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ORIGINAL ARTICLE

Ameliorative effects of *Mentha aquatica* on diabetic and nephroprotective potential activities in STZ-induced renal injury

Prabhakar Yellanur Konda[1] · Janardhan Yadav Egi[2] · Sreenivasulu Dasari[3] · Raju Katepogu[4] · Krishna Kumar Jaiswal[5] · Prabhusaran Nagarajan[6]

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Abstract

Diabetes is a chronic metabolic disorder characterized by chronic hyperglycemia which causes secondary pathophysiological changes in multiple organ systems. Clinically used oralhypoglycemic agents are associated with a lot of side effects and high cost of treatment. As per ethnobotanical relevance, traditional medicines and natural products offer a valuable alternative to the oralhypoglycemic drugs. This study was hypothesized to evaluate the antidiabetic and nephroprotective activities of *Mentha aquatica* in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ at a dosage of 40 mg/kg bw. At the end of the study, overnight-fasted rats were dissected, and the blood and kidney samples were analyzed for biochemical and histopathological analysis. Oral administration of aqueous extract of leaves of *Mentha aquatica* (AELMA) at a dose of 100 mg/kg bw/day for 90 days significantly decreased the level of fasting blood glucose, HbA1c, TC, TG, plasma urea, creatinine, urine albumin, and kidney lipid peroxidation and increased the body weight, insulin, HDL cholesterol, plasma albumin, urinary urea, urinary creatinine, and antioxidant enzyme activities. The present study demonstrates that aqueous extract leaves of *Mentha aquatica* exert significant antidiabetic activity by stimulating secretion of insulin and nephroprotective potential activity by reducing the lipid peroxidation and enhancing the scavenging ability of antioxidant defense system in the body.

Keywords *Mentha aquatica* · Streptozotocin · Antidiabetic activity · Nephroprotective activity

Introduction

Diabetes mellitus (DM) is a chronic endocrine metabolic disorder and a very rapidly growing epidemic around the world, which associated with persistent increased levels of glucose in the blood (Kulkarni and Garud 2016). DM is a global distribution, affecting all ages, as indicated by the International Diabetes Federation, approximately 425 million adults were living with diabetes; by 2045, this will rise to 629 million. Almost 79% of adults were suffering from diabetes in low- and middle-income countries (IDF 2017). The long-term increased level of glucose in the blood leads chronic hyperglycemic condition which switches the development of various complications like diabetic retinopathy, diabetic neuropathy, diabetic myopathy, stroke, and diabetic nephropathy degenerative changes because of the uncontrolled level of glucose in the blood (Kumar et al. 2016). Hyperglycemia is central to the pathogenesis of diabetic nephropathy (DN). DN results chronic kidney disease (CKD) ultimately leads end stage renal disease (ESRD) which requires renal replacement therapy (RRT) or kidney transplantation (Pruthia et al. 2012). Clinical renal involvement is usually seen at the age of 15 to 25 years after the onset of type 1 and 2 diabetes.

Oxidative stress is a factor contributing to kidney damage by increasing the production of oxidants due to insufficiency of endogenous antioxidants. In diabetes, hyperglycemia leads

✉ Prabhusaran Nagarajan
prabhusaransrm@gmail.com

[1] Department of Biochemistry, Krijan Biotech, Malleshwaram, Bangalore 560 003, India

[2] Department of Biotechnology, Sri Venkateswara University, Tirupati 517 502, India

[3] Department of Biochemistry, Sri Venkateswara University, Tirupati 517 502, India

[4] Department of Science and Humanities, SV College of Engineering (JNTUA), Tirupati 517 507, India

[5] Centre for Green Energy Technology, Pondicherry University, Puducherry 605 014, India

[6] Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, Tiruchirapalli, Tamilnadu 621 105, India

to the production of reactive oxygen species (ROS) which acts as a mediator for the pathophysiology of diabetic nephropathy. Increase in oxidative stress activates glycation and production of advanced glycation end products (AGEs), cytokines, and growth factors through the mechanisms of increased polyol pathway flux, formation of AGEs, protein kinase C (PKC) activation, increased activity of aldose reductase (AR) enzyme, and toxic products (TGFβ) production (Tang et al. 2012). Mainly AR enzyme involves in the conversion of glucose to sorbitol, and long-term hyperglycemia increases the enzyme activity and sorbitol production, which increases the extracellular matrix. The accumulation of extracellular matrix in the mesangium and glomerular basement membrane (GBM) increases mesangium volume and thickening of GBM. TGFβ is one of these toxic products caused by hyperglycemia, and it increases the production of extracellular matrix in the glomerular mesangium. Also, with inhibition of collagenases, synthesis causes decreased extracellular matrix removal. These changes cause thickening of the glomerular basement membrane and obstruction of arteries and increased glomerular permeability (Chen and Miner 2012).

Diabetes also causes damage to the nerves which causes difficulty in emptying the bladder. The pressure resulting from the bladder can back up and injure the kidneys. Sometimes, urine remains in the bladder for a long time and can develop an infection from the rapid growth of bacteria in urine that has a high sugar level. So, in a diabetic condition, hyperglycemia plays a central role in the pathogenesis of DN. The principle of treatment of diabetic nephropathy is based on tight control of hyperglycemia. Medicinal plants are the great source of a wide range of biologically active constituents for many centuries, and they have been used extensively as crude plant extracts or as pure components for treating diabetes (Konda et al. 2019; Prabhakar et al. 2013). Unlike allopathic drugs which are single active compounds that can specifically target one pathway, herbal remedies work in a way that depends on a synergistic approach and act on targeted elements of the cellular complex pathways (Ramya et al. 2014). When compared to synthetic ones, most natural remedies generally have less side effects and toxicity. So, presently, the use of herbal remedies has increased when compared to allopathic drugs (Arif et al. 2009).

