), where Voucher Specimen (No. *RRCBI/Mus/3*) of the plant was deposited. 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-Ciocalteau 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/ pharmacopoieal grade purchased from E. Merck India Ltd and Ranbaxy respectively.

1.2. Plant material and extraction

Samples were collected manually, washed thoroughly under running tap water and shade dried at room temperature (25-30°C) for 15 days. The dried root barks were subjected to pulverized using hammer mill to a coarse powder and thereafter screened through 40 # mesh sieve. Powdered mass (500 g) was soxhlet extracted at 45-65°C using analytical grade methanol solvent (2.0 L, 72 h). Resulting extracts was concentrated into viscous semisolid mass (dark greenish to brown colored) in rotary evaporator at 40^0 C under reduced pressure and dried using hot air oven (45^0C) and then stored in airtight containers in refrigerator between 4-8°C till subsequent uses [yield: 12.4%]. Further, there was prepared a fresh suspension of 0.3% v/v carboxymethylcellulose (CMC) in distilled water. Thereafter, the methanol was mixed with 0.3% CMC separately to form methanol suspension (MAG) before administration to rats.

1.3. 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. About 100 µl of plant extract (1 mg ml^{-1}) were prepared in a 100 ml Volumetric flask and added 0.75 ml of Folin-Ciocalteu reagent (previously diluted in 1:10 ratio with deionised water) was thoroughly mixed and allowed to incubate for 5-8 min at room temperature (25±1°C) condition. Thereafter, 0.75 ml of Na_2CO_3 solution (60 g/L) was added to mixture and allowed it to stand at room temperature (25±1°C) for 2 h intervals. Absorbance was measured against blank using Double-Beam UV-Vis Spectrophotometer (Simadzu-1800) at λmax 725 nm respectively. Gallic acid was used as a standard to construct calibration curve (0.02-0.1 mg ml^{-1}) and readings taken in triplicate to get accurate results. The total phenolic content was expressed as milligrams of gallic acid equivalent/ g extract.

1.4. 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 housed in groups of six in polypropylene cages, acclimatized for a period of 7 days before experimentation was kept under standard conditions of 10:14 hr light: dark cycle, relative humidity 50-60% RH, temperature 22±3°C, were fed with rat pellet diet (Gold Moher, Lipton India Ltd) and water *ad libitum*. All the experimental protocol was approved by the institute animal ethics committee (Regd. No.1169/ac/08/CPCSEA) by following prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

1.5. Oral toxicity studies

Organization for Economic Co-operation and Development (OECD) guidelines (Guidelines 420; Fixed Dose Procedure) was followed for acute oral toxicity test to plant extract. Before experimentation rats (n=6) were fasted overnight with water *ad libitum* and was oral administered with fixed extracts dose of 5, 50, 300 and 2000 mg kg^{-1} body weight respectively by gavage using intubation canula. Administered dose was found tolerable as no death was found. Therefore, three dose levels of 50, 100 and 200 mg kg^{-1} b.w were selected for antidiabetic activity. Animals 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.

1.6. Induction of diabetes in rats

Induction of diabetes was performed by a single dose subcutaneous injection of freshly prepared alloxan monohydrate (120 mg/kg, Sigma chemicals, USA) dissolved in normal saline (0.9% w/v NaCl in distilled water) to overnight fasted male CF albino rats [27]. Blood glucose level was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats showed fasting blood glucose levels more than 250 mg/dl were considered to be diabetic and were selected for experimentation.

1.7. Experimental Design

1.7.1. Study on Oral Glucose Tolerance Test (OGTT)

Initially, OGTT of plant extracts was carried out in overnight fasted normal rats, which were equally divided into five groups of six rats each. During testing normal control group 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.), whereas group from third to fifth were administered with 1 ml of MAG (50, 100 and 200 mg/kg, p.o.) respectively. After 30 min of post extract administration all the animals were fed with glucose (2 g/kg). Then blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. Estimation for FBG was carried out using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, GmbH, Germany).

1.7.2. 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. 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 MAG (50, 100 and 200 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.

