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SYSTEMATIC STUDY TO EVALUATE ANTI-DIABETIC POTENTIAL OF *Amaranthus spinosus* ON TYPE-1 AND TYPE-2 DIABETES

J. H. BAVARVA[1,2] AND A. V. NARASIMHACHARYA[1]✉

[1] Department of Biosciences, Sardar Patel University, Satellite Campus, Vadtal Road, Vallabh Vidyanagar – 388 120, Gujarat, India.

[2] Virginia Bioinformatics Institute, Washington Street (0477), Virginia Tech, Blacksburg, VA 24061 USA.

Abstract

The present study was designed to systematically investigate the antidiabetic potential of *Amaranthus spinosus* leaves which are traditionally known to have various medicinal properties and used for the treatment of diabetes mellitus. The ethanolic extract of leaves of *Amaranthus spinosus* was administered (150, 300 and 450mg/kg bw) to type-1 and type-2 diabetic rats. Standard drugs, glibenclamide and metformin were used as a positive control for comparison. Changes in carbohydrate and lipid metabolism and antioxidants were assessed and compared with control and standard drug treated animals. Among the standardized extract doses tested (150, 300 and 450 mg/kg bw), higher doses significantly decreased plasma glucose levels ($p<0.01$ and $p<0.001$), hepatic glucose-6-phophatase activity ($p<0.01$ and $p<0.001$) and increased the hepatic glycogen content ($p<0.01$) with a concurrent increase in hexokinase activity in both type 1 and 2 diabetic rats ($p<0.01$ and $p<0.001$). Besides, the higher doses also significantly lowered the plasma and hepatic lipids, urea, creatinine levels ($p<0.001$) and lipid peroxidation with an improvement in the antioxidant profiles ($p<0.001$) of both type-1 and type-2 diabetic rats. It is concluded that *Amaranthus spinosus* has potential antidiabetic activity and significantly improves disrupted metabolisms and antioxidant defense in type-1 and type-2 diabetic rats.

Key words: Alternative medicine, antidiabetic, *Amaranthus spinosus,* type-1 diabetes, type-2 diabetes, metabolism.

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✉ **Corresponding author**
Tel: + 91 02692 234412
Fax: + 91 02692 231041
E-mail: narasimhacharya@spuvvn.edu, jasmin.spu@gmail.com

INTRODUCTION

Diabetes mellitus (DM) has become one of the major health problems affecting a significant segment of the population worldwide (48) and it is one of the major causes of premature illness and death worldwide. It is established that hyperglycemia is the principal cause of diabetic complications and, both insulin-dependent and non-insulin-dependent diabetes mellitus (type 1 and type 2) share most common symptoms i.e., chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both, leading to macro- and micro-vascular complications (12). An effective control of blood glucose levels therefore, is an important step in controlling or reducing the risk of diabetic complications. As the drugs presently used for treatment of DM have a number of limitations causing adverse side effects and high rates of secondary failure (29, 41), plants with antidiabetic potential are gaining importance and, a number of plants from India are known for their antidiabetic properties (8).

Amaranthus spinosus Linn. (Family- Amaranthaceae), a folk-lore medicinal plant (a weed species, growing abundantly in India) is used traditionally to treat various ailments such as indigestion, blood diseases, stomach troubles, gonorrhea, kidney complaints and diarrhea and, is also known to be a diuretic, antimalarial and hepatoprotective (3, 13, 24, 47). Various compounds are identified from this plant such as β-sitosterol, stigmasterol, campesterol, α-spinasterol, hentriacontane, glycosides, flavonoids, tannins and phenolics (30). *Amaranthus spinosus* is traditional used to treat diabetes mellitus (14). In preliminary laboratory investigations, it is reported that the extracts of this plant possesses glucose and lipid lowering properties (22, 38). However, there are no further reports for systematic evaluation of effects of this plant on type-1 and type-2 diabetic conditions on comparative basis as well how does it compare to standard drugs. The present investigation therefore was an attempt to evaluate comprehensive systematic effect/s of standardized ethanol leaf extract of *Amaranthus spinosus* leaves on carbohydrate, lipid metabolism and antioxidant profiles in both insulin dependent (type-1) and non-insulin dependent diabetes mellitus (type-2).

MATERIALS AND METHODS

Plant material, extract preparation and phytochemical quantitation

Amaranthus spinosus plants were identified in the University Botanical Gardens and authenticated by our faculty taxonomist Dr. A. S. Reddy (Voucher specimen number: JHB-04). The leaves of the plants were collected, air-dried, ground to powder and stored in an airtight container. The leaf powder was soxhlet-extracted with 95% ethanol, filtered and dried at room temperature and the residue (ASLEt) was stored at 4°C until used. The residue (ASLEt) was analyzed quantitatively for the presence of polyphenols and flavanoids, phytosterols and saponins (15, 23, 42). The ASLEt doses- 150, 300 and 450 $mgkg^{-1}$ bw were decided upon the results of the preliminary experiments that confirmed the non- toxic nature of these doses.

