ORIGINAL ARTICLE

Protective role of *Excoecaria agallocha* L. against streptozotocin-induced diabetes and related complications

Ganesh N. Sharma[1], G. Kiran[2], A. M. S. Sudhakar Babu[3], Birendra Shrivastava[4]

[1]Department of Pharmacology, Jaipur National University, Jaipur, Rajasthan, India, [2]Department of Pharmacology, MRM College of Pharmacy, Hyderabad, Telangana, India, [3]Department of Pharmaceutics, A.M Reddy Memorial College of Pharmacy, Narasaraopet, Andhra Pradesh, India, [4]Department of Pharmaceutics, Jaipur National University, Jaipur, Rajasthan, India

Abstract

Aim: The present study was designed to investigate the effect of different extracts of *Excoecaria agallocha* L. in the treatment of diabetes and its complications. Diabetes was induced by intraperitoneal administration of streptozotocin (65 mg/kg) for the development of diabetic nephropathy and neuropathy. Diabetic nephropathy was evaluated by assessing levels of fasting blood glucose, glycated hemoglobin (Hb A1C), serum insulin level, albuminuria, serum urea, uric acid, creatinine, and blood urea nitrogen along with altered lipid profile (total cholesterol, triglycerides, low-density lipoprotein [LDL], very LDL, and high-density lipoprotein-cholesterol). **Materials and Methods:** Oral treatment of diabetic rats with extracts of *E. agallocha* L. improved renal dysfunction indicated by a significant decrease in urinary albumin; serum creatinine, urea, and uric acid. Moreover, a significant increase in body weight along with a significant decrease in fasting blood glucose and Hb A1C in treated groups was observed. Hyperglycemia-induced oxidative stress in diabetic control rats indicated by a significant decrease in superoxide dismutase and reduced glutathione level with a significant increase in malondialdehyde (thiobarbituric acid reactive substances) levels was also ameliorated by treatment with extracts. **Results and Discussion:** Development of neuropathy was evident from marked hyperalgesia (thermal as well as mechanical) and tactile allodynia along with reduced Motor nervous conduction velocity (MNCV). An elevated level of nitrite, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and interleukin-1β (IL-1β) were also assessed in the sciatic nerve of diabetic neuropathy animals. Treatment with different extracts of *E. agallocha* L. significantly attenuated elevated thermal and mechanical hyperalgesia. Elevated tactile allodynia assessed using flexible Von Frey filaments was also reversed by extracts. Treatment with both extracts significantly attenuated the parameters of oxidative stress in sciatic nerve of diabetic neuropathy rats. Furthermore, level of nitrite, TNF-α, TGF-β, and IL-1β significantly increased in the sciatic nerve of diabetic neuropathy animals that were ameliorated by treatment with *E. agallocha* L. extracts. **Conclusion:** Histopathological changes in kidney, pancreas, liver, and sciatic nerve of diabetic rats were also reversed by the treatment. These findings suggested that treatment with *E. agallocha* L. ameliorated diabetes and its complications (diabetic nephropathy and diabetic neuropathic pain).

Key words: *Excoecaria agallocha,* high-density lipoprotein-cholesterol, low-density lipoprotein, total cholesterol, triglycerides, very low-density lipoprotein

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence, blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes,

Address for correspondence:
Ganesh N. Sharma, Department of Pharmacology, Jaipur National University, Jaipur, Rajasthan, India. Phone: +91-9966949285.
E-mail: kirn1987@gmail.com

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nerves, heart, and blood vessels. Complications in some of these organs can lead to death.[1]

Diabetes mellitus is a constitutional disease. It is also known as "Disease of Civilization" (Urbanization) seen more in cities than villages. However, it is now making inroads into Indian villages. Diabetes is an important human ailment afflicting many from various walks of life in different countries. It is an outcome of sedentary lifestyle and incorrect food habits. Number of people afflicted by diabetes mellitus is increasing each day. About 9–12% population of the world has either established diabetes mellitus or tendency of contracting it in the near future. 18 Millions of Indians are suffering from diabetes mellitus. Although it is rampant today, it is not a new disease. It is well-known from historic times. Well-known ayurvedic physicians Maharshi Charaka (600BC) and Sushrutha (400BC) correctly described almost all the symptoms of this disease and called it as "Madhu Meha" (a shower of honey) and explained in ayurvedic literature called "Sushrutha Samhitha."

After the discovery of insulin, people had started believing that diabetes mellitus will soon be banished from the earth. However, this belief has turned out to be a dream and proved fallacious. With vigorous treatment, short-term complications of diabetes mellitus can be checked; but its long-term complications can be hardly be prevented.

Suspicion of Diabetes[2]

Suspicion of diabetes under the following circumstances:
- Sudden weight gain after the age of 45 years
- Coronary heart disease
- Cerebral hemorrhage.

Types

Types of diabetes mellitus

The WHO classification of diabetes introduced in 1980 and revised in 1985 was based on clinical characteristics. The two most common types of diabetes were insulin-dependent diabetes mellitus (IDDM) or (type I) and non-IDDM or (type II). The WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes. Malnutrition-related diabetes was omitted from the new classification because its etiology is uncertain, and it is unclear whether it is a separate type of diabetes.

Type I diabetes mellitus

It is a result of cellular mediated autoimmune destruction of the insulin-secreting β-cells of the pancreas, which results in an absolute deficiency of insulin for the body. Patients are more prone to ketoacidosis. It occurs in children and young, usually before 40 years of age, although disease onset can occur at any age. The patient with type I diabetes must rely on insulin medication for survival. It may account for 5–10% of all diagnosed cases of diabetes. Autoimmune, genetic, and environmental factors are the major risk factors for type I diabetes.

Diabetic ketoacidosis is caused by reduced insulin levels, decreased glucose use, and increased gluconeogenesis from elevated counter-regulatory hormones, including catecholamines, glucagon, and cortisol. Primarily, it affects patients with type I diabetes but also may occur in patients with type II diabetes. Patients with diabetic ketoacidosis usually present with polyuria, polydipsia, polyphagia, and weakness.

Type II diabetes mellitus

Two key features in the pathogenesis of type II diabetes mellitus are a decreased ability of insulin to stimulate glucose uptake in peripheral tissues, insulin resistance, and the inability of the pancreatic β-cell to secrete insulin adequately, β-cell failure. The major sites of insulin resistance in type II diabetes are the liver, skeletal muscle, and adipose tissue.

Both defects, insulin resistance and β-cell failure, are caused by a combination of genetic and environmental factors. Environmental factors such as lifestyle habits (i.e., physical inactivity and poor dietary intake), obesity, and toxins may act as initiating factors or progression factors for type II diabetes. The genetic factors are still poorly understood.