1.7.3. Study on Alloxan-induced diabetic rats

Diabetes in normal rats was induced using single intraperitoneal injection of alloxan monohydrate (120 mg/kg b.w.; i.p.) in 0.9% w/v NaCl solution (normal saline) and then, allowed for free access to food and water. Thereafter 72 h of injection, rats shown increased hyperglycemia (fasting

blood glucose level >250 mg/dl) was selected for further experimental studies. Animals selected were randomly divided into six 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.; Group-IV: Diabetic rats administered with MAG (50 mg kg^{-1} b.w. day^{-1}); Group-V: Diabetic rats administered with MAG (100 mg kg^{-1} b.w. day^{-1}) and Group-VI: Diabetic rats administered with MAG (200 mg kg^{-1} b.w. day^{-1}) respectively. Treatment was continued for a period of 21 days following oral administration to the experimental animals by gastric intubation. Quantitatively, plasma glucose was estimated by withdrawing blood samples 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 estimations.

1.8. Biochemical estimations:

Serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), glutamate oxaloacetate transaminases (SGOT), glutamate pyruvate transaminases (SGPT), alkaline phosphate (ALP), urea, creatinine, protein and albumin were estimated in an Hitachi Auto analyzer using commercial kit (Ecoline, E-Merck, India; Roche Diagnostics, GmbH, Germany) using standard procedures as markers for lipid profile. Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boerhringer Mannheim, Germany). The level of hemoglobin (Hb) and glycosylated hemoglobin (HbA_{1C}) were estimated using Drabkin reagent.

1.9. Estimation of lipid peroxidase

The extent of lipid peroxidation was estimated by measuring the plasma malondialdehyde (MDA) content using the method described by Uchiyama and Mihara, 1978 [28] with minor modification by Sunderman et al., 1985 [29] 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 95^0C 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.

1.10. Estimation of superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity in plasma was determined by the method of Ukeda et al., 1997 [30]. 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-[(phenyl amino)-carbonyl]-3,4-tetrazolium} -bis(4-methoxy-6-nitro)-benzene sulfonicacid hydrate) dissolved in buffer at 50^0C 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 (25^0C) by the degree of inhibition of this reaction.

1.11. Estimation of catalase (CAT)

Catalase (CAT) activities were assayed by following the method of Aebi, 1984 [31]. 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 4^0C. 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.

1.12. 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

1.13. Effect of MAG (50, 100 and 200 mg/kg) on oral glucose tolerance test (OGTT) and plasma glucose level in normoglycemic and diabetic rats:

Table-1 illustrates the effect of MAG (50, 100 and 200 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) = 0.33, P>0.05]. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups [F (4, 25) = 3.77, P<0.05]. Post-hoc test revealed that GL (0.25 mg/kg) and MAG (100 and 200 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) = 7.52, P<0.05].

Table-1: Hypoglycemic effect in oral glucose tolerance test (OGTT)

Groups	0 min	30 min	60 min	90 min	120 min
CON	87.5±3.62	134.9±3.16	125.2±1.65	117.1±2.29	112.3±4.27
GL	83.7±1.51	102.8±3.54[a]	97.7±4.71[a]	81.7±1.79[a]	62.3±3.17[a]
MAG-50	82.7±1.75	112.7±3.49	101.7±2.75	92.4±4.86	81.6±1.75
MAG-100	84.5±1.71	110.6±2.65[a]	100.7±3.35[a]	86.3±4.72[a]	84.6±2.60[a]
MAG-200	84.3±1.53	115.4±3.35[a]	105.1±2.56[a,c]	91.4±3.45[a,c]	87.3±1.56[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).

Post-hoc by Student Newmann Keuls test revealed that GL and MAG (100 and 200 mg/kg) showed significant decrease in the plasma sugar level compared to control. Furthermore, MAG (200 mg/kg) showed significant increase in the sugar level ($P<0.05$) compared to GL and this trend was similar at 90 min [$F(4, 25) = 16.81$, $P<0.05$] and 120 min [$F(4, 25) = 18.92$, $P<0.05$]. Time dependant effect of MAG (50, 100 and 200 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 [$F(4, 25) = 0.18$, $P>0.05$]. Similarly, statistical analysis at 7 day showed that there was significant difference among the groups [$F(4, 25) = 3.32$, $P<0.05$]. Post-hoc test revealed that GL and MAG (200 mg/kg) showed significant decrease in the plasma sugar level compared to control. Further, MAG (200 mg/kg) showed significant reduction in blood sugar levels compared to GL, MAG (50 and 100 mg/kg). Furthermore, the similar trend like at 7 day was observed at 14 day [$F(4, 25) = 11.93$, $P<0.05$] and 21 day [$F(4, 25) = 15.82$, $P<0.05$].