Animals

Colony bred male Albino rats (*Charles Foster*) were used in the present investigation. The animals were provided standard diet (Pranav Agro Industries, Vadodara, India), water *ad libitum* and were housed individually in well-ventilated animal unit (26 ± 2 °C, humidity 62%, and 12-h light/dark cycle). The animals' food intake was monitored throughout the experimental period along with the

body weights. The experiment was approved by Institutional Animal Ethics Committee (MoEF/CPCSEA/Reg.337).

Induction of insulin dependent diabetes (type-1)

Overnight fasted three-month-old rats weighing 200-250g were given a single intraperitoneal injection of alloxan monohydrate (120 mg/kg bw) dissolved in normal saline. Rats exhibiting fasting blood glucose concentrations (FBG) more than 140 mg/dl after two weeks were considered type-1 diabetic animals.

Induction of non-insulin dependent diabetes mellitus (type-2)

Neonate males aged 48 ± 2 h were injected intraperitoneally with a single dose of streptozotocin (100 mg/kg bw) in citrate buffer (pH 4.5) (9). The animals showing FBG levels >140 mg/dl after twelve weeks were considered type-2 diabetic animals.

Experimental design

The animals were distributed into eleven groups of six rats each and treated as followed: Normal control (normal rats without treatment), type -1 control (type-1 diabetic rats given only vehicle treatment), type -1-AS I (type -1 diabetic rats given ASLEt 150 mg/kg bw), type -1-AS II (type -1 diabetic rats given ASLEt 300 mg/kg bw), type -1-AS III (type -1 diabetic rats given ASLEt 450 mg/kg bw), Type -1-GB (type -1 diabetic rats given glibenclamide 600 µg/kg bw, Aristo Pharmaceuticals, Mumbai, India) (4), type -2 control (type -2 diabetic control rats given only vehicle treatment), type -2-AS I (type -2 diabetic rats given ASLEt 150 mg/kg bw), type -2-AS II (type -2 diabetic rats given ASLEt 300 mg/kg bw), type -2-AS III (type -2 diabetic rats given ASLEt 450 mg/kg bw), type -2-ME (type -2 diabetic rats given metformin 500 mg/kg bw, USV Ltd., Daman, India) (32).

The plant extract, glibenclamide and metformin were constituted in 1ml of 2% (v/v) Tween 80 and administered daily using an intragastric tube for thirty days. At the end of experimental period, the animals were deprived of food overnight, weighed and sacrificed under mild anesthesia; blood and liver tissue were collected immediately. After recording the liver weights, the tissue was stored at -20˚ C for further biochemical analyses.

Plasma glucose, hepatic glycogen, hexokinase and glucose-6-phosphatase

Plasma glucose levels were measured by o-toluidine method (43). Hepatic glycogen was extracted with 30% KOH and the yield was determined by anthrone–sulphuric acid method (40). Hepatic hexokinase (EC 2.7.1.1) and glucose-6-phosphatase (EC 3.1.3.9) activities were determined following the methods of Brandstrup et al. (10) and Baginsky et al. (5), respectively.

Plasma and hepatic lipid profiles

Plasma and hepatic cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were estimated by Wybenga and Pileggi's method with a cholesterol kit (Eve's Inn Diagnostics, Baroda, India). Plasma and hepatic triglycerides (TG) were estimated by GPO method with a Triglyceride kit (Eve's Inn Diagnostics, Baroda, India). Plasma low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) were calculated by Friedewald formula (21). Plasma and hepatic total lipids (TL) were extracted by the method of Folch et al., (19) and were estimated by gravimetric method. The same extract was also used for estimating total cholesterol