Mentha aquatic L. (water mint) is a traditional medicinal plant (genus, *Mentha*; family, Lamiaceae), commonly called Neeti Pudina (Telugu). *Mentha aquatica* (MA) is a perennial plant characterized by their volatile oils which are used by the flavor, fragrance, and pharmaceutical industries. MA is a culinary and medicinal plant, used as traditional medicine for the treatment of depression and age-related illnesses (Stafford et al. 2008), colds and respiratory problems, and also used to treat arthritic diseases (Miyazawa et al. 1998). This plant was reported for antimicrobial activity (Mimica-Dukić et al. 2003), lipid peroxidation (Dorman et al. 2003), anti-inflammatory activity, and antioxidant activities (Conforti et al. 2008). The isolated monoamine oxidase (MAO)-inhibitor naringenin from MA is responsible for the MAO-inhibitory activity and also used as traditional medicine for depression-like conditions (Olsen et al. 2008). Based on traditional properties of MA, this plant was evaluated for antidiabetic and nephroprotective activity in STZ-induced diabetic rats.

Materials and methods

Procurement of *Mentha aquatica*

The leaves of *Mentha aquatic* L. (LMA) were collected from the stream areas of Talakona waterfall forest, Chittoor, India. It was identified and authenticated by the botanist (Herbarium Voucher no: 2001). The leaves of MA were dried in a shade and powdered, and the powder was used for the preparation of different solvent extracts.

Preparation of crude powder aqueous suspension

To prepare aqueous crude suspension, 100 g of shade-dried leaves powder of MA was dissolved in 1 l of water and soaked in a glass jar for 48 h at room temperature and the solvent was collected until it gave no coloration. Crude aqueous suspension is a direct raw water suspension which was concentrated and used. Crude aqueous suspension is the blend of whole compounds of plant materials. The solvent was concentrated to dryness under reduced pressure in Buchi Rotavapor R-200 and finally freeze-dried. The yield of the crude suspension was 40% (w/w). From this yield, 50 mg, 100 mg, and 150 mg of different crude suspension doses were used for the screening of the antihyperglycemic activity in STZ-induced diabetic rats.

Preparation of ethanol and aqueous extracts

Ethanol and aqueous extracts were prepared by successive solvent extraction of leaves of MA powder in Soxhlet apparatus at 68–70 °C in increasing order of polarity which provides serial dilution of phytoconstituents in extracts. It means that phytoconstituents were dissolved according to the polarity of the solvent. In this case, we get limited molecules which show a significant antidiabetic activity. The filtrates obtained were distilled and concentrated under reduced pressure at low temperature (40–45 °C) in Buchi Rotavapor R-200 and finally freeze-dried. The yield of the ethanol extract was 40% w/w, respectively. Finally, to prepare aqueous extract, the leaves powder was soaked in a glass jar for 48 h at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the filtrate gave no coloration. The filtrate was concentrated to dryness under reduced pressure in Buchi Rotavapor R-200 and finally freeze-dried. The yield of the extract was

60% (w/w). All the extracts were stored at − 20 °C in airtight containers until needed for further studies.

Phytochemical analysis

The freshly prepared different extracts of leaves of MA were qualitatively tested for the presence of different phytochemical constituents by using a standard method (Harbone 1998).

Animal management

Male albino Wistar rats weighing 180–200 g were housed in clean cages with a temperature of 22–24 °C, 12-h light/12-h dark cycle, and relative air humidity 40–60%. Rats had continuous access to food and tap water. The rats were randomly divided into different groups according to the experimental design, each group consisting of six rats ($n = 6$).

Induction of diabetes with streptozotocin

Single intraperitoneal administration of STZ at a dose of 40 mg/kg bw dissolved in freshly prepared 0.01 M citrate buffer with pH 4.5 and administered to normoglycemic rats starved for 16 h. After 48 h, rats with marked hyperglycemia (fasting blood glucose higher than 250 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

Effect of antihyperglycemic activity of crude powder suspension of LMA

The rats were divided into five groups and consisting of six rats in each group ($n = 6$), a total of 30 adult male rats:

G 1. Control rats (C)
G 2. Diabetic control rats (DC)
G 3. DC rats treated with 50 mg of crude aqueous suspension of LMA
G 4. DC rats treated with 100 mg of crude aqueous suspension of LMA
G 5. DC rats treated with 150 mg of crude aqueous suspension of LMA

After an overnight fast, the diabetic-treated rat group received the crude aqueous suspension of LMA (in 1 ml of distilled water) by gastric intubation using a force-feeding needle, while the normal and untreated diabetic rats were fed distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein after the administration of crude suspension, and blood glucose levels were determined by using Dextrostix (Glucose oxidase-peroxidase method) with a basic one-touch Accu-Chek Glucometer.

Effect of ethanol and aqueous extracts of LMA on antihyperglycemic activity

The rats were divided into four groups and consisting of six rats in each group ($n = 6$), a total of 24 adult male rats:

G 1. Control rats (C)
G 2. Diabetic control rats (DC)
G 3. DC rats treated with 100 mg ethanol extract of LMA
G 4. DC rats treated with 100 mg aqueous extract of LMA

After an overnight fast, the rats of group 1 and group 2 received only distilled water, whereas group 3 and group 4 diabetic rats received ethanol and aqueous extracts each at a dosage of 100 mg/kg bw, respectively. Blood samples were collected for the measurement of blood glucose from the tail vein, and the results were compared with control and diabetic control groups.