Table-2: Hypoglycemic effect of MAG (50, 100 and 200 mg/kg) in fasted normoglycemic rats

Groups	Day 0	Day 7	Day 14	Day 21
CON	74.8±3.73	75.6±2.45	70.2±4.32	71.5±2.11
GL	71.7±1.35	53.8±3.28[a]	39.6±3.67[a]	54.4±1.12[a]
MAG-50	74.6±3.75	63.4±3.21	63.2±5.81	62.1±1.45
MAG-100	72.5±1.71	61.1±2.46	62.4±2.96	57.9±1.32
MAG-200	71.2±2.11	49.7±5.39[a,c,d,e]	41.7±3.45[a,c,d,e]	53.6±3.15[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 MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

Table-3 showed time dependant effect of MAG (50, 100 and 200 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 [$F(5, 30) = 41.49$, $P>0.05$].

Table 3: Hypoglycemic effect of MAG (50, 100 and 200 mg/kg) in Alloxan induced animals

Groups	Day 0	Day 7	Day 14	Day 21
CON	77.1±2.13	75.7±1.81	72.1±3.32	70.1±10.11
DM	313.2±2.25	412.6±4.16[a]	366.3±9.25[a]	343.3±10.23[a]
GL	316.3±1.15	233.2±8.71[a,b]	185.6±11.55[a,b]	99.6±11.56[a,b]
MAG-50	319.3±1.12	367.1±11.25[a,c]	282.7±13.25[a,c]	246.7±13.21[a,c]
MAG-100	329.7±5.41	253.5±12.21[a,b,c,d]	243.5±12.51[a,b,c,d]	141.5±11.55[a,b,c,d]
MAG-200	306.5±5.21	223.7±10.41[a,b,d,e]	226.6±10.77[a,b,d,e]	110.6±11.21[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 MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

Further, statistical analysis at 7 day showed that there was significant difference among the groups [$F(5, 30) = 21.04$, $P<0.05$]. Post-hoc test revealed that DM, GL and MAG (50, 100 and 200 mg/kg) showed significant increase in the plasma sugar level compared to control. Further, GL and MAG (100 and 200 mg/kg) groups showed significant decrease in the blood glucose

level compared to DM. The MAG (50 and 100 mg/kg) groups showed significant increase in sugar level compared to GL. Further, MAG (100 and 200 mg/kg) groups showed significant difference compared to MAG (50 mg/kg). In addition, MAG (200 mg/kg) group showed significant decrease in blood sugar level compared to MAG (100 mg/kg). Furthermore, the similar trend like at 7 day was observed at 14 day [F (4, 25) = 23.93, P<0.05] and 21 day [F (4, 25) = 74.82, P<0.05].

1.14. Effect of MAG (50, 100 and 200 mg/kg) on body weight, food and fluid intake of diabetic rats:

The effect of MAG (50, 100 and 200 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) = 0.61, P>0.05]. Further, statistical analysis revealed that there was significant difference among the groups during final body weight estimation [F (5, 30) = 1.73, P<0.05]. Post-hoc test revealed that DM and MAG (50, 100 and 200 mg/kg) showed significant decrease in body weight compared to control. GL and MAG (100 and 200 mg/kg) groups showed significant increase in body weight compared to DM. MAG (50 and 100 mg/kg) groups showed significant decrease in body weight compared to GL. The body weight was significantly increased in MAG (200 mg/kg) compared to MAG (50 and 100 mg/kg).