Type II diabetes is increasingly being diagnosed at any age nowadays, and it accounts for 90–95% of all diagnosed cases of diabetes. It is associated with old age, obesity, family history of diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity.

Gestational diabetes mellitus[3]

Gestational diabetes, blood glucose elevation during pregnancy, is a significant disorder of carbohydrate metabolism due to hormonal change during pregnancy, which can lead to elevated blood glucose in genetically predisposed individuals. It is more common among obese women and women with a family history of diabetes. It usually resolves once the baby is born, however, after pregnancy, 5–10% of women with gestational diabetes are found to have type II diabetes and 20–50% of women have a chance of developing diabetes in the next 5–10 years.

Other forms of diabetes mellitus include:
- Congenital diabetes: Which is due to genetic defects of insulin secretion
- Cystic fibrosis: Related diabetes, steroid diabetes induced by high doses of glucocorticoids
- Monogenic diabetes.

Pre-diabetes is a common condition related to diabetes. In people with pre-diabetes, the blood sugar level is higher than normal but not high enough to be considered diabetic.
- Pre-diabetes can typically be reversed without insulin or medication by losing a modest amount of weight and

increasing your physical activity. This weight loss can prevent, or at least delay, the onset of type II diabetes.
- An international expert committee of the American Diabetes Association redefined the criteria for pre-diabetes, lowering the blood sugar level cutoff point for prediabetes. Approximately 20% more adults are prediabetes increases your risk of developing type II diabetes and of heart disease or stroke.
- Now believed to have this condition and may develop diabetes within 10 years if they do not exercise or maintain a healthy weight.

About 17 million Americans (6.2% of adults in North America) are believed to have diabetes. About one-third of diabetic adults do not know they have diabetes.
- About 1 million new cases occur each year, and diabetes is the direct or indirect cause of at least 200,000 deaths each year.[4]
- The incidence of diabetes is increasing rapidly. This increase is due to many factors, but the most significant is the increasing incidence of obesity and the prevalence of sedentary lifestyles.
- "Diabetes is one of the most costly of chronic diseases, accounting for $174 billion in medical care each year in the United States, with the cost of care for patients with diabetes averaging 2.3 times higher than similar patients without diabetes," said Robert Gab bay, who led the investigation. He added that the model of care could help control costs.

Diagnosis of diabetes mellitus[5]

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia and is diagnosed by demonstrating any one of the following:
- Fasting plasma glucose level ≥7.0 mmol/L (126 mg/dL)
- Plasma glucose ≥11.1 mmol/L (200 mg/dL) 2 h after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥11.1 mmol/L (200 mg/dL)
- Glycated hemoglobin ≥6.5%.

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes 2 h to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/L) are considered diagnostic for diabetes mellitus. People with fasting glucose levels from 100 to 125 mg/dL (5.6–6.9 mmol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), 2 h after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular, are a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease [Table 1].

Diabetes is first detected under various circumstances. The symptoms of acute diabetes are so dramatic that it is almost to miss the diagnosis. However, in a number of patients, diabetes develops so gradually and silently that it is revealed only by the methodical examination of urine and the blood. It is believed that undiagnosed diabetics probably outnumber known diabetics. It is precisely for this reason that all persons above 35 years of age, especially if they are obese or have a family history of diabetes, should get their urine and blood examined at regular intervals of time, to ascertain the presence or absence of diabetes. Actually, most of these tests are utterly simple and can be learned or performed by every interested.

MATERIALS AND METHODS[6]

Identification and Authentification of Plant Materials

The plant *Excoecaria agallocha* L. identified and authenticated by Dr. Madhava Chetty, Department of Botany, S.V. University, Tirupati and preserved in the herbarium for further identification.

Collection of Plant Materials

The leaves of *E. agallocha* L. collected in the month of April–May at the coastal region of Andhra Pradesh. The collected materials are washed and dried in the shade. The dried materials are powered using a mixer. The powdered drug is subjected to solvent extraction by Soxhlet apparatus.

Plant Part Morphology

Fresh leaves of *E. agallocha* were studied for morphological characteristics such as color, odor, taste, size, shape of lamina, apex, base, margin, surface, and texture.

Preparation of Petroleum, Alcoholic, and Hydroalcoholic Extracts

The collected leaves were shade dried for 7–14 days under room temperature (27–37°C) the dried leaves were coarsely powdered mechanically using commercial electrical stainless-steel blender. Plant material was then sequentially extracted with solvents of increasing polarity as petroleum ether, alcohol, and hydroalcohol (40%) using Soxhlet apparatus. The extracts were then filtered and dried under vacuum. Crude extracts were dissolved in water or solvent and use for the assessment of *in vitro* and *in vivo* assays.

Qualitative Analysis[7,8]

Preliminary phytochemical screening

Both extracts of *E. agallocha* are subjected to preliminary phytochemical screening for the detection of various phytoconstituents present in the plant such as alkaloids, glycosides, flavonoids, proteins, amino acids, carbohydrates, and tannins.

In vitro Antioxidant Assay

Estimation of total phenolic content[9]

According to the Folin–Ciocalteu method, the total phenolic (soluble) content was estimated using the Folin–Ciocalteu reagent. This method based on the oxidation reaction. Gallic acid was used as a standard reagent in this procedure Liu *et al*. (2013).[246] Extract solution (1.0 g/ml) was taken in the flask, and then dilution of the extract was made up to 46 ml with distilled water. After dilution, Folin–Ciocalteu reagent (1 ml) was added and mixed. After proper mixing, the solution was stand for 3 min. Further sodium carbonate was mixed into the above mixture solution and allowed to stand for 180 min by occasional shaking. Blue color developed was then noted at 760 nm. Phenolic compounds in the extract were determined as µg of gallic acid equivalent.

Total antioxidant capacity (TAOC)[10]

Different concentrations (25, 50, 100, 200, and 400 µg/ml) were prepared by dissolving the extracts in distilled water. 0.3 ml of the extract mixed with 3 ml of reagent. Reagent solution comprises mixture of various reagents such as sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). After mixing both extract and reagent solution, the mixture was incubated for 90 min (95°C) after covering the test tubes with aluminum foil. Absorbance was recorded at 695 nm after cooling the solution at room temperature, against blank. Similar procedure was carried out with ascorbic acid which is used as standard. The values of TAOC were expressed as equivalents of ascorbic acid.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity[11,12]

The activity of scavenging of DPPH free radical was evaluated for the estimation of antioxidant property of any substance.[248] DPPH solution (0.1 mM) was prepared in ethanol and 1.0 ml of this solution was added to 3.0 ml of extract solution prepared in water at different concentrations (1–5 µg/ml). The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. The result of this method was expressed as percentage DPPH scavenging effect and was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was absorbance of blank and A_t absorbance in the presence of extract.