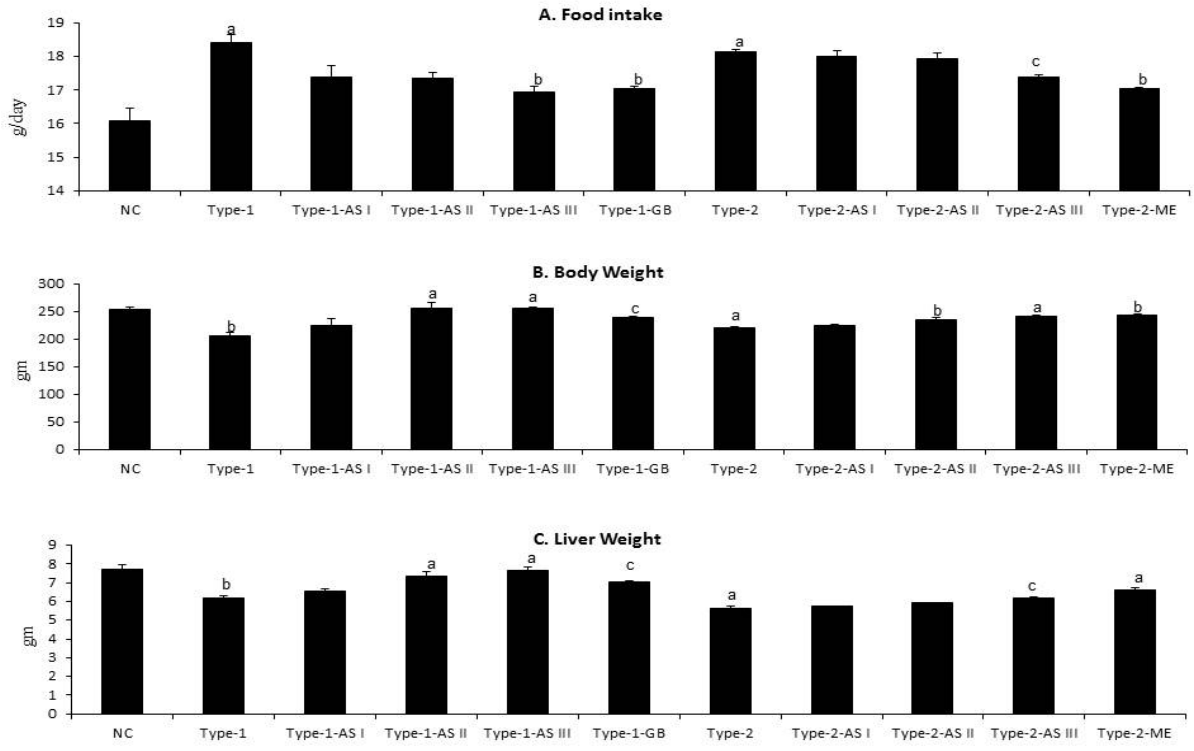

Figure 1. Effect of ASLEt on food intake (a), body weight (b) and liver weight (c) in all experimental groups. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

and triglyceride content using appropriate kits (Eve's Inn Diagnostics, Baroda, India).

Blood urea and creatinine

Blood urea and creatinine levels were determined (using diacetyl monoxime and alkaline picrate reagents) with urea and creatinine kits (Eve's Inn Diagnostics, Baroda, India).

Lipid peroxidation and enzymatic and non-enzymatic antioxidants

The hepatic lipid peroxidation (LPO) was determined by TBARS assay (31). Total ascorbic acid was assayed by the method described by Schaffert and Kingsley (39). Antioxidant enzymes-Superoxide dismutase (SOD) (EC 1.15.1.1) and Catalase (EC 1.11.1.6) were estimated according to the methods described by Kakkar et al. (28) and Aebi (1), respectively. Reduced Glutathione (GSH) was measured by the method of Jollow et al. (27). The glutathione peroxidase (GPx) (EC 1.11.1.9) activity was determined by the method of Flohe and Gunzler (18).

Statistical analysis

Results are expressed as means ± SEM. Significant differences among the groups were determined by one-way ANOVA using 12th version of SPSS with Tukey's test as post hoc analysis. Differences were considered significant if $p<0.05$.

RESULTS

Food intake, body and liver weights

Both type-1 and type-2 rats exhibited a significant increase in food consumption compared to that of controls. However, the body and liver weights of these animals registered a significant decline in both types of diabetic rats. ASLEt administration at 450 mg/kg bw dose significantly increased body and liver weights in both types of diabetic rats (Fig. 1).

Plasma glucose, lipid profiles, urea and creatinine

The plasma glucose concentrations were elevated in both type-1 and type -2 rats. ASLEt administration significantly decreased plasma glucose levels in type-1 with 300 and 450 mg/kg bw doses while in type-2 rats with 450 mg/kg bw dose alone. Both type-1 and type-2 rats exhibited elevated plasma TL, TC, TG, LDL-C and VLDL-C and reduced HDL-C when compared to controls. ASLEt at 450 mg/kg bw dose significantly reduced plasma lipid profiles of both type-1 and type-2 rats. The elevated levels of urea and creatinine in both types of diabetic rats were lowered significantly with both 300 and 450 mg/kg bw doses of ASLEt administration (Figs. 2-3).

Hepatic glycogen and lipid profiles

A significant decline in hepatic glycogen content was observed in both the types of diabetic rats. ASLEt administration with both 300 and 450 mg/kg bw doses increased glycogen content in both diabetic models. Both types of diabetes also exhibited increased hepatic TL, TC and TG. ASLEt administration (all three doses) to type-1 rats significantly reduced TL, TC and TG. However, ASLEt at only higher doses (300 and 450 mg/kg bw) brought about a significant reduction in hepatic TL, TC and TG in type-2 rats (Fig. 4).