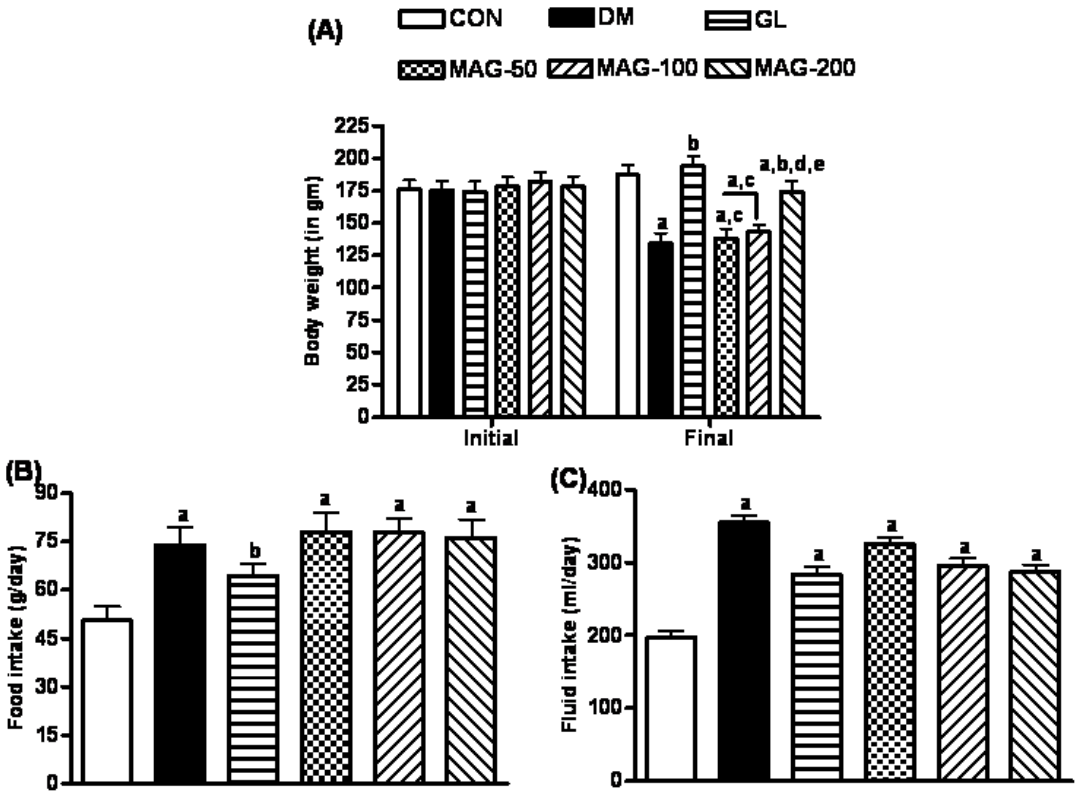

Fig-1. Effect of MAG (50, 100 and 200 mg/kg) on the body weight (A), food (B) and fluid (C) intake. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to DM, [c]P<0.05 compared to GL, [d]P<0.05 compared to MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

The effect of MAG (50, 100 and 200 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) = 4.41, 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 MAG (50, 100 and 200 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) = 71.40, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in fluid intake compared to control. However, all the treated groups did not show any significant change in fluid intake among themselves.

1.15. Effect of MAG (50, 100 and 200 mg/kg) on plasma lipid profile:

The effect of MAG (50, 100 and 200 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) = 8.36, P<0.05]. Post-hoc test revealed that DM and MAG (50 and 100 mg/kg) showed significant increase in TC level compared to control. The TC levels were decreased significantly in GL and MAG (200 mg/kg) compared to DM. The groups MAG (50 and 100 mg/kg) showed significant increased in TC levels compared to GL. Further, MAG (200 mg/kg) showed significant decrease in TC levels compared to MAG (50 and 100 mg/kg).

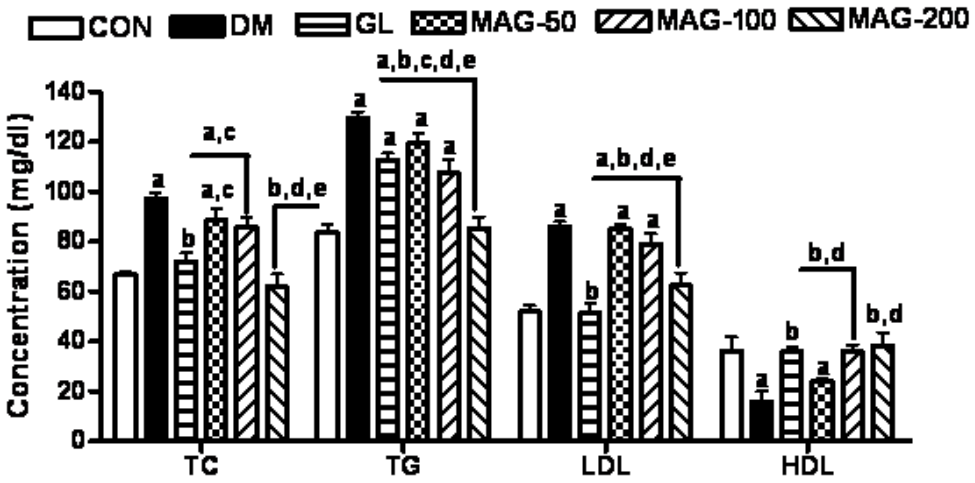

Fig-2. Effect of MAG (50, 100 and 200 mg/kg) on plasma TC (A), TG (B), LDL (C) and HDL (D) levels. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to DM, [c]P<0.05 compared to GL, [d]P<0.05 compared to MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

Furthermore, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 10.36, P<0.05]. Post-hoc test revealed that the TG levels were significantly elevated in all the groups compared to control. Further, MAG (200 mg/kg) showed significant decrease in TG levels compared to DM, GL, and MAG (50 and 100 mg/kg) groups.

