Pharmacological evaluation of antidiabetic and antihyperlipidemic activity of Chenopodium album root extract in male Wistar albino rat models

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Abstract

Aim: The roots of Chenopodium album Linn (C. album) are traditionally used for the treatment of diabetes. In the present communication, we investigated the antidiabetic effect of methanolic extract of C. album roots in male Wistar albino rat models. Materials and Methods: Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (60 mg/kg body weight). Blood glucose and plasma insulin levels were measured at the end of the study. Glucometer and enzyme-linked immunosorbent assay method were used for the determination of blood glucose and plasma insulin levels, respectively. At the end of treatments, various biochemical parameters such as triglyceride, cholesterol, insulin, low-density lipoproteins (LDL), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) were examined. The liver and pancreas were isolated for histopathology examinations. Body weight measurements were done on a weekly basis to determine the effect of treatments. Results and Discussions: The phytochemical screening of C. album methanolic extract indicated the presence of proteins, alkaloids, saponin glycosides, amino acids and flavonoids. C. album extract showed a significant decline in fasting blood glucose level. Further, a high dose (HD) of C. album extract significantly normalized insulin level. The results revealed that the methanolic extract of C. album roots was effective in normalizing plasma lipid status and decreased cholesterol, triglyceride, and LDL levels. The results revealed that HD of C. album extract had a positive effect on body weight of treated rats. A nonsignificant increase in SGPT and SGOT enzyme activity was observed in STZ treated animals when compared to the control animals. Treatment with glibenclamide (10 mg/kg) and all the doses of C. album extract showed a decreased pattern of these liver enzymatic activities as compared to the diabetes group. Conclusion: The results suggest that the methanolic extract of C. album roots is effective in the prevention of experimentally induced diabetes substantiate its traditional claim.

Key words: Antidiabetic, Chenopodium album, histopathology, methanolic extract, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase

INTRODUCTION

Diabetes mellitus (DM) is characterized by the deficiency in insulin production by the pancreas, which leads to prolonged hyperglycemia with disturbances in most metabolic processes in the human body.[1] DM is one of the oldest diseases known to humans.[2,3] Based on disease etiology, the term Type 1 DM (T1DM) and Type 2 DM (T2DM) are widely used to describe insulin dependent DM and non-insulin dependent DM, respectively.[1,4,4] The estimated number of worldwide diabetes cases by 2030 is approximately 434 million.[7] North America, Europe, and Japan have the highest prevalence of diabetes and India is supposed to surpass them with 79 million cases of diabetes by 2030.[9] In most cases, the onset of T2DM

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occurs after age of 50 and 60 years and develops gradually. In recent years, there has been a steady increase in the number of younger individuals, some <20 years old, with T2DM. This trend happens to be mainly related to the increasing prevalence of obesity; the most important risk factor for T2DM in children as well as adults. T2DM is known to cause hyperlipidemia through various metabolic derangements. Insulin deficiency stimulates lipolysis in adipose tissue and gives rise to hyperlipidemia and fatty liver, thus in diabetes hypercholesterolemia and hypertriglyceridemia often occurs. Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of the various lipid and lipoprotein fractions, which is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death. The current antihyperlipidemic therapy includes statins and fibrates. Statins correct the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and fibrates acts by enhancing the clearance of triglyceride-rich lipoproteins.

Traditionally, medicinal plants are being used as an alternate means of treatment without any adverse effects. Various medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesterolemic properties. Wide arrays of plant-derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic and hypolipidemic activity. The WHO has in fact recommended the use of indigenous plants as an alternative remedy especially in developing countries. Traditional medicines have advocated the use of herbs to treat diabetes and hyperlipidemia. There are about 800 plants reported to show antidiabetic potential. Chenopodium album Linn (C. album) is an annual shrub from Chenopodiaceae family widely grown in Africa, Asia, Europe, and North America. The plant is used in folk medicine in different parts of the world as diuretic, laxative, sedative, hepatoprotective, antidiabetic, and antiparasitic.

Hence, the purpose of the present study was to isolate the flavonoids and saponins from C. album (C. album) and to investigate the antidiabetic activity against diabetic rat models.

**MATERIALS AND METHODS**

**Plant Material**

The C. album plant material was collected from the local area of Sangrur, Punjab, India.

**Preparation of Methanolic Extract**

The roots were collected from mature C. album plants, thoroughly cleaned, and shade dried at 25–30°C for a week. The dried roots were subjected to a fine powder using a dry grinder. The powdered crude material (1 kg) was defatted with petroleum ether and extracted successively with methanol using Soxhlet extractor followed by cold maceration (7 days) with 50% methanol. The extracts were concentrated using rotary vacuum evaporator (RE300, Yamato, Japan) to obtain a dark brown powder extract. The flask was rotated at 125 ± 5 rpm, and the temperature was maintained at 45 ± 5°C. Powder extract was stored in a desiccator until further use. The concentrated extract was subjected to qualitative phytochemical screening.