Hepatic hexokinase and glucose-6-phosphatase

A significant decrease in hepatic hexokinase and an elevation in glucose-6-phosphatase were found in both types

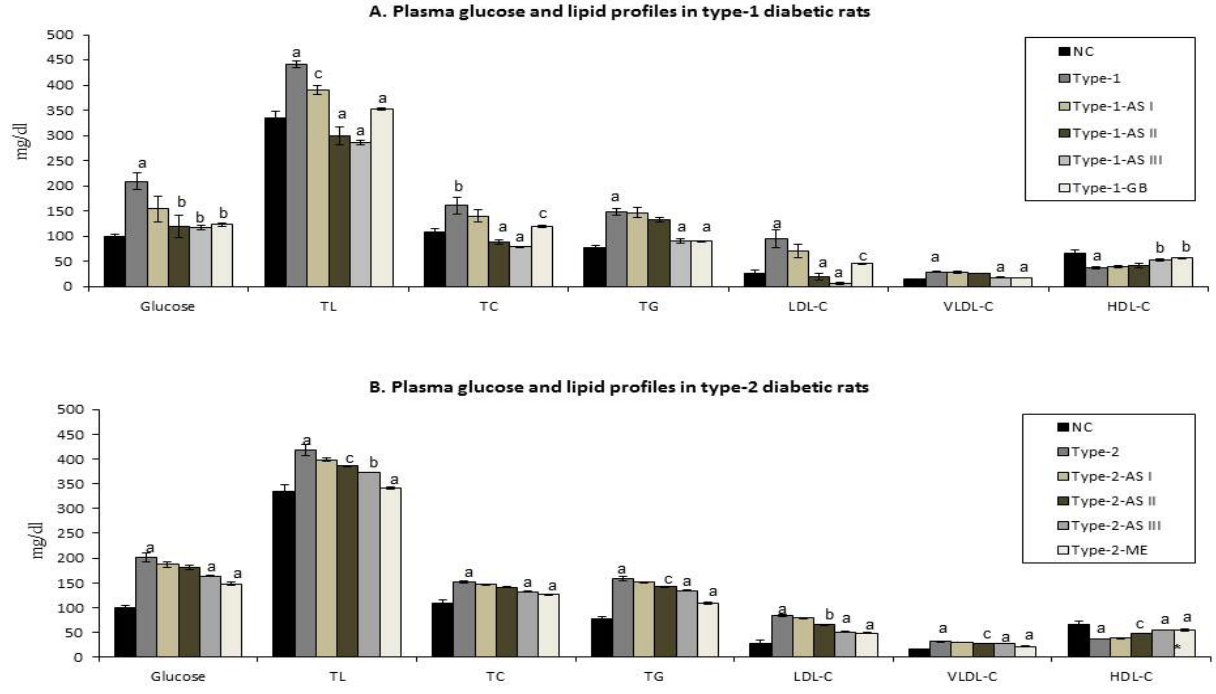

Figure 2. Effect of ASLEt on plasma glucose and lipid profiles in type-1 (a) and type-2 (b) diabetic rats. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: $P< 0.001$, b : $P<0.01$, c: $P <0.05$).