Similarly, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 11.20, 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 MAG (200 mg/kg) showed significant decrease in LDL levels compared to DM. MAG (200 mg/kg) showed significant decrease in LDL levels compared to MAG (50 and 100 mg/kg) groups.

Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (5, 30) = 16.38, P<0.05]. Post-hoc test revealed that DM and MAG (50 mg/kg) showed significant decrease in HDL levels, however there was no change in HDL levels of GL and MAG (50 and 100 mg/kg) compared to control. Further, GL and MAG (100 and 200 mg/kg) showed significant increase in HDL levels compared to DM. MAG (100 and 200 mg/kg) showed significant increase in HDL levels compared to MAG (50 mg/kg).

1.16. Effect of MAG (50, 100 and 200 mg/kg) on liver function and plasma antioxidant profile:

The effect of MAG (50, 100 and 200 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) = 17.77, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and MAG (200 mg/kg) showed significant decrease in SGOT levels compared to DM. MAG (50 mg/kg) showed significant increase in SGOT levels compared to GL. MAG (200 mg/kg) showed significant decrease in SGOT levels compared to MAG (50 mg/kg) group.

The effect of MAG (50, 100 and 200 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) = 19.81, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in SGPT levels compared to control. GL and MAG (100 and 200 mg/kg) showed significant decrease in SGPT levels compared to DM. MAG (50 mg/kg) showed significant increase in SGPT levels compared to GL. MAG (200 mg/kg) showed significant decrease in SGOT levels compared to MAG (50 mg/kg) group.

The effect of MAG (50, 100 and 200 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) = 82.16, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in ALP levels compared to control. GL and MAG (200 mg/kg) showed significant decrease in ALP levels compared to DM. MAG (50 and 100 mg/kg) showed significant increase in SGOT levels compared to GL.

The effect on LPO of MAG (50, 100 and 200 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) = 19.37, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in LPO levels compared to control. GL and MAG (100 and 200 mg/kg) showed significant decrease in LPO levels compared to DM. MAG (50 mg/kg) showed significant increase in LPO levels compared to GL. MAG (100 and 200 mg/kg) showed significant decrease in LPO levels compared to MAG (50 mg/kg) group.