The test was carried out in triplicate.

Assay of Hydrogen Peroxide (H_2O_2) Scavenging Activity[13,14]

The H_2O_2 scavenging activity was determined according to the method of Ruch *et al*. (1989)[249] with some modifications. The mixture containing sample (1 ml; 10–320 µg/ml), phosphate-buffered solution (PBS) (2.4 ml; 0.1 M, pH 7.4), and H_2O_2 solution (0.6 ml; 40 mM) was shaken vigorously and incubated at room temperature for 10 min. The absorbance of the reaction mixture was determined at 230 nm. Ascorbic acid was used as a positive control. The H_2O_2 scavenging activity was calculated.

Where, A_0 is the absorbance of the control (water instead of the sample), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The half-maximal inhibitory concentration value represented the concentration of the compounds that caused 50% inhibition of H_2O_2.

Reducing Power Assay[15]

According to Oyaizu (1986),[250] reducing power was evaluated as follows, 2.5 ml volume of various concentrations of extracts (10–320 µg/ml) was mixed with sodium phosphate buffer (2.5 ml, 200 mM) and potassium ferricyanide (2.5 ml, 1%) at pH 6.6. After mixing, the solution was incubated (50°C for 20 min) and then trichloroacetic acid (2.5 ml of 10% w/v) was added into the above solution. After proper stirring and mixing this solution was centrifuged (8 min at 1000 rpm) for separation of layers. After centrifugation of 8 min, the upper layer was separated. This upper layer was taken for estimation. 5 ml of the upper layer was added into deionized water (5 ml) and ferric chloride (1 ml, 0.1%). After proper mixing, absorbance was measured at 700 nm using double beam spectrophotometer. This procedure was repeated 3 times and means values ± standard deviation were calculated. Half-maximal effective concentration value was calculated from concentration-absorbance graph, and ascorbic acid was used as a standard analytical agent.

Estimation of Superoxide Radical-scavenging Activity[16]

The scavenging was estimated using nitroblue tetrazolium (NBT) as given by Sabu and Ramadas an (2002). Different concentrations of extract (10–320 µg/ml) were taken in a test tube. 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT, and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube, and immediately absorbance of the mixture was measured at 560 nm.

Table 1: 2006 WHO diabetes criteria

Condition	2-h glucose, mmol/l (mg/dl)	Fasting glucose, mmol/l (mg/dl)
Normal	<7.8 (<140)	<6.1 (<110)
Impaired fasting glycemia	<7.8 (<140)	≥6.1 (≥110) and <7.0 (<126)
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)

Then, 0.4 ml of 1 mM of hydroxylamine hydrochloride was added to initiate the reaction. Finally, the reaction mixture was incubated at 25°C for 15 min and the reduction of NBT was measured at 560 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. Ascorbic acid was used as the reference compound.

Determination of Nitric Oxide Radical-scavenging Activity[17]

5 mM of sodium nitroprusside in PBS was mixed with 3.0 ml of different concentrations (10–320 µg/ml) of the extracts. This reaction mixture was incubated for 2.5 h (25°C). Further mixture of sulfanilamide (1%), H_3PO_4 (2%), and naphthyl ethylenediamine dihydrochloride (0.1%) (Griess reagent) was added to the above reaction mixture. Diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine generates a chromophore. The absorbance of the chromophore formed was noted at 546 nm.[252] Ascorbic acid was used as the reference compound. Percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_o was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the test (extract).

Extraction

Size reduced powder of leaves of *E. agallocha* were extracted successively by Soxhlet extraction technique with solvents down the eluotropic series, namely, petroleum ether (60–80°C), and alcohol and hydroalcohol (40%). The extractive yield of plants from the respective solvents is mentioned in Table 2.

Qualitative Phytochemical Screening[18]

The qualitative analysis of extracts from leaves of *E. agallocha* showed the presence of various phytochemical constituents [Table 3].

Physical Parameters[19]

The physical parameters of extracts from leaves of *E. agallocha* are shown in Table 4.

Quantitative Phytochemical Analysis[20]

The quantitative phytochemical of extracts from leaves of *E. agallocha* is shown in Table 5.

RESULTS AND DISCUSSION

Effect of AEEA and HEAA on Body Weight (BW) (g) in Diabetic Nephropathy Wistar Rats

In Figure 1, it is shown that the administration of AEEA (200 and 400 mg/kg) produced a significant increase in reduced BW by 12.72 and 16.82%, respectively, in comparison to DN control group by the end of the study. Different doses of HEAA (200 and 400 mg/kg) also produced a significant elevation in reduced BW 15.88 and 21.96%, respectively, in comparison to DN control rats. Glimepiride at a dose of 10 mg/kg significantly increased BW by 16.43%

Effect of AEEA and HEAA on Food Intake in Diabetic Nephropathy Wistar Rats

AEEA and HEAA ameliorated food and water intake in a dose-dependent manner. AEAA (200 and 400 mg/kg) reduced food intake to 41.26 ± 0.88 and 30.69 ± 0.31 g/rat/day, respectively, in comparison to DN control rats (66.73 ± 1.01 g/rat/day), whereas HEAA at 200 and 400 mg/kg reduced food intake to 30.79 ± 0.54 and 27.69 ± 0.17 g/rat/day, respectively, by the end of study is shown in Figure 2.

Effect of AEEA and HEAA on Water Intake in Diabetic Nephropathy Wistar Rats

In Figure 3, it is shown that the AEAA (200 and 400 mg/kg) reduced water intake to 117.30 ± 0.63 and 110.85 ± 0.81 ml/rat/day respectively, whereas HEAA at 200 and 400 mg/kg reduced water intake to 102.79 ± 0.70, and 97.85 ± 0.56 ml/rat/day respectively by the end of study in comparison to DN control rats (150.65 ± 0.56 ml/rat/day; $P < 0.001$).

Effect of AEEA and HEAA on Urine Output in Diabetic-nephropathy Wistar Rats

Urine output in DN rats significantly increased in comparison to normal rats.

AEEA (200 and 400 mg/kg) reduced urine output to 74.62 ± 1.44 and 70.18 ± 1.26 ml/rat/day, respectively, whereas HEAA at 200 and 400 mg/kg reduced urine output to 60.62 ± 1.44 and 54.18 ± 1.26 ml/rat/day, respectively, by the end of study in comparison to DN control rats (103.90 ± 0.94 ml/rat/day; $P < 0.001$) is shown in Figure 4.