Effect of different doses of aqueous extract of leaves of *Mentha aquatica* (AELMA): dose fixation study

The rats were divided into six groups and consisting of six rats in each group ($n = 6$), a total of 36 adult male rats:

G 1. Control rats (C)
G 2. Diabetic control rats (DC)
G 3. DC rats treated with 50 mg of AELMA
G 4. DC rats treated with 100 mg of AELMA
G 5. DC rats treated with 150 mg of AELMA
G 6. DC rats treated with 20 mg of glibenclamide

After an overnight fast, group 1 and group 2 rats received only distilled water, whereas groups 3, 4, and 5 diabetic rats received AELMA at a dosage of 50, 100, and 150 mg/kg bw, respectively. Group 6 rats received glibenclamide at a dosage of 20 mg/kg bw as a reference drug. Blood samples were collected for the measurement of blood glucose after feeding the extract, and the results were compared with those of control and diabetic control groups.

Effect of long-term treatment of AELMA (90 days) in STZ-induced diabetic rats

The rats were divided into four groups and each group consisting of six rats ($n = 6$), a total of 24 adult male rats:

G 1. Control rats (C)
G 2. Diabetic control rats (DC)

G 3. DC rats treated with 100 mg of AELMA
G 4. DC rats treated with 20 mg of glibenclamide

Group 1 animals were served as control. Group 2 animals were left as untreated control and served as diabetic control. Group 3 animals were treated with 100 mg aqueous extract of leaves of MA, and the last, group 4 animals were treated with glibenclamide 20 mg/kg bw, a reference antidiabetic drug. All treatments were done orally, twice daily at 8-h interval for a period of 90 days.

Biochemical methods

Blood samples were collected from each group through the retro-orbital plexus and then centrifuge at 4000 rpm for 20 min. Thereafter, the plasma was carefully separated and used to determine various parameters. Fasting blood glucose, HbA1c, insulin, and lipid profile markers such as total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were assessed. Kidney functional markers such as plasma albumin, urea, and creatinine levels were analyzed by using commercially available kits (Biosystems, Spain).

Urinary parameters

Urinary albumin, urea, and creatinine levels were estimated by using commercially available kits (Biosystems, Spain).

Effect of AELMA on oxidative stress of kidney (lipid peroxidation)

The kidney lipid oxidative degradation was determined by the method of Fraga et al. (1998) through measuring the concentration of thiobarbituric acid reactive substances (TBARS) which were expressed regarding malondialdehyde (MDA) content in tissues.

Effect of AELMA on antioxidant activities of the kidney

The activity of catalase (CAT) was measured by the method of Sinha (1972), the activity of superoxide dismutase (SOD) was measured by the method of Kakkar et al. (1984), glutathione peroxidase (GPx) activity was analyzed by the method of Rotruck et al. (1973), and glutathione *S*-transferase (GST) activity was estimated by the method of Habig et al. (1974).

Histopathology of kidney

The kidney histopathological changes were assessed by hematoxylin and eosin staining (H&E). The kidney specimens from all groups were fixed in 10% formalin solution and processed for paraffin embedding. The specimens were sectioned 5 μm thick and finally stained with the hematoxylin and eosin (H&E dye). The effect of MA on kidney sections was observed under a light microscope.

Statistical analysis

Data values were expressed as mean ± SEM. The effect of the treatment was evaluated using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test.

Results

Phytochemical analysis

Phytochemical screening of ethanol and aqueous extracts of LMA showed the presence of alkaloids, flavonoids, phenols, glycosides, saponins, steroids, tannins, and volatile oils. Phytoconstituents of ethanol and aqueous extracts of LMA are shown in Table 1.

Evaluation of crude powder aqueous suspension of LMA for antihyperglycemic activity

The effect of crude powder aqueous suspension of LMA on FBG levels of diabetic and diabetic treated rats is shown in Fig. 1a. The FBG levels of diabetic rats (group 2) were significantly higher than those in control rats (group 1). A significant decrease in the FBG was observed after administration of 100 mg of crude powder aqueous suspension (group 4) when compared to 50 mg and 150 mg of aqueous suspension of LMA (Fig. 1a).

Table 1 Phytochemical constituents of ethanol and aqueous extracts of leaves of *Mentha aquatica*

Constituents	Ethanol extract	Aqueous extract
Alkaloids	++	+
Flavonoids	–	+++
Phenols	–	+++
Glycosides	–	++
Saponins	+	++
Steroids	+	++
Tannins	–	+++
Volatile oils	+	++

+++, present in high amount (positive within 2 min); ++, moderately present (positive within 5 min); +, trace amounts (positive within 10 min); –, completely absent