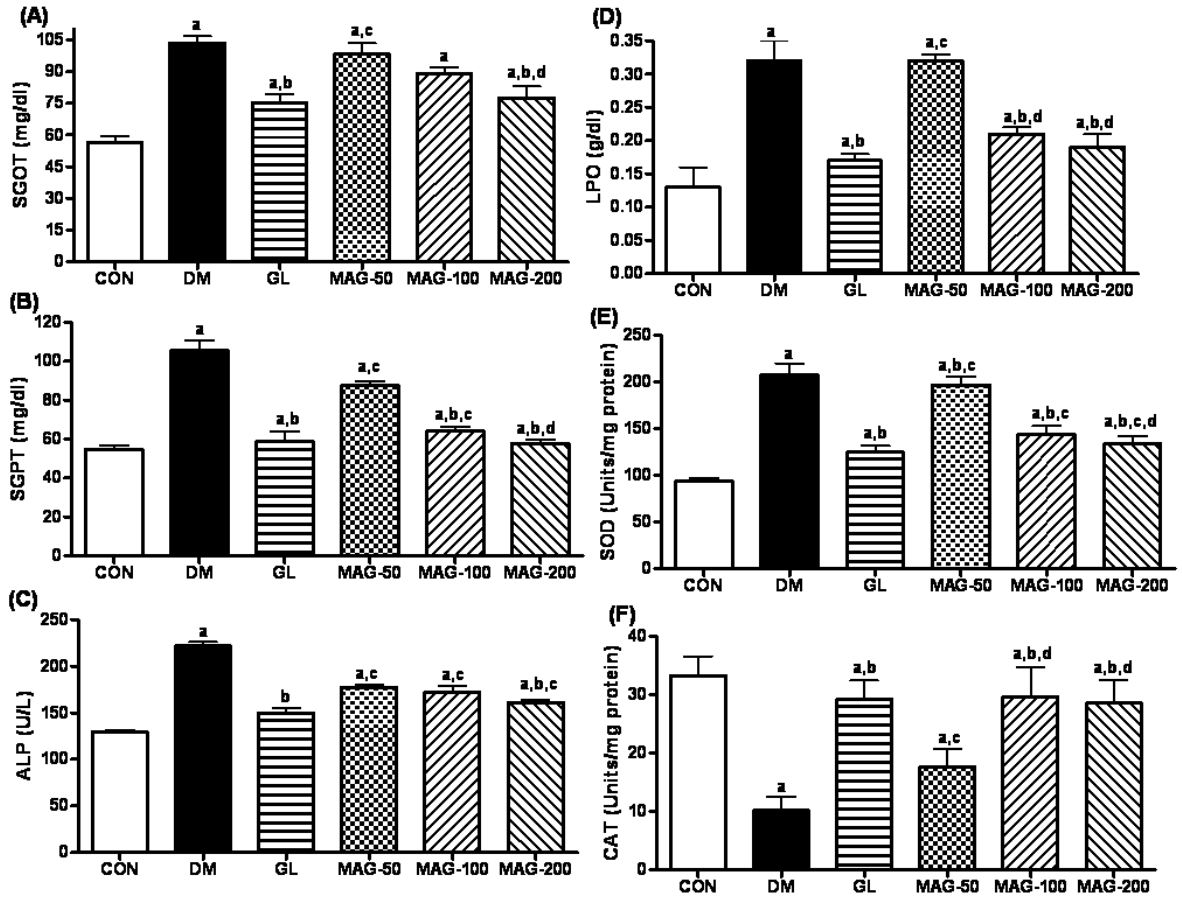

Fig-3. Effect of MAG (50, 100 and 200 mg/kg) on the levels of SGOT (A), SGPT (B), ALP (C), LPO (D), SOD (E) and CAT (F) intake. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to DM, [c]P<0.05 compared to GL, [d]P<0.05 compared to MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

The effect of MAG (50, 100 and 200 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) = 23.15, P<0.05]. Post-hoc test revealed that all the groups showed significant elevation in SGOT levels compared to control. GL and MAG (50, 100 and 200 mg/kg) showed significant decrease in SOD levels compared to DM. MAG (50 and 100 mg/kg) showed significant increase in SOD levels compared to GL. MAG (200 mg/kg) showed significant decrease in SOD levels compared to MAG (50 mg/kg).

The effect of MAG (50, 100 and 200 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) = 22.11, P<0.05]. Post-hoc test revealed that all the groups showed significant reduction in CAT levels compared to control. GL and MAG (100 and 200 mg/kg) showed significant increase in CAT levels compared to DM. MAG (50 mg/kg) showed significant decrease in CAT levels compared to GL. MAG (100 and 200 mg/kg) showed significant decrease in CAT levels compared to MAG (50 mg/kg).

1.17. Effect of MAG (50, 100 and 200 mg/kg) on Total Hb, HbA1c, plasma insulin, urea, creatinine and albumin:

The effect of MAG (50, 100 and 200 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) = 0.81, P<0.05]. Post-hoc test revealed that DM showed significant reduction and no other groups did not show any change in total Hb levels compared to control. The GL and MAG (50, 100 and 200 mg/kg) groups showed significant increase in total Hb levels compared to DM.