Table 2: Extractive yield of plants

Extract	% w/w extractive yield of *Excoecaria agallocha*
Petroleum ether	1.24
Alcohol	11.28
Hydroalcoholic (40%)	14.89

Effect of AEEA and HEAA on Kidney Index in Diabetic Nephropathy Wistar Rats

In Figure 5, it is shown that the kidney index (kidney weight/BW ratio) was increased in DN control rats (1.05 ± 0.02%) in comparison to normal control (0.49 ± 0.01%). Treatment with AEEA and HEAA at 200 and 400 mg/kg significantly reduced the kidney index to 0.67 ± 0.002 and 0.59 ± 0.004% (AEEA); 0.58 ± 0.006 and 0.51 ± 0.004% (HEAA), respectively. Glimepiride at a dose of 10 mg/kg also reduced serum urea level to 0.48 ± 0.006%.

Table 3: Qualitative analysis of leaves of *Excoecaria agallocha*

Plant extracts	Petroleum ether extract	Alcohol extract	Hydroalcoholic extract
Alkaloids			
Mayer's reagent	–	+	+
Hager's reagent	–	+	+
Wagner's reagent	+	+	+
Dragendorff's reagent	+	+	+
Phenol phenolic compounds			
$Fecl_3$	+	+	+
Lead acetate test	–	–	+
Saponin			
Frothing test	–	–	+
Carbohydrate			
Molisch test	–	+	+
Fehling's solution	–	+	+
Benedict's test	–	+	+
Barfoed's test	–	+	+
Protein and amino acids			
Millon's test	–	–	–
Biuret test	–	–	–
Ninhydrin test	–	–	–
Glycosides			
Borntrager's test	–	+	+
Legal's test	–	+	+
Flavonoids			
Lead acetate solution test	–	+	+
Shinoda test	–	+	+
Phytosterols			
Liebermann–Burchard test	–	–	+
Tri terpenoids	+	+	+
Tannins	–	+	+
Anthraquinones	–	–	+

+ : Present , – : Absent

Table 4: Physical parameters of leaves of *Excoecaria agallocha*

Physical parameter	% (with reference to air-dried drug)
Alcohol soluble extractive	6
Water-soluble extractive	8.5
Ether soluble extractive	8.3
Chloroform soluble extractive	2.5
Total ash	11
Acid insoluble ash	4
Water-soluble ash	7
Loss on drying	15

Table 5: Quantitative phytochemical analysis of leaves of *Excoecaria agallocha*

Phytochemicals	Leaves (W/w)
Alkaloids	14.71
Flavonoids	11.29
Phenol	21.93
Carbohydrates	10.93

Effect of AEEA and HEAA on Serum Very Low-density Lipoprotein (VLDL) Level in Diabetic Nephropathy Wistar Rats

In Figure 6, it is shown that the level of serum VLDL decreased significantly, i.e., 27.43 ± 0.25 and 23.16 ± 0.55 mg/dL, respectively, in the DN rats treated with 200 and 400 mg/kg of AEEA, whereas HEAA (200 and 400 mg/kg) decreased VLDL level to 23.06 ± 0.52 and 22.1 ± 0.27 mg/dL, respectively, in comparison to DN control groups. Glimepiride at a dose of 10 mg/kg has substantially reduced the serum VLDL level, i.e., 20.76 ± 0.36 mg/dL.

Effect of AEEA and HEAA on Glutathione (GSH) in Diabetic Nephropathy Wistar Rats

AEAA increased the level of GSH in kidney, liver, and pancreas in a dose-dependent manner to 40.90 ± 0.81, 38.53 ± 0.31, and 41.72 ± 0.48 µM/mg protein, respectively, at 200 mg/kg; 50.08 ± 0.64, 47.53 ± 0.47, and 52.14 ± 0.63 µM/mg protein at 400 mg/kg, respectively. HEAA increased the level of GSH in kidney, liver, and pancreas to 59.78 ± 0.68, 57.03 ± 0.52, and 59.05 ± 0.32 µM/mg protein, respectively, at 200 mg/kg; 62.58 ± 1.02, 60.19 ± 0.54, and 64.81 ± 0.66 µM/mg protein, respectively, at 400 mg/kg in comparison to DN control group (35.26 ± 0.44, 37.36 ± 0.34, and 40.40 ± 0.44 µM/mg protein, respectively). Glimepiride increased the level of GSH in kidney, liver, and pancreas to 60.50 ± 0.48, 56.59 ± 0.55, and 62.77 ± 0.61 µM/mg protein, respectively, it is shown in Figure 7.

Effect of AEEA and HEAA on Superoxide Dismutase (SOD) in Diabetic Nephropathy Wistar Rats

In Figure 8, it is shown that the treatment with AEEA and HEAA elevated the level of SOD in kidney, liver, and pancreas of DN rats in a dose-dependent manner. AEEA increased SOD level in kidney, liver, and pancreas to 3.2 ± 0.020, 3.2 ± 0.015, and 3.1 ± 0.020 U/mg protein at 200 mg/kg; 2.00 ± 0.017, 2.1 ± 0.053, and 2.1 ± 0.037 U/mg protein at 400 of AEEA, whereas HEAA increased SOD level in kidney, liver, and pancreas to 2.50 ± 0.004, 2.5 ± 0.013, and 3.0 ± 0.019 U/mg protein at 200 mg/kg; 3.7 ± 0.054, 3.7 ± 0.054, and 3.8 ± 0.021 U/mg protein at 400 mg/kg.

Effect of AEEA and HEAA on Thiobarbituric Acid Reactive Substances (TBARS) in Diabetic Nephropathy Wistar Rats

In Figure 9, it is shown that the administration of AEAA and HEAA significantly reduced the level of TBARS. AEAA decreased the level of TBARS in kidney, liver, and pancreas in a dose-dependent manner to 1.6 ± 0.037, 1.6 ± 0.017, and 1.6 ± 0.012 nmol/mg protein, respectively, at 200 mg/kg; 1.7 ± 0.028, 1.4 ± 0.018, and 1.4 ± 0.031 nmol/mg protein at 400 mg/kg, respectively. HEAA decreased the level of TBARS in kidney, liver, and pancreas to 1.8 ± 0.033, 1.6 ± 0.023, and 1.5 ± 0.033 nmol/mg protein, respectively, at 200 mg/kg; 1.6 ± 0.012, 1.5 ± 0.017, and 1.3 ± 0.027 nmol/mg protein, respectively, at 400 mg/kg.