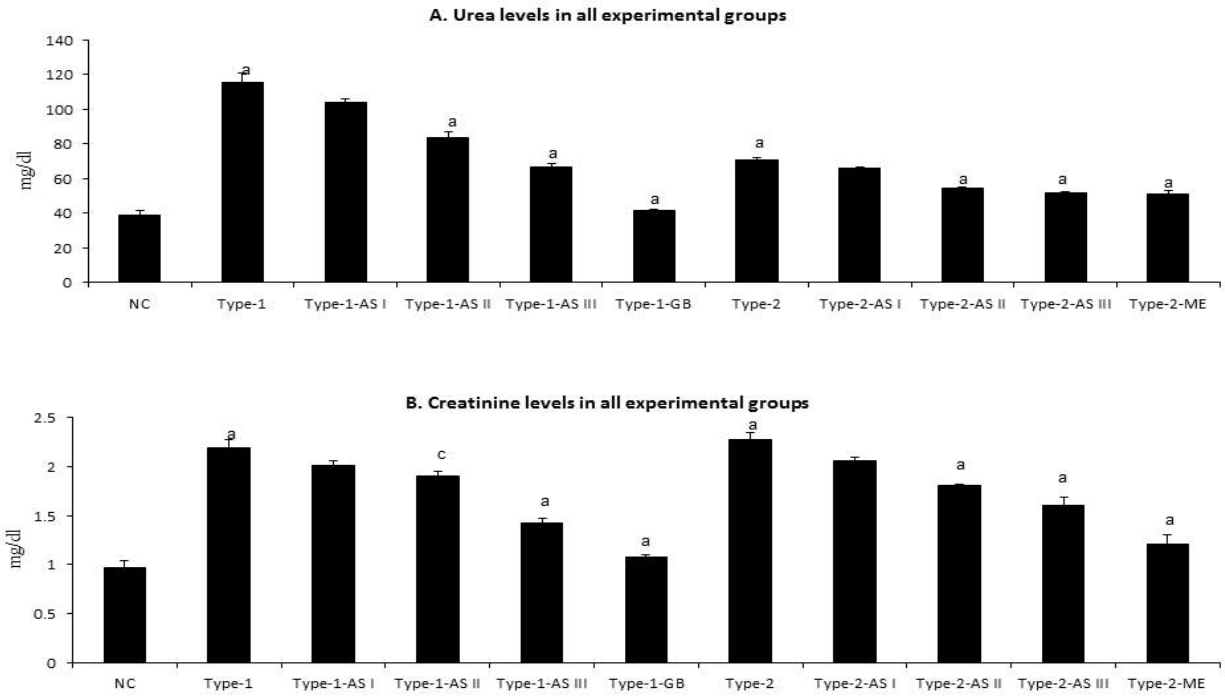

Figure 3. Levels of urea and creatinine in type-1 (a) and type-2 (b) rats. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

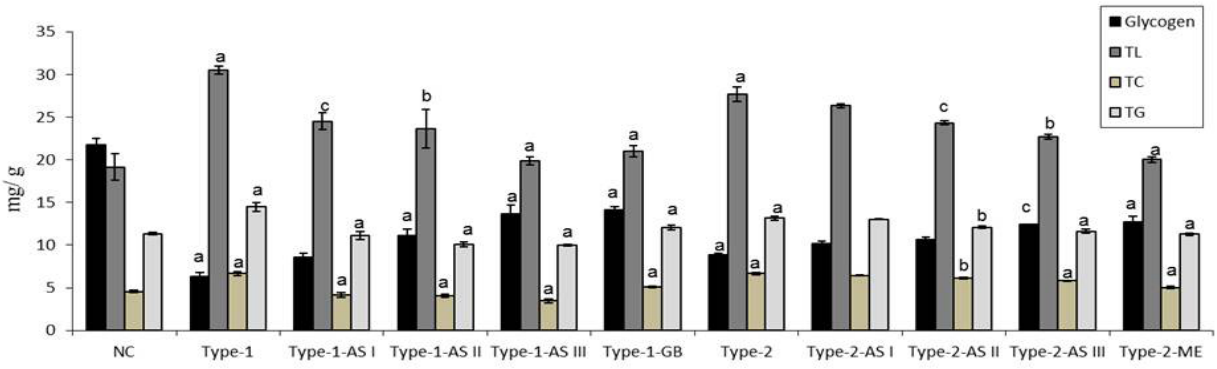

Figure 4. Hepatic glycogen and lipid profiles in all experimental groups. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

of diabetic rats. ASLEt (300 and 450 mg/kg bw) administration significantly increased the hexokinase activity with a concomitant decrease in glucose-6-phosphatase levels in both types of diabetic rats (Fig. 5).

Hepatic lipid peroxidation and antioxidants

Both type-1 and type-2 control group registered significant reduction in ascorbic acid, SOD, catalase, reduced glutathione and glutathione peroxidase activities with an increase in lipid peroxidation. While 300 and 450 mg/kg bw doses increased the ascorbic acid, catalase, glutathione and glutathione peroxidase activities, the SOD activity increased significantly only with 450mg/kg bw dose in both type-1 and type-2 diabetic rats. While the type-2 rats responded positively to 300 and 450 mg/kg bw doses of ASLEt, the type-1 rats registered a significant decline in lipid peroxidation with 450 mg/kg bw alone (Figs. 6 and 7).

Phytochemical analysis

The quantitation of major phytochemicals in ASLEt

(yield 30.62% w/w) revealed 5.51 mg/g of polyphenols, 35.92 mg/g phytosterols, 17.35 mg/g flavanoids and 66.6 mg/g saponins.

DISCUSSION

The present study provides an evidence for the beneficial effects of *Amaranthus spinosus* leaf extract on glucose and lipid metabolism and oxidative defense system in both types of diabetes (type-1 and type-2). The effects in type-1 and type-2 diabetic rats were found to be dose dependent i.e., 450 mg/kg bw dose was maximally effective compared to 150 or 300 mg/kg bw doses. While diabetic animals significantly lost their body and liver weights, the food intake increased. This decline in liver weight could be attributed to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis due to lack of / low levels of insulin (7, 45). These antihyperglycemic actions of ASLEt (300 and 450 mg/kg bw to type 1; 450 mg/kg bw to type 2) were seen in both diabetic groups with an increase in hepa-