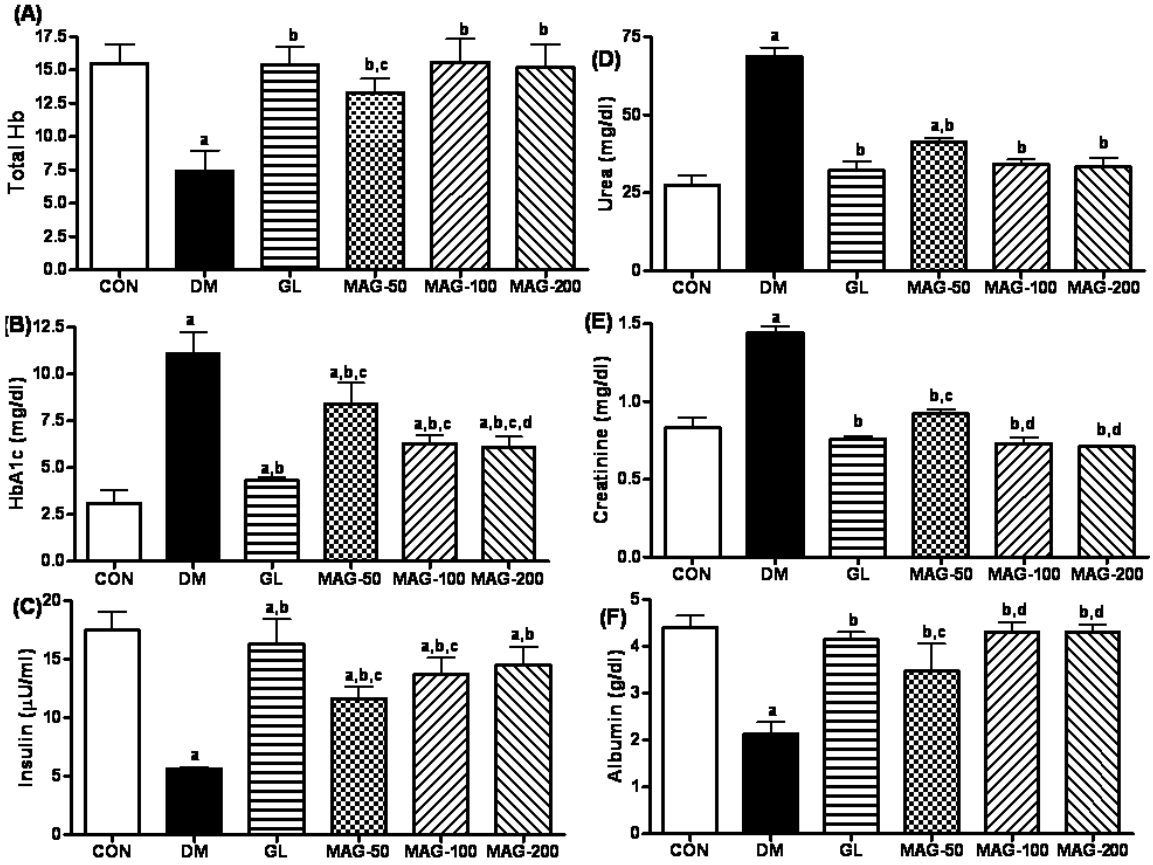

Fig-4. Effect of MAG (50, 100 and 200 mg/kg) on the levels of Total Hb (A), HbA1c (B), Insulin (C), Urea (D), Creatinine (E) and Albumin (F) intake. All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to DM, [c]P<0.05 compared to GL, [d]P<0.05 compared to MAG-50 and [e]P<0.05 compared to MAG-100 (One-way ANOVA followed by Student Newmann keuls test).

The effect of MAG (50, 100 and 200 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) = 17.19, P<0.05]. Post-hoc test revealed that DM, GL and MAG (50, 100 and 200 mg/kg) showed significant elevation in HbA1c levels compared to control. GL and MAG (50, 100 and 200 mg/kg) showed significant decrease in HbA1c levels compared to DM. MAG (50 and 100 mg/kg) showed significant increase in HbA1c levels compared to GL.

The effect of MAG (50, 100 and 200 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) = 35.69, P<0.05]. Post-hoc test revealed that DM, GL and MAG (50, 100 and 200 mg/kg) showed significant reduction in plasma insulin levels compared to control. GL and MAG (50, 100 and 200 mg/kg) showed significant increase in plasma insulin levels compared to DM. MAG (50 and 100 mg/kg) showed significant decrease in insulin levels compared to GL.

The effect of MAG (50, 100 and 200 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) = 41.39, P<0.05]. Post-hoc test revealed that DM and MAG (50 mg/kg) showed significant increase in plasma urea levels compared to control. The GL and MAG (50, 100 and 200 mg/kg) groups showed significant decrease in plasma urea levels compared to DM.

The effect of MAG (50, 100 and 200 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) = 76.91, P<0.05]. Post-hoc test revealed that DM showed significant increase in plasma creatinine levels compared to control. GL and MAG (50, 100 and 200 mg/kg) showed significant decrease in plasma creatinine levels compared to DM. MAG (50 mg/kg) showed significant increase in creatinine levels compared to GL. MAG (100 and 200 mg/kg) showed significant decrease in creatinine levels compared to MAG (50 mg/kg).