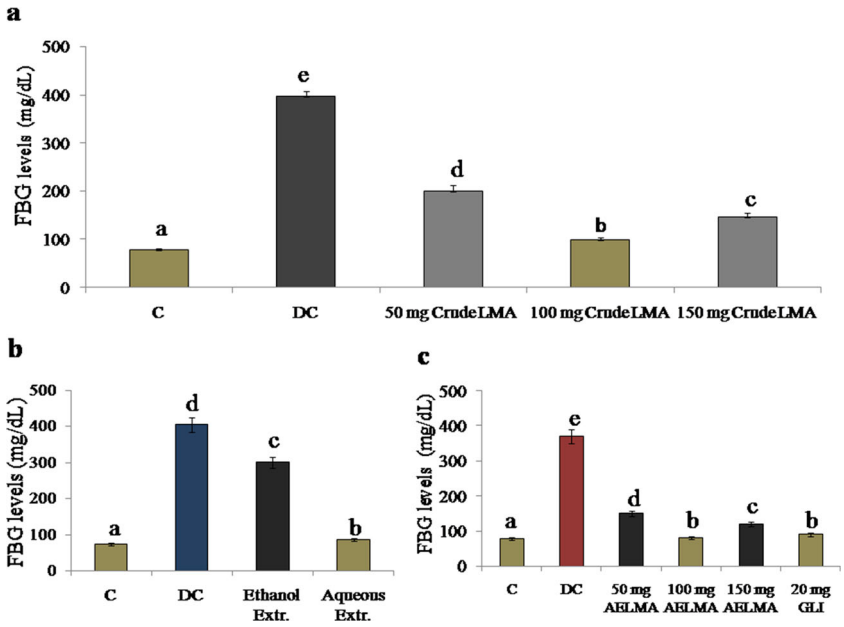

Fig. 1 Evaluation of antidiabetic activity in STZ-induced diabetic rats. **a** Effect of crude aqueous suspension of LMA. **b** Effect of ethanol and aqueous extracts. **c** Effect of different doses of AELMA on diabetes. C control, DC diabetic control, FBG fasting blood glucose, LMA leaves of *Mentha aquatica*, Extr extract, AELMA aqueous extract of leaves of *Mentha aquatica*, GLI glibenclamide, STZ streptozotocin. Values are mean ± SEM of triplicate. Superscript letters (a, b, c, d, and e) indicate significant difference ($p < 0.05$)

Evaluation of ethanol and aqueous extracts of LMA for antihyperglycemic activity

The effects of ethanol and aqueous extracts on the FBG levels of diabetic and diabetic treated rats are shown in Fig. 1b. The FBG levels of diabetic rats (group 2) were significantly higher than those of control rats (group 1); when ethanol and aqueous extracts were tested for their blood glucose-lowering effects, the aqueous extract at a dosage of 100 mg/kg bw produced significant and maximum fall in the FBG levels compared to the ethanolic extract (Fig. 1b).

Evaluation of different doses of AELMA for antihyperglycemic activity

The effect of different doses (50 mg, 100 mg, and 150 mg) of AELMA on FBG levels of diabetic (group 2) and diabetic treated rats are shown in Fig. 1c. Among the different doses, the aqueous extract at the dose of 100 mg/kg bw has produced a significant fall in the FBG level of diabetic rats after the treatment when compared to other doses. The treatment with glibenclamide at a dosage of 20 mg/kg bw also showed a fall in FBG levels (Fig. 1c).

Evaluation of long-term treatment of AELMA for FBG, HbA1C, body weights, and insulin

The FBG levels of control rats (group 1) were found to be normal before and after the treatment, but the levels of diabetic rats (group 2) were significantly increased before starting the treatment (Fig. 2a). However, at the end of the 90 days of treatment, there was a significant decrease in FBG levels that were observed in diabetic rats treated with AELMA (group 3), while there was a further increase in the FBG levels of diabetic untreated rats that was observed (Fig. 2a). The treatment with glibenclamide (group 4) also produced a significant decrease in blood glucose levels.

$HbA1_C$ levels of the diabetic rats (group 2) were found to be increased compared to control group (group 1), but the treatment with AELMA significantly reduced the HbA_{1C} levels (Fig. 2b) in diabetic treated rats (group 3) and treatment with glibenclamide (group 4) also showed similar results (Fig. 2b).

The body weights of all groups of rats were the same before starting the treatment (Fig. 2c). But, the body weights of diabetic rats (group 2) were significantly decreased than those of control rats (group 1) which is due to increased muscle wasting and due to the loss of tissue protein after STZ induction (Fig. 2c). After the treatment with AELMA, the body weights of diabetic treated rats (group 3) were significantly increased and the treatment with glibenclamide also showed similar results (Fig. 2c).

Insulin levels of diabetic rats (group 2) were found to be decreased (Fig. 2d) than those of control rats (group 1), but after the treatment with AELMA, the insulin levels of diabetic treated rats (group 3) were significantly increased and treatment with glibenclamide also showed similar results (Fig. 2d).

Evaluation of AELMA for cholesterol, triglycerides, and HDL cholesterol

In diabetic rats (group 2), the levels of cholesterol and triglycerides were found to be increased (Fig. 3a, b) whereas the levels of HDL were reduced (Fig. 3c) when compared to control rats (group 1). But after the treatment with AELMA, the levels of cholesterol and triglycerides were reduced