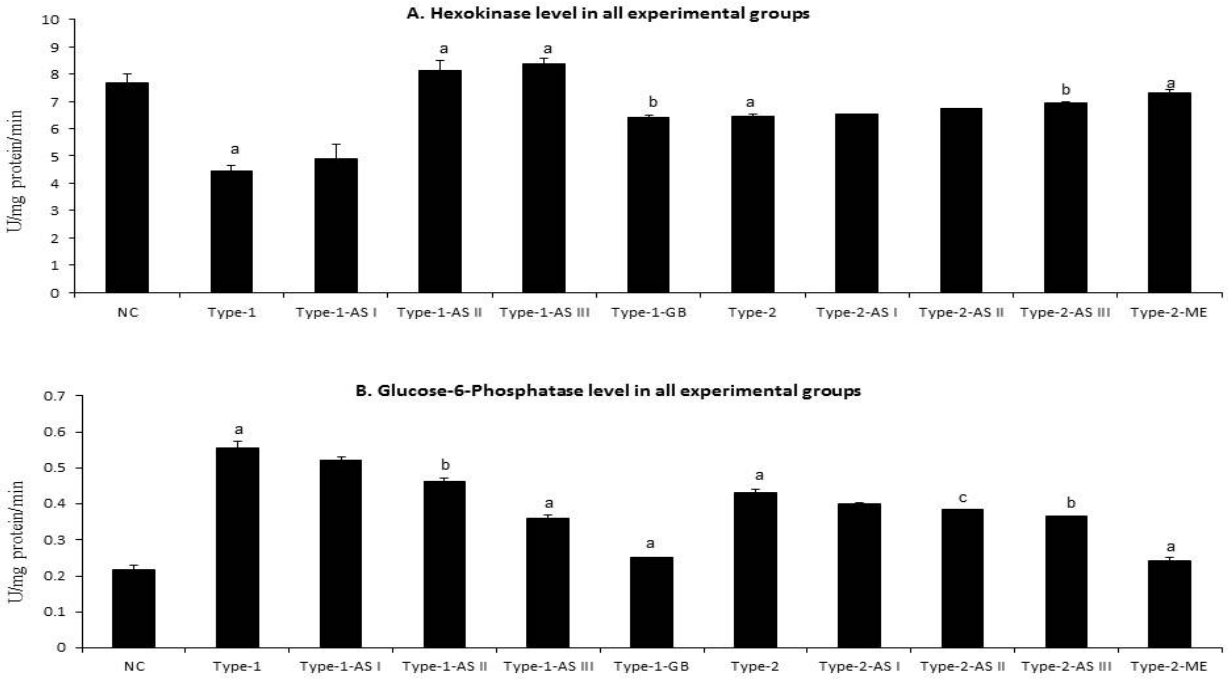

Figure 5. Levels of hepatic hexokinase (a) and glucose-6-phosphatase (b) in all experimental groups. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

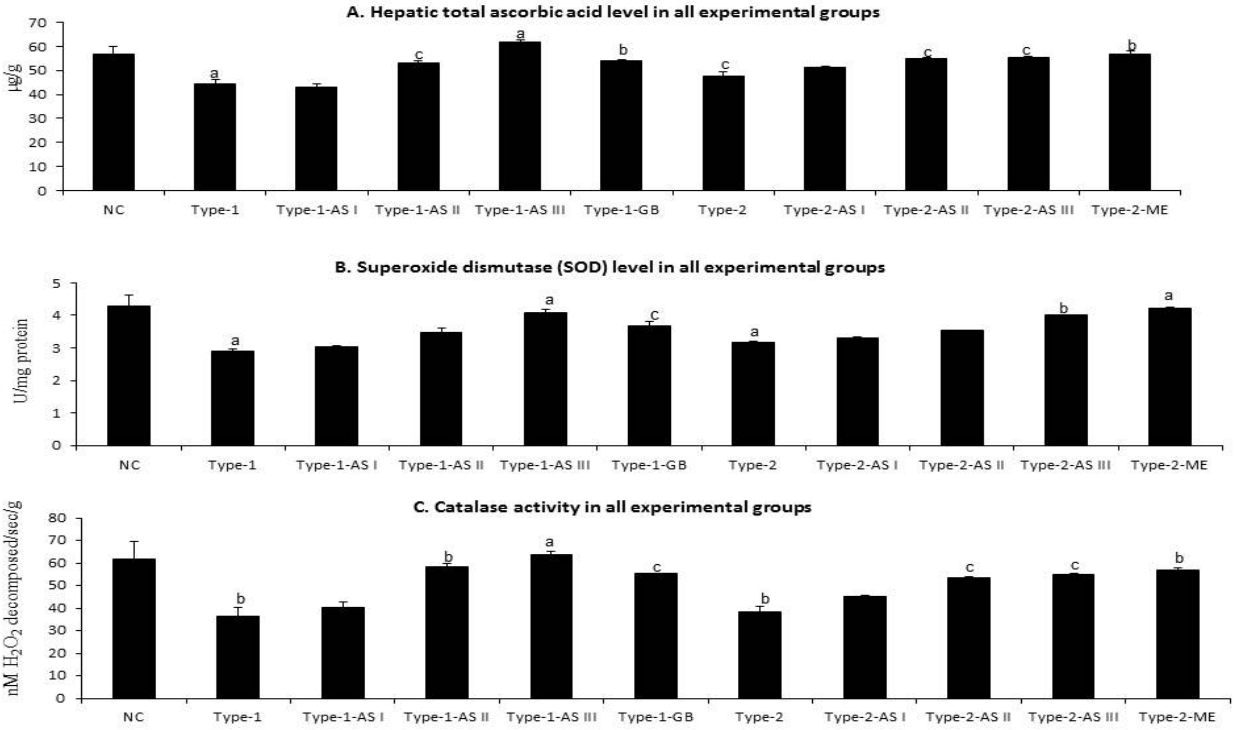

Figure 6. Hepatic total ascorbic acid (a), superoxide dismutase activity (b) and catalase (c) in all experimental groups. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