Effect of AEEA and HEAA on AGEs in Diabetic Nephropathy Wistar Rats

In Figure 10, it is shown that the induction of DN in rats led to a substantial increase in level of AGEs in kidney in comparison to normal animals administration of AEEA and HEAA significantly ($P < 0.001$) ameliorated AGEs level in kidney as compared to DN control rats (4.2 ± 0.27 RFU/mg protein). AEAA at 200 and 400 mg/kg reduced AGEs to 3.1 ± 0.039 and 2.5 ± 0.076 RFU/mg protein, respectively, whereas HEAA (200 and 400 mg/kg) reduced AGEs level to 2.2 ± 0.052 and 1.8 ± 0.031 RFU/mg protein, respectively. Glimepiride also reduced AGEs level to 3.5 ± 0.05 RFU/mg protein.

Histopathology

Kidney of normal control animals showed normal renal parenchyma with renal glomeruli as a glomerulus and Bowman's capsule and surrounded by proximal and distal tubules. Kidney of DN rats showed mesangial expansion and thickening of glomerular capillaries. Glomeruli infiltrated by inflammation cells along with infiltration seen in cortex and medulla area. Atrophy of glomeruli was seen in streptozotocin-induced diabetic rats. In glimepiride treatment group, the necrotic condition was reduced in convoluted

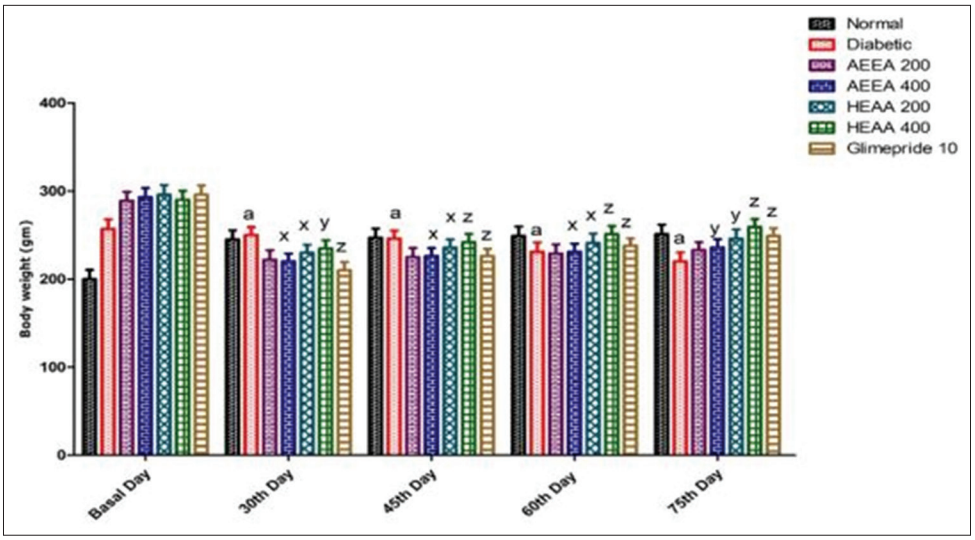

Figure 1: Effect of AEEA and HEAA on body weight (g) in diabetic nephropathy Wistar rats

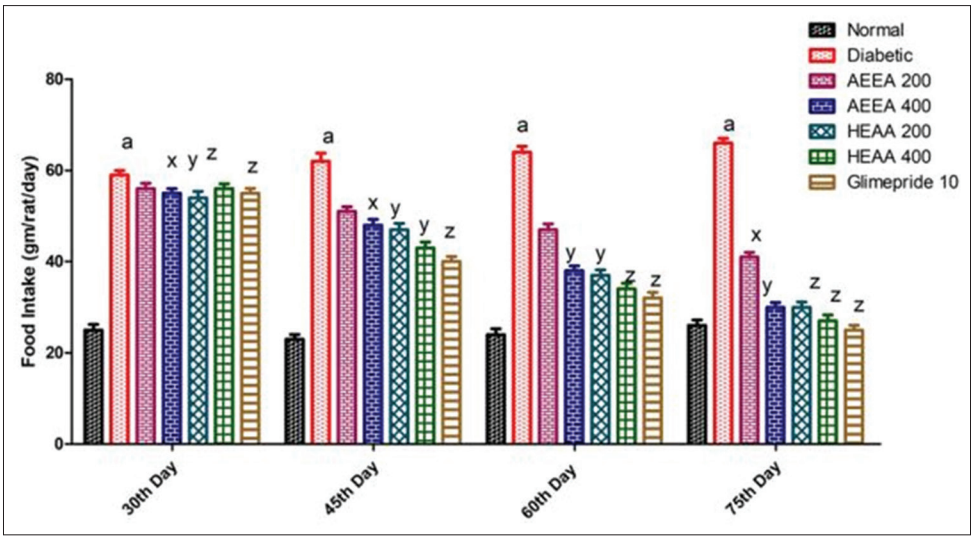

Figure 2: Effect of AEEA and HEAA on food intake in diabetic nephropathy Wistar rats

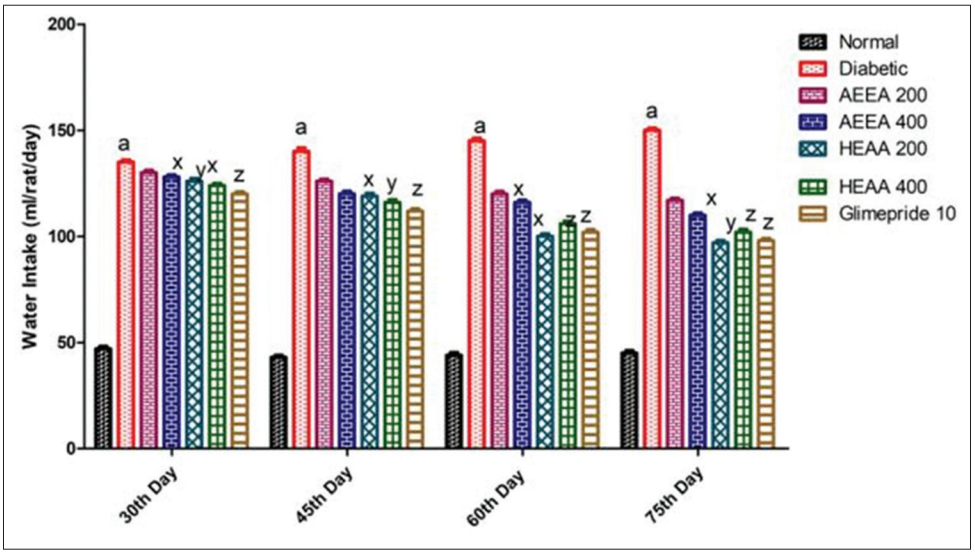

Figure 3: Effect of AEEA and HEAA on water intake in diabetic nephropathy Wistar rats