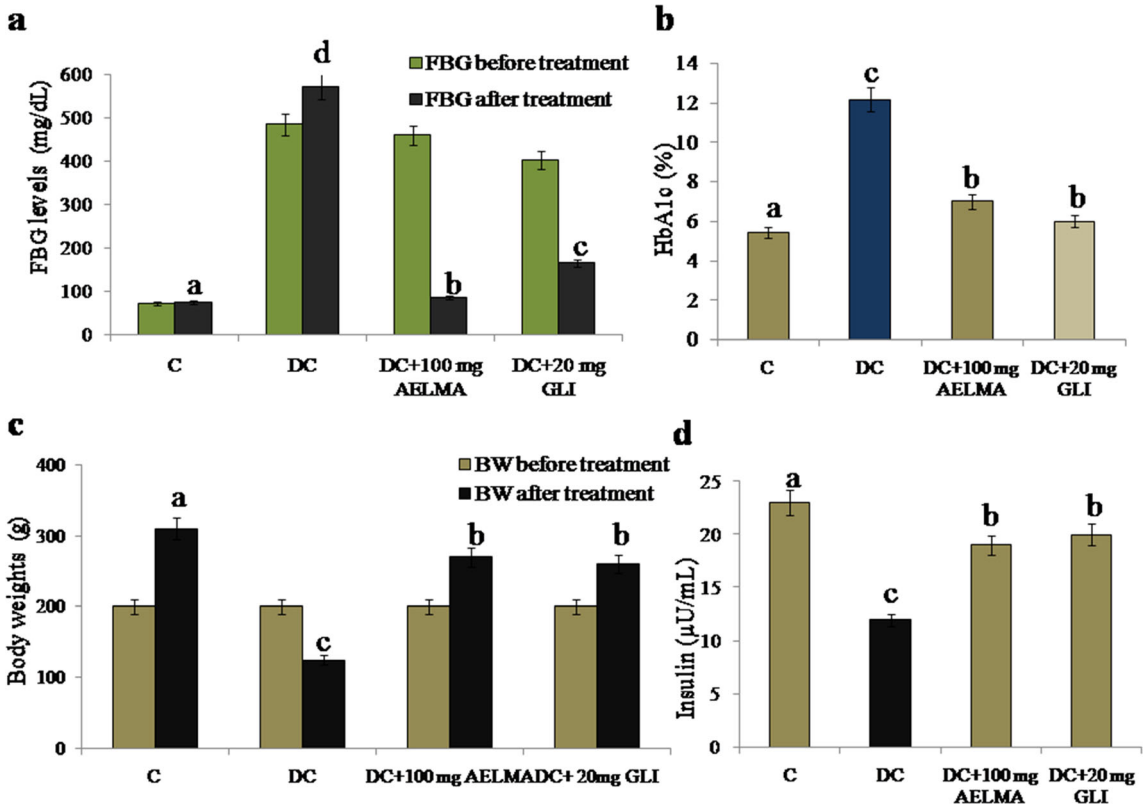

Fig. 2 Long-term treatment effect of AELMA on **a** FBG levels, **b** HbA1c, **c** body weights, and **d** insulin levels in all of the experimental groups. HbA1c glycosylated hemoglobin. Values are mean ± SEM of triplicate. Superscript letters (a, b, c, and d) indicate significant difference ($p < 0.05$)

(Fig. 3a, b), and the levels of HDL were significantly increased (Fig. 3c) and treatment with glibenclamide also showed similar results.

Evaluation of AELMA for plasma albumin, urea, and creatinine

Palma albumin levels of diabetic rats (group 2) were significantly decreased than those of control rats (group 1). But after the treatment with AELMA, the plasma albumin levels of diabetic treated rats (group 3) were significantly increased (Fig. 4a). But the levels of urea and creatinine were found to be increased in diabetic rats (group 2); however, the treatment with AELMA considerably reduced (Fig. 4b, c) and treatment with glibenclamide also showed similar results.

Evaluation of AELMA for urinary albumin, urea, and creatinine

Urinary albumin levels of diabetic rats (group 2) were significantly increased than those of control rats (Fig. 4d). But after the treatment with AELMA, the urinary albumin levels of diabetic treated rats (group 3) were significantly decreased (Fig. 4d). But the levels of urea and creatinine were found to be decreased in diabetic rats (Fig. 4e, f); however, the treatment with AELMA considerably increased (Fig. 4e, f) and treatment with glibenclamide also showed similar results.

Evaluation of AELMA for oxidative stress of the kidney

The hyperglycemia-induced oxidative degradation of lipids in kidney homogenates was assessed by evaluating the concentration of thiobarbituric acid reactive substances (TBARS) which expressed regarding malondialdehyde (MDA) content. The concentration of MDA level in the kidney was found to be increased in the diabetic rats in comparison to control rats (Fig. 5a), but in diabetic treated rats, TBARS formation was significantly influenced by AELMA and the concentration of MDA level significantly decreased than in diabetic rats (Fig. 5a) and treatment with glibenclamide also showed similar results.

Evaluation of AELMA for antioxidant activities of the kidney

The ability of the AELMA to boost the capacity of antioxidant enzymes was evaluated by determining the activity of endogenous antioxidant enzymes. The activities of CAT, SOD, GPx,