The effect of MAG (50, 100 and 200 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) = 15.09, P<0.05]. Post-hoc test revealed that DM showed significant decrease in plasma albumin levels compared to control. GL and MAG (50, 100 and 200 mg/kg) showed significant increase in plasma albumin levels compared to DM. MAG (50 mg/kg) showed significant decrease in albumin levels compared to GL. MAG (100 and 200 mg/kg) showed significant increase in albumin levels compared to MAG (50 mg/kg).

DISCUSSION

In the present study, the continuous post-treatment for 21 days with the MAG showed potential hypoglycemic activity in OGTT and normoglycemic rats and antidiabetic activity in alloxan induced rat model in terms of significant hypolipidemic and antioxidant activity.

Preliminary phytochemical screening revealed that MAG showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the MAG showed total phenolic content (198.8 mg/g). The decreased level of total hemoglobin in diabetic control group indicates the significant increased level of glycosylated hemoglobin (HbA1c). HbA1c is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus [32]. HbA1c is the product of non-enzymatic condensation reaction between excess glucose present in the blood and free amino groups on the globin component of hemoglobin [33]. Its concentration is abnormally high in chronic hyperglycemia which reflects long-term glycemic status and also correlates with risk for complications development such as retinopathy, nephropathy or neuropathy related to chronic diabetes stage [34]. However on oral administration of MAG significantly decreased the HbA1c

level possibly due to improved glycemic control mechanisms in experimental diabetic rats. The above results are in agreement with the previous carried out studies [35].

Diabetes mellitus is often linked with hyperlipidaemia or dyslipidemaia due to abnormal lipid metabolism which leads to profound alteration in the composition of lipid [36,37]. The diabetic rats had significant increased TC, TG, LDL and reduction of HDL level in comparison to normal control group. Abnormal lipid composition is due to impairment of insulin secretion at diabetic state which resulted into uninhibited actions of sensitive lipolytic hormones on the peripheral fat depots of which enhanced mobilization of free fatty acids occurred [38]. Lipid abnormalities are associated with hypercholesterolemia and hypertriglyceridemia which contributes to major risk factor for cardio vascular diseases [39,40]. During normal condition insulin hydrolyses triglycerides on activating the enzyme lipoprotein lipase but failure promotes to liver conversion of free fatty acids to phospholipids and cholesterol and discharged into blood [41]. On administration of experimental diabetic animals with the MAG (200 mg/kg) repeatedly for 21 days had significantly ($P<0.05$) reduced TC and TG level and raised HDL level near to normal range which implies that plant may posses insulin-like activity which would be helpful to reduce the incidence of lipid born complications [42]. It has been reported that the significant control of the serum lipids may definitely reduce the incidence of morbidity and mortality of diabetes [43].

Elevated level of sensitive qualitative biomarker enzymes viz. SGOT, SGPT and ALP was recorded in circulation of diabetic rats when compared with normal control rats reflecting hepatocellular damage and/ or indicative of liver mitochondrial injury [44-46]. During diabetes the insulin deficiency contributes to increased serum level of transaminase enzymes due to increased availability of amino acids which leads to enhanced occurrence of gluconeogensis and ketogenesis processes [47]. Treatment of experimental groups with MAG significantly reversed the elevated concentration of marker enzymes suggesting that the extract might hepatoprotective properties and restoration of normal functioning of liver organ. SOD and CAT are parameters for prediction of total antioxidant status which provides protection of membranes and biological structures against oxidative damage by Reactive Oxygen Species (ROS) [48]. SOD is a first metallo-protein enzyme involved in conversion of super oxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2) while CAT in association with GPx normally detoxifying H_2O_2 and decomposition into H_2O and O_2 [49]. Alloxan treated diabetic rats were observed with decreased serum concentration of total antioxidant enzymes indicating an imbalance between ROS production and antioxidant scavenging systems [50]. Living organisms consisting of reduced glutathione (GSH) as major non-protein thiol that acts as co-substrate for GPx and important key role in coordinating the body's defense processes [51].

MDA (CH_2 (CHO) $_2$) is commonly used as redox marker and in quantification of LPO end products which determines the oxidant/ antioxidant balance in body during diabetes [52]. Due to impaired antioxidant defenses and excessive generation of ROS the MDA leads to proteolysis by attacking on lysine amino acids in protein molecules [53]. Earlier reports shown that wide variety of plants possess tannins, saponosides, alkaloids, flavonoids, phenolic acids and poly phenols etc. as natural antioxidant constituents may be responsible to ameliorate change in

antioxidant enzymes and enhances free radical scavenging activities and also possibly attributes for treatment of related complications during diabetes.

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

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