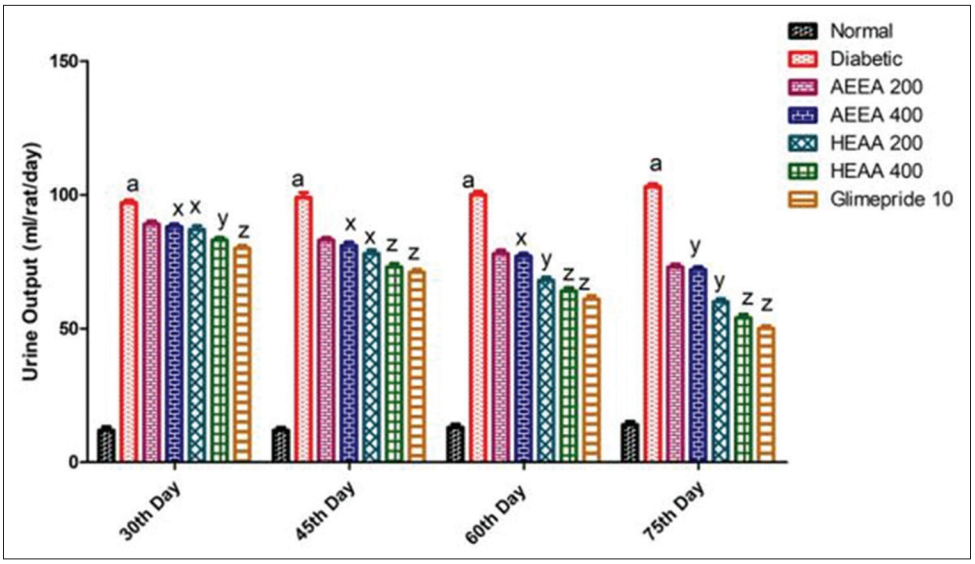

Figure 4: Effect of AEEA and HEAA on urine output in diabetic nephropathy Wistar rats

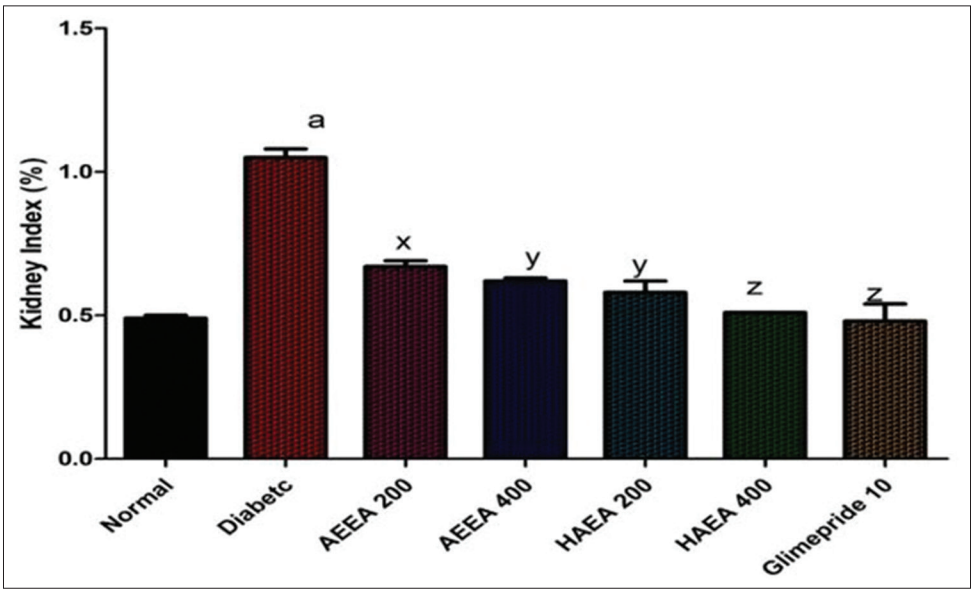

Figure 5: Effect of AEEA and HEAA on kidney index in diabetic nephropathy Wistar rats

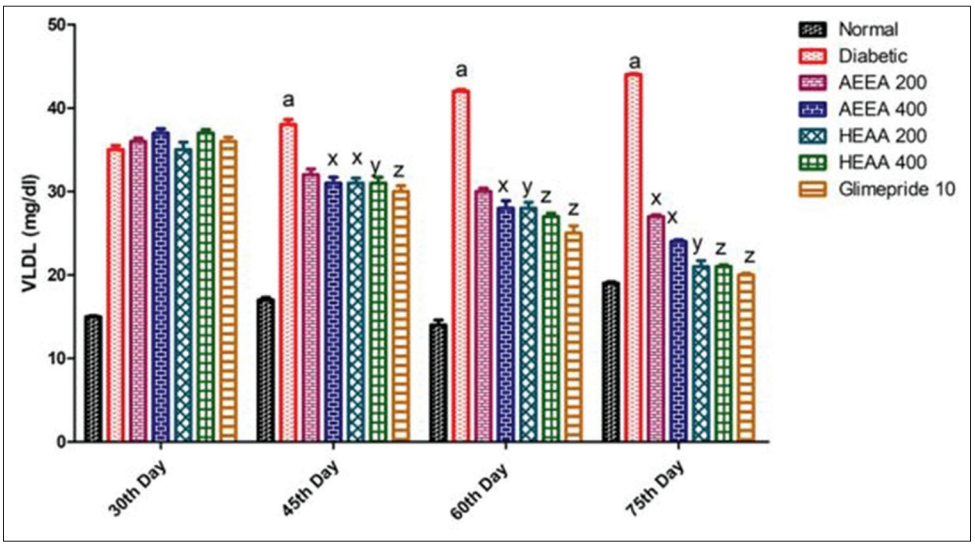

Figure 6: Effect of AEEA and HEAA on serum very low-density lipoprotein level in diabetic nephropathy Wistar rats