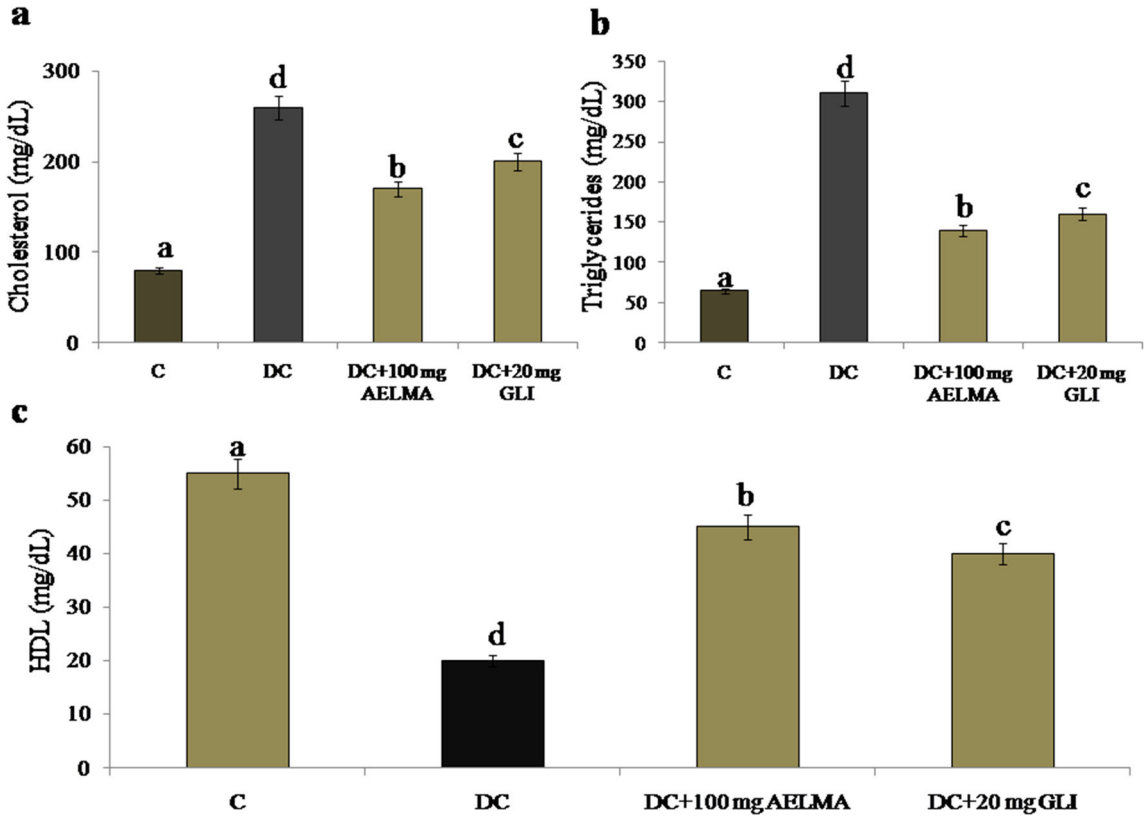

Fig. 3 Long-term treatment effect of AELMA on lipid profiles **a** cholesterol, **b** triglycerides, and **c** HDL levels in all of the experimental groups. HDL high-density lipoproteins. Values are mean ± SEM of triplicate. Superscript letters (a, b, c, and d) indicate significant difference ($p < 0.05$)

and GST were found to be decreased in the kidney of diabetic rats (Fig. 5b–e). But the treatment with AELMA significantly increased activities of the antioxidant enzymes in diabetic treated rats (Fig. 5b–e). Similar effects were noticed with glibenclamide, but they were fewer in magnitude in comparison to those of the MA.

Evaluation of AELMA for kidney histopathological studies

The kidney of control rats showed normal architecture with normal glomeruli and normal tubular epithelial cells (Fig. 6a). But the kidney of diabetic control rats showed renal hypertrophy of the glomeruli, necrotic tubular epithelial cells, and hemorrhage was seen within the Bowman's space due to glomerular damage and degeneration of glomeruli with wider Bowman's spaces and diffuse vacuolation of the tissues (Fig. 6b). Diabetic rats treated with AELMA at a dose of 100 mg/kg bw/day showed normal glomeruli, normal intertubular vessels, and tubular epithelial cells indicating regenerative changes in the kidney (Fig. 6c). This study suggests that the aqueous extract of leaves of *Mentha aquatica* could be used to ameliorate the renal structural changes caused due to STZ-induced toxicity.

Discussion

The practice of screening of medicinal plants has been performed increasingly for the last few decades with the hope of finding an effective remedy for various ailments (Atanasov et al. 2015). Most of the disorders related to the excessive oxidation of cellular substrates which cause oxidative stress include diabetes, neurodegenerative diseases, and some types of cancer. In diabetes, hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to kidney cell damage (Forbes et al. 2008). In our investigation, STZ-induced diabetic rats were treated orally with the aqueous extract of leaves of *Mentha aquatica* at the dose of 100 mg/kg bw/day for 90 days. At the end of the study, its effect on fasting blood glucose levels, insulin levels, lipid profile, kidney function tests, TBARS assay for kidney oxidative stress, antioxidant enzyme activities, and kidney histopathology was evaluated. This study was performed to find the long-term effect of AELMA on hyperglycemia and diabetic nephropathy.

This investigation confirms the antidiabetic property of the leaves of MA in STZ-induced diabetic rats. Administration of crude powder aqueous suspension of LMA showed a significant decrease in FBG levels. So, the leaves of MA were used for the preparation of different solvent extracts and prepared