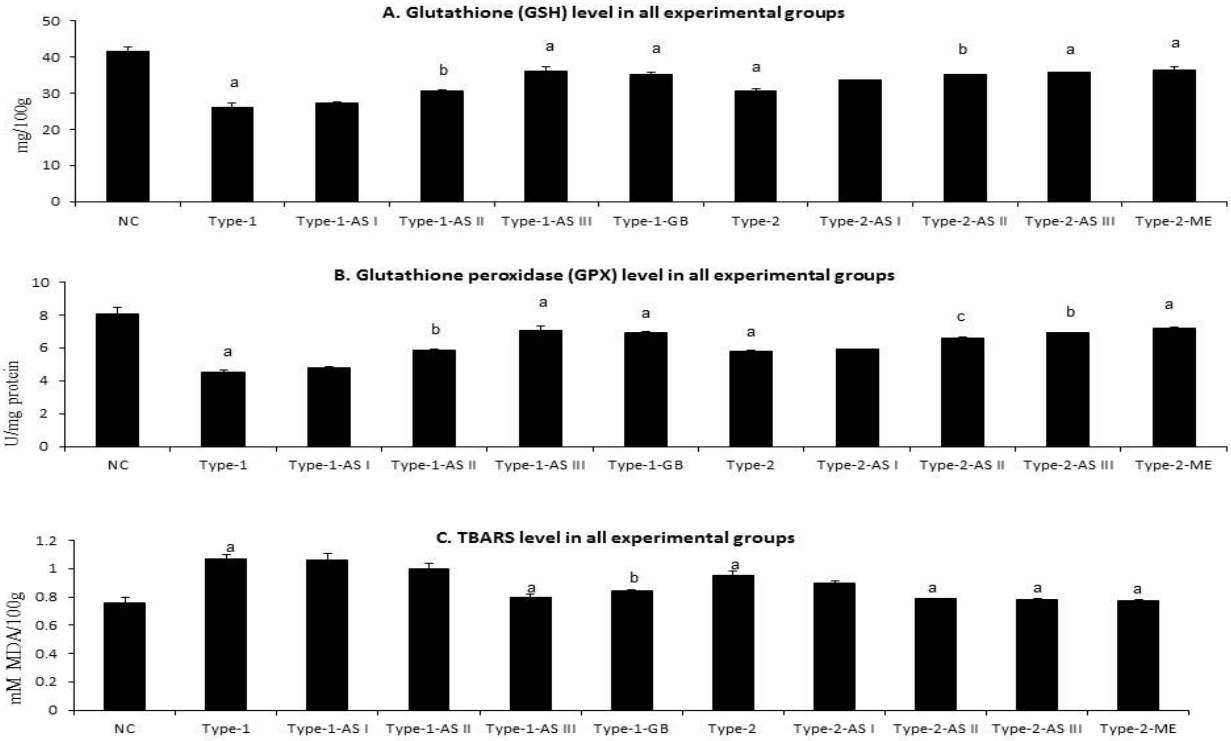

Figure 7. Hepatic glutathione content (a), Glutathione peroxidase (b) and TBARS (c) in all experimental groups. Values are mean ±SEM. Both diabetic controls were compared with normal controls; treated groups were compared with corresponding diabetic controls. a/ b/ c values represents statistical significance differences (a: P< 0.001, b : P<0.01, c: P <0.05).

tic hexokinase activity and glycogen content followed by a decrease in glucose-6-phosphatase activity. This reduction in blood glucose levels in both type-1 and type-2 diabetic rats administered ASLEt could be due to its synergetic effects of various phytochemical constitution i.e., polyphenols, flavanoids, phytosterols and saponins. For instance, polyphenols are reported to improve blood glucose levels, ameliorate insulin resistance and protect pancreatic β cells (49). Flavonoids are found to be antidiabetic, antioxidative and cardioprotective (46) and phytosterols along with saponins have also been shown to possess hypoglycaemic properties (20). However, the antihyperglycemic activity of ASLEt was more potent in type-1 compared to type-2 rats and the higher doses of ASLEt (450 mg/kg bw) were more potent than the lower doses (Figs. 2, 4, 5) owing to the higher concentrations of the phytoconstituents in 450 mg/kg bw dose.