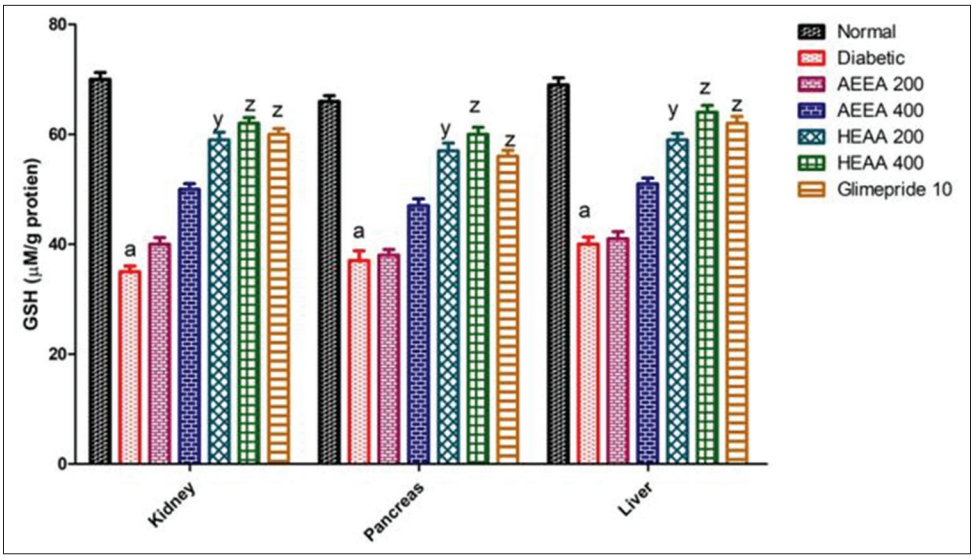

Figure 7: Effect of AEEA and HEAA on glutathione in diabetic nephropathy Wistar rats

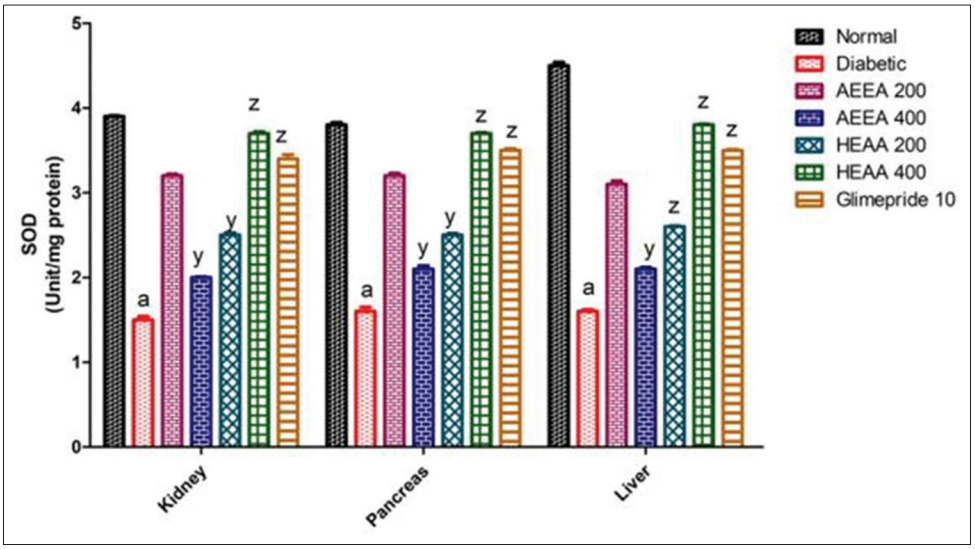

Figure 8: Effect of AEEA and HEAA on superoxide dismutase in diabetic nephropathy Wistar rats

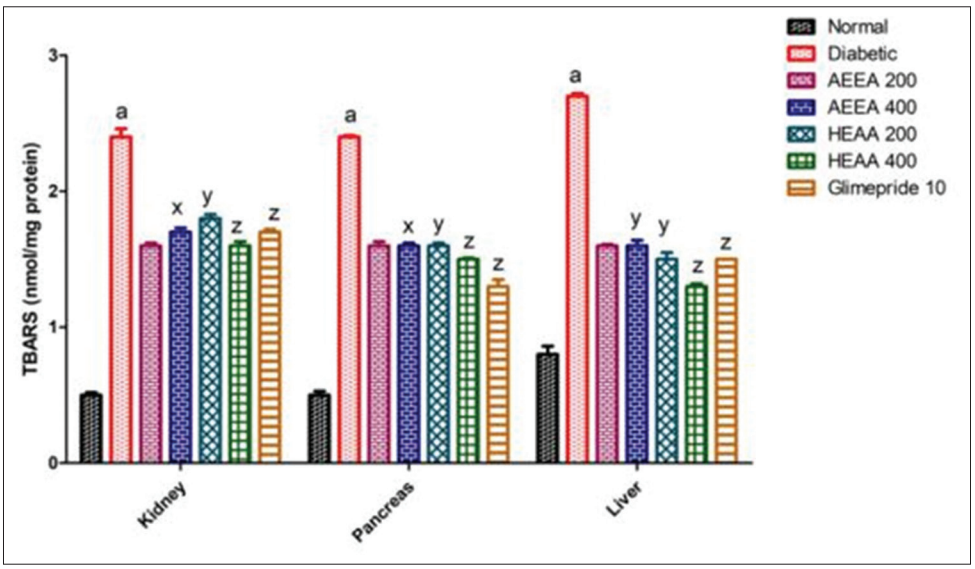

Figure 9: Effect of AEEA and HEAA on thiobarbituric acid reactive substances in diabetic-nephropathy Wistar rats

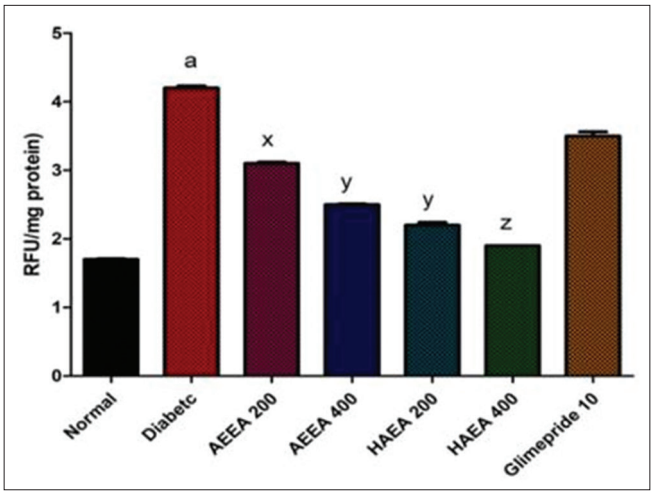

Figure 10: Effect of AEEA and HEAA on AGEs in diabetic nephropathy Wistar rats

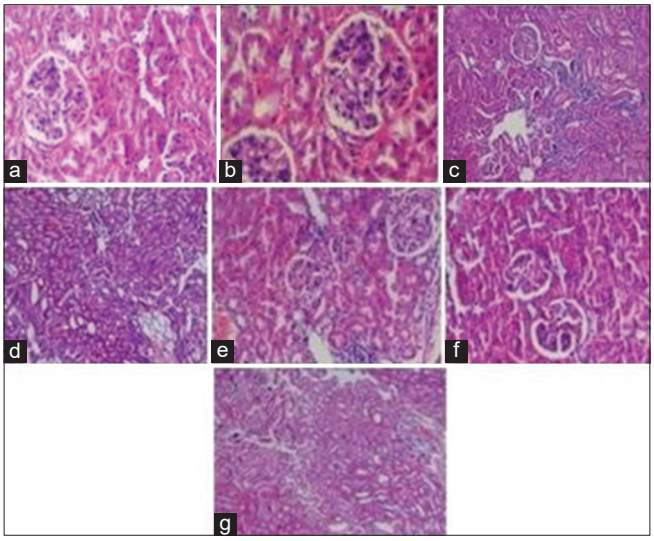

Figure 11: Histopathological changes in kidney of normal and treated rats (H&E ×10). (a) Normal, (b) diabetic nephropathy control, (c) glimepiride, 10 mg/kg, (d) AEEA 200 mg/kg treated, (e) AEEA 400 mg/kg, (f) HEAA 200 mg/kg treated, (g), HEAA 400 mg/kg treated