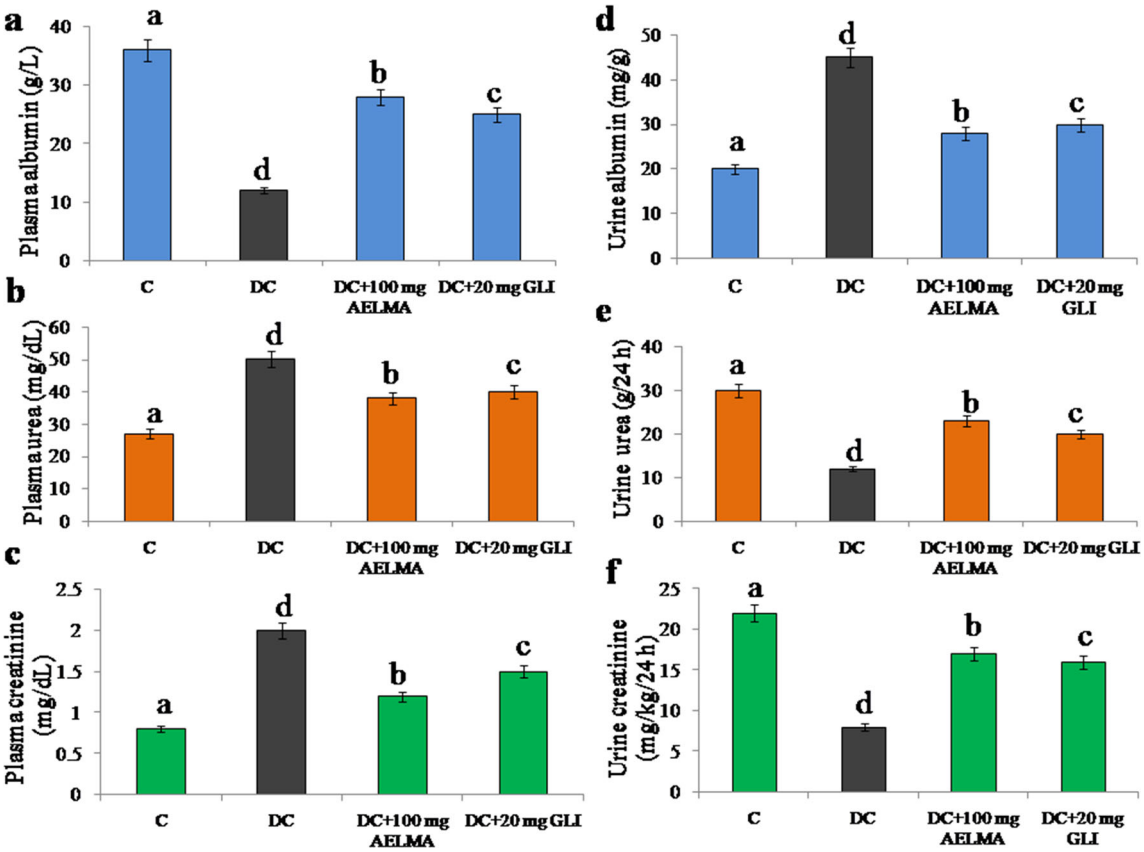

Fig. 4 Long-term treatment effect of AELMA on kidney functional markers **a** plasma albumin, **b** plasma urea, **c** plasma creatinine, **d** urine albumin, **e** urine urea, and **f** urine creatinine levels in all of the experimental groups. Values are mean ± SEM of triplicate. Superscript letters (a, b, c, and d) indicate significant difference ($p < 0.05$)

solvent extracts were used for the evaluation of antidiabetic property. Among these solvent extracts, the aqueous extract at a dosage of 100 mg/kg bw tended to bring blood glucose levels towards near normal levels. Hence, the aqueous extract may be considered to have good antidiabetic effect without causing any hypoglycemia unlike insulin and other synthetic drugs. In long-term treatment, there was a significant decrease in FBG levels and a significant increase in body weights, and insulin levels were observed in diabetic treated rats with AELMA. $HbA1_C$ levels were monitored as a reliable index of glycemic control in diabetes. Elevated HbA1C levels were observed in diabetic rats which might be due to the increased formation of glycosylated hemoglobin, but after the treatment, a significant decrease in $HbA1_C$ levels was observed.

The increase in cholesterol and triglycerides and a decrease in HDL levels were noted in diabetic rats, but after the treatment with AELMA, there was a significant decrease in cholesterol and triglyceride levels and a significant increase in HDL levels were observed. Decrease in lipid profiles and increase in HDL levels could be due to inhibition of hepatic cholesterol biosynthesis by phytoconstituents present in AELMA. The effect of AELMA on lipid profile in diabetic rats exhibited significant ameliorating effects on TC, TG, and HDL cholesterol. The decreased levels of plasma albumin and elevated levels of plasma urea and creatinine have been attributed to impair the structural integrity of the kidney, because these are cytoplasmic in location and are released into the circulation after cellular injury. In our study, we observed acute renal damage in the diabetic group. The increased levels of urinary albumin and decreased levels of urinary urea and creatinine are also specific markers for kidney damage. But, after the treatment with AELMA significantly ameliorated above parameters which were found in normal level and responsible for proper maintenance, functioning of kidney, and change in the glomerular filtration rate.

Oxidative stress is known to play an important role in the development of various complications during diabetes. Activities of antioxidative and detoxicant enzymes play important protective roles in the kidney. ROS can damage all the major cellular components which lead to a state of kidney oxidative stress (Wang et al. 2012). The antioxidant enzymes which are responsible for the protection from ROS are catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione *S*-transferase (GST) and metabolize toxic electrophiles and reduce oxidative stress (Prabhakar et al. 2013). This investigation before treatment confirms that