ASLEt administration to both types of diabetic animals appeared to improve the renal functions in terms of excretion of urea and creatinine. Nephropathy is a characteristic feature of diabetes where the increased levels of glucose in kidneys result in enhanced blood urea and creatinine levels (45). While the diabetic controls exhibited significantly higher amounts of urea and creatinine, the ASLEt administered diabetic rats registered significant decreases in both urea and creatinine levels. This reduction could be due to an improvement in renal function owing to a reduced blood glucose concentration in both ASLEt administered diabetic groups.

Both types of diabetic rats showed elevation of TL, TC, TG, LDL-C, and VLDL-C and a reduction in HDL-C. The ASLEt administration reversed these effects i.e., ASLEt reduced TL, TC, TG, LDL-C, VLDL-C and increased HDL-C in both types of diabetic groups at a higher dose. The abnormally high concentrations of plasma and hepatic lipids in diabetes is primarily due to an increase in the mobilization of free fatty acids from the peripheral depots due to unavailability of insulin/ insulin resistance and also as a consequence of the uninhibited actions of lipolytic hormones (glucagon and catecholamines) on the fat depots (2, 7, 35). Presently observed significant decline in plasma and hepatic lipid profiles in ASLEt administered diabetic rats could be attributed to an insulin-like effect of ASLEt or its stimulatory effect on insulin release. These antihyperlipidemic effects could also be due to the phytoconstituents of ASLEt: as polyphenols are found to regulate lipid profiles, increase bile acid excretion and prevent adiposity and obesity and improve insulin secretion (49). Flavanoids are found to inhibit not only G-6-Pase activity but also lipid biosynthesis and are antihypercholesterolemic (16, 33). Phytosterols are reported to inhibit the cholesterol absorption and decrease its circulation (25, 34) and saponins precipitate cholesterol and prevent its absorption from the intestines leading to reduction in plasma cholesterol levels. Thus the ASLEt's effects on the lipid metabolism in diabetic rats appeared to be due to polyphenol, flavanoid, phytosterol and saponin contents Lipid peroxidation is one of the characteristic features of chronic diabetes and it is known to mediate tissue damage (17). The significant reduction of lipid peroxides in the liver of diabetic rats administered with ASLEt could be due to a reduced glucose oxidation and LDL-C concentration. Ascorbic acid (AA) levels in hepatic tissues of both types of diabetic rats were found to be significantly lower than in controls (7). The ASLEt administration (300 and 450 mg/kg bw) improved the AA levels substantially in hepatic tissues of both types of diabetic rats. Glutathione (GSH) a tripeptide, normally present at high concentrations in cells constitutes the major reducing capacity of the cytoplasm and protects the cells against toxic effects of lipid peroxidation (44). The diabetic animals in the present study registered lowered levels of GSH while a significant elevation of GSH levels

in ASLEt administered diabetic rats coincided with significant decline in lipid peroxidation (6, 7). A reduced GPx activity in diabetic controls with a concurrent reduction in GSH indicates that GPx activity is dependent on GSH content (26). The ASLEt administration to the diabetic rats particularly at higher doses (300 and 450 mg/kg bw) significantly increased the GPx activity which could be due to an increase in GSH concentration.

The antioxidant enzymes- SOD and catalase play important roles in reducing the cellular stress. SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen (36) while catalase brings about the reduction of hydrogen peroxides and protects tissues from the highly reactive hydroxyl radicals (11). Both these enzymes in the present context, registered low levels in type-1 and type-2 diabetic rats as noted earlier (7), while the ASLEt administered diabetic rats showed a clear improvement in both SOD and catalase activities substantially reflecting the antioxidant potency of ASLEt. These effects of ASLEt (450 mg/kg bw) on antioxidants (GSH, GPx, SOD, catalase and ascorbic acid) were found to be better than those of glibenclamide and metformin administered diabetic rats. This finding is in agreement with previously reported antioxidant activity of *A. spinosus* (47). The increased antioxidant potential in ASLEt administered diabetic rats could also be due to the presence of polyphenols, flavanoids, phytosterols and saponins as these compounds are not only antihyperglycemic, antihyperlipidemic but they are also shown to be antioxidant agents (20, 37, 46).

In conclusion, results of present systematic study clearly demonstrates the potential of *Amaranthus spinosus* in regulation of glucose and lipid metabolism and improved oxidative defense in type-1 and type-2 diabetes mellitus that could be due to the synergetic influence of phytocompounds present. The fact that diabetes is a metabolic disorder that is difficult to cure with a single agent, approach to tackle the symptoms and improve the quality of life for a patient with multiple beneficial compounds is probably the better way for overall beneficial and less toxic medication. A further study is required to understand molecular mechanism behind anti-diabetic action of *Amaranthus spinosus* and to isolate major and minor compounds present that influence the synergetic effects.

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