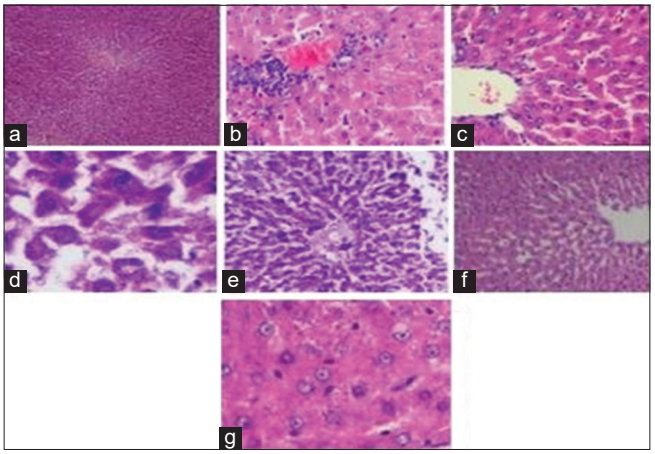

Figure 12: Histopathological changes in liver of normal and treated rats (H&E ×10). (a) Normal, (b) diabetic nephropathy control, (c) glimepiride, 10 mg/kg, (d) AEEA 200 mg/kg treated, (e) AEEA 400 mg/kg, (f) HEAA 200 mg/kg treated, (g) HEAA 400 mg/kg treated

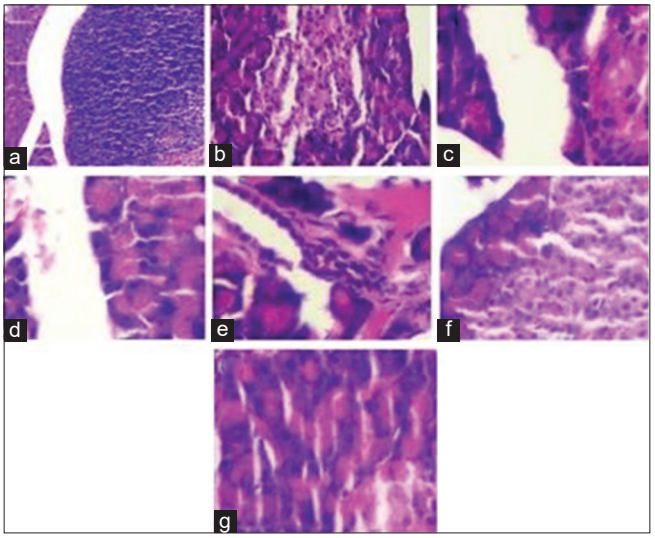

Figure 13: Histopathological changes in the pancreatic islet of normal and treated rats (H&E ×10). (a) Normal, (b) diabetic nephropathy control, (c) glimepiride, 10 mg/kg, (d) AEEA 200 mg/kg treated, (e) AEEA 400 mg/kg, (f) HEAA 200 mg/kg treated, (g) HEAA 400 mg/kg treated

tubules with reduced infiltration of inflammation cells in cortex and medulla. Administration of *E. agallocha* extracts (AEEA and HEAA) and glimepiride showed protection, namely, reduced mesangial expansion, membrane thickness, and atrophy, as shown in Figure 11.

In Figure 12, it is shown that the liver of normal rats, normal central vein with radiating sinusoid cords were present. There was no sinusoid congestion; swelling and necrotic cells. DN rats demonstrated perivenular inflammatory collection and hyperplasia of Kupffer cell with condensed nuclei and fatty infiltration. These pathological changes were reversed by *E. agallocha* extracts (AEEA and HEAA) administered DN rats.

In Figure 13, it is shown that the pancreatic cells of the normal control group showed normal architecture with normal acini and islets cells with no signs of edema and inflammation. In DN rats, inflammation, and disorganization of the islets and steatosis were observed. Cell infiltration was seen in the acinar cells along with necrosis and shrinkage of islet cells. Treatment with *E. agallocha* extracts (AEEA and HEAA) showed a protective effect on islets of Langerhans and acinar cells as compared to diabetic rats and further reduction in edema, inflammation, and shrinkage of islets.

CONCLUSION

The results obtained in the present study suggested that *E. agallocha* L. has higher amount of phenols, which are known

to scavenge free radicals. These plant extracts were also found to be effective in scavenging of DPPH, H_2O_2, NO, and SOD. Further, the study reflects the ability of these plants to inhibit the formation of AGEs and sorbitol accumulation and inhibition of enzyme ALR which has been recognized as an important strategy in the attenuation of long-term diabetic complications. Findings in the present study suggest that supplementation with *E. agallocha* L. might be beneficial in diabetic nephropathy since they exhibited beneficial effects on the blood glucose level, renal parameters, namely, urinary albumin excretion, serum urea, uric acid, creatinine, blood urea nitrogen, kidney index, and lipid parameters, namely, total cholesterol, triglycerides, LDL, VLDL, and HDL. Furthermore, these plant extracts inhibited the formation of AGE's in kidney, increased the level of antioxidant enzymes such as SOD, GSH and decreased the level of TBARS (marker of Lipid peroxidation). Moreover, supplementation with *E. agallocha* L. reversed the pain response assessed by thermal (hot plate and tail immersion assay) and mechanical hyperalgesia (Randall Selitto analgesiometer and Von Frey filament) and improvement of MNCV in diabetic neuropathy rats. Thus, these plants modulated diabetic neuropathic pain through reducing the formation of AGEs and amelioration of oxidative/nitrosative stress in peripheral nerves. Finally, it can be concluded that *E. agallocha* L. ameliorated diabetes and its complications (diabetic nephropathy and diabetic neuropathic pain).

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