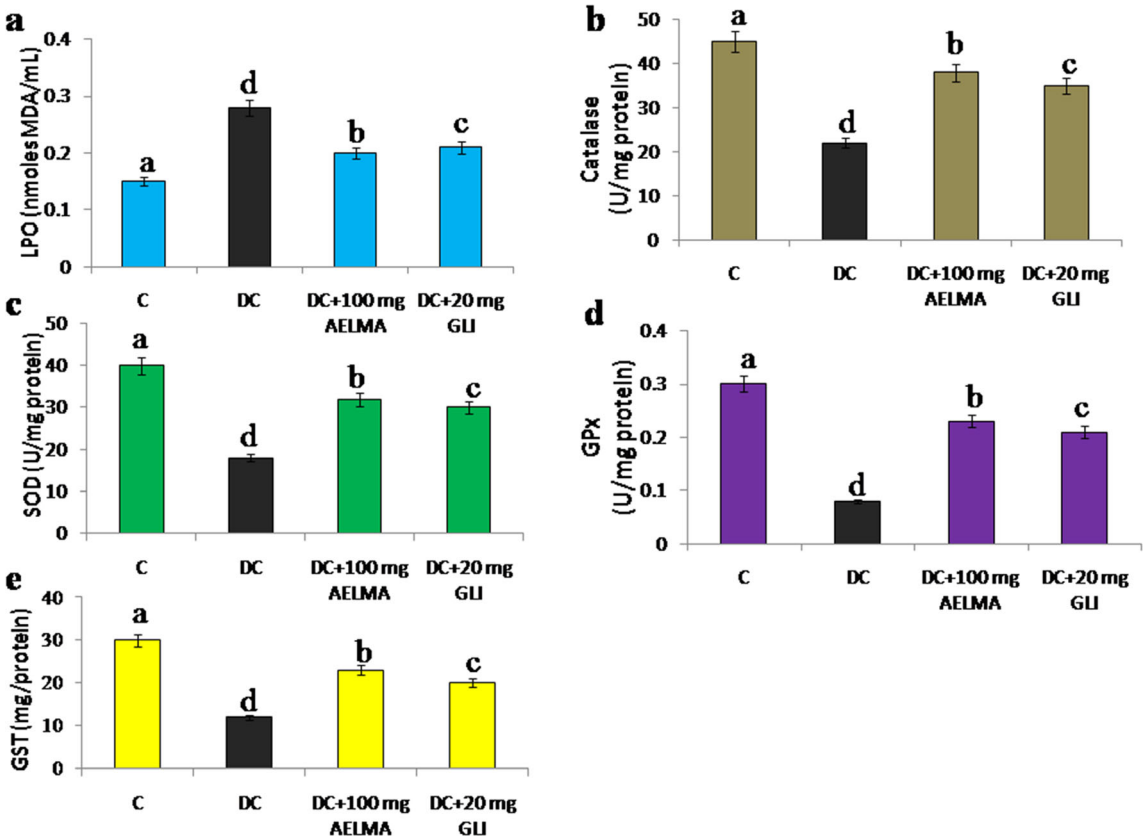

Fig. 5 Long-term treatment effect of AELMA on kidney oxidative stress and antioxidant enzymes activities in STZ-induced diabetic rats. **a** LPO, **b** catalase, **c** SOD, **d** GPx, and **e** GST activities in all of the experimental groups. LPO lipid peroxidation, SOD superoxide dismutase, GPx glutathione peroxidase, GST glutathione *S*-transferase. Values are mean ± SEM of triplicate. Superscript letters (a, b, c, and d) indicate significant difference ($p < 0.05$)

there is a reduced antioxidant enzyme protection and increased oxidative stress observed with a significant increase in kidney lipid peroxidation and was evidenced by increased MDA levels resulted from decreased activities of CAT, SOD, GPx, and GST in diabetic rats. The administration of AELMA at a dose 100 mg/kg bw/day significantly decreased oxidative stress by increasing the level of catalase, SOD, GPx, and GST in the kidney. This shows the antioxidant potential activity of AELMA.

Histopathological study of the kidney also supports that the long-term administration of AELMA at a dose of 100 mg/kg bw/day could significantly decrease the renal hypertrophy, reduces the enlargement of renal cortex and medulla, and protects the kidney from damage. This investigation suggests that

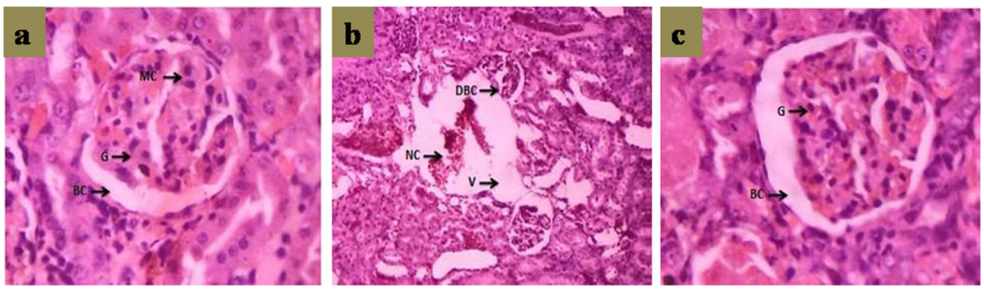

Fig. 6 Sections of the kidney with hematoxylin-eosin staining. **a** Control rats. **b** Diabetic control rats. **c** Diabetic rats treated with AELMA at a dose of 100 mg/kg. MC mesangial cells, G glomeruli, BC Bowman's capsule, DBC distractive Bowman's capsule, NC necrotic change, V vacuolization. Control rats specimen showed normal architecture of the kidney with normal glomeruli and normal tubular epithelial cells. But the diabetic control rats specimen showed atrophy of the glomeruli, necrotic tubular epithelial cells, and hemorrhage was seen within Bowman's space due to glomerular damage and degeneration of glomeruli with wider Bowman's spaces and diffuse vacuolation of the tissues. Diabetic rats treated with AELMA showed normal glomeruli, normal intertubular vessels, and tubular epithelial cells indicating regenerative changes in the kidney

AELMA could be used to ameliorate the renal structural changes caused by STZ-induced toxicity. So, this investigation confirmed that the treatment with AELMA showed significant improvement in renal functions and change in the glomerular filtration rate.

From the overall results of these biochemical and histological examinations, it could be concluded that the aqueous extract of leaves of *Mentha aquatica* showed the beneficial effects on hyperglycemia and renal functions in STZ-induced diabetic rats due to the presence of phytoconstituents like flavonoids, phenols, tannins, glycosides, saponins, steroids, and volatile oils which may be responsible for the antidiabetic and nephroprotective potential effects. The leaves of *Mentha aquatica* has a potential effect on glycemic control, dyslipidemia, and kidney antioxidant enzyme activities in STZ-induced diabetic rats by improving insulin secretion through B cell restoration capacity. *Mentha aquatica* which mostly possess kidney antioxidant properties have shown to be effective in the prevention and treatment of oxidative stress-related renal injury. The antioxidant activities of AELMA have been related to phytochemicals present in it; however, phenolic compounds, flavonoids, saponins, glycosides, steroids tannins, and volatile oils were mostly involved. The antioxidant status elaborates endogenous antioxidant capacity to protect from renal damage by a reduction of lipid peroxidation. *Mentha aquatica* ameliorates kidney dysfunction associated with diabetes due to increased urinary Na^+ outputs and reduction of plasma creatinine with a concomitant increase in glomerular filtration rate (GFR).

Conclusion

The report of this investigation concluded that the oral administration of AELMA at a dose of 100 mg/kg bw/day has antidiabetic and nephroprotective properties against oxidative stress-induced kidney damage by decreasing the lipid peroxidation and increasing endogenous antioxidants by an enhancement of the scavenging ability of the antioxidant defense system. The antidiabetic and nephroprotective potential activities of the extract may be mainly attributed to the presence of phytoconstituents such as flavonoids, phenols, tannins, glycosides, saponins, steroids, and volatile oils and their synergistic effects.

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Author contributions PN designed the study and wrote the original draft. YKP, JYE, SD, RK, and KKJ performed the experiments, are involved in data curation, and wrote the manuscript. YKP and PN reviewed and edited the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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