**Preliminary Phytochemical Screening and Quantitative Estimation of Phytoconstituents**

Preliminary phytochemical screening of the extract was carried out to detect the presence of carbohydrates, alkaloids, glycosides, steroids, saponins, and tannins. The total phenolic content was determined spectrometrically and expressed as milligrams of tannic acid equivalents per gram of extract. Total flavonoid content was measured by aluminum chloride colorimetric assay and expressed as milligram of quercetin equivalent per gram of extract. Saponin content was estimated using petroleum ether.

**Test for Carbohydrates**

*Molisch’s test*

To 2–3 ml methanolic dispersion of extract, few drops of alpha-naphthol solution in alcohol were added and shaken well. Concentrated sulfuric acid was added from sides of the test tube and formation of the violet ring was observed at the junction of two liquids.

**Barfoed’s test**

Equal volume of Barfoed’s reagent and test dispersion was mixed and heated for 1–2 min in boiling water bath. Formation of red color precipitate was observed.

**Test for Alkaloids**

The aqueous dispersion of extract was evaporated, and the residue was collected. To the residue dilute hydrochloric acid was added and filtered. Filtrate was collected, and following tests were performed.

*Murexide test for purine alkaloids*

To 3–4 ml test dispersion, 3–4 drops of concentrated sulfuric acid were added; and evaporated to dryness. Residue was cooled, two drops of ammonium hydroxide were added and observed for the appearance of purple color.

**Wagner’s test**

To 2–3 ml filtrate, few drops of Wagner’s reagent were added and observed for the appearance of a reddish brown color precipitate.
Hager’s test

To 2–3 ml filtrate, few drops of Hager’s reagent were added and observed for the appearance of a yellow color precipitate.

Mayer’s test

To 2–3 ml filtrate, few drops of Mayer’s reagent were added and observed for the appearance of a precipitate.

Dragendorff’s test

To 2–3 ml of filtrate, few drops of Dragendorff’s reagents were added and observed for the appearance of orange-brown precipitate.

Test for Glycosides

Cardiac glycoside

Baljet’s test

A dispersion of the extract was observed for the appearance of yellow to orange color with sodium picrate.

Anthraxinone glycosides

Bornträger’s test

To 3 ml dispersion of extract, an equal volume of dilute hydrochloric acid was added, boiled, and filtered. To cold filtrate, an equal volume of chloroform was added and shaken well. Then, the organic layer was separated, and ammonia was added to it. The appearance of pink or red color in ammonia layer confirms the presence of glycosides.

Test for steroids

Salkowski reaction

To 2 ml of extract dispersion, chloroform (2 ml) and concentrated sulfuric acid (2 ml) were added and shaken well. The reaction mixture was observed for the separation of chloroform layer and greenish-yellow fluorescence in the acid layer.

Test for Tannins

FeCl₃ (5%) solution

To 2–3 ml of an alcoholic dispersion of extract, few drops 5% ferric chloride solution were added, and the reaction mixture was observed for the appearance of deep blue-black color.

Test for Phenolic Content

One milliliter of C. album extract (500 µg/ml) and standard gallic acid solutions (25, 50, 75, 100, and 120 µg/ml) were placed into the test tubes. Freshly prepared distilled water (5 ml) and Folin Ciocalteu’s reagent (0.5 ml) were mixed to the above mixture and shaken for 5 min. After 5 min, 20% sodium carbonate (1.5 ml) was added to each test tubes, and the final volume was made up to 10 ml with freshly prepared distilled water and mix well. The mixture was incubated for 2 h at room temperature (25°C) which resulted in an intense blue color formation. After 2 h of incubation, the absorbance was measured spectrophotometer using ultraviolet (UV) visible spectrophotometer (UV 3000°, Lab India Instruments, Mumbai, India) at 750 nm. The analysis was conducted in triplicate. Gallic acid was used as a standard.[16]

Test for Flavonoid Content

Aluminum chloride colormetric assay method was used to determine flavonoid content in the extract. One milliliter of the extract (500 µg/ml) and standard quercetin solutions (25, 50, 75, 100, and 120 µg/ml) were placed into test tubes. Freshly prepared distilled water (4 ml) and 5% sodium nitrite solution (0.3 ml) were added to each test tube and mixed for 5 min. To this mixture, 10% aluminum chloride (0.3 ml) was added followed by the addition of 1 M sodium hydroxide (2 ml). The final volume was made up to 10 ml with freshly prepared distilled water and mix well. This leads to the formation of orange yellowish color, the absorbance was measured spectrophotometer using UV visible spectrophotometer (UV 3000°, Lab India Instruments, Mumbai, India) at 510 nm. The analysis was conducted in triplicate. Quercetin was used as a standard.[16]

Test for Saponin Content

The extract (2 g) was taken in a beaker containing 50 ml of petroleum ether. The mixture was heated on water-bath to 40°C for 5 min with gentle shaking. The petroleum ether was filtered and repeated the operation twice with further 50 ml of petroleum ether. The marc obtained was extracted with 4 × 60 ml of methanol with gentle heating. The methanol layer was concentrated to approximately 25 ml on water-bath, and 150 ml of dry acetone was added to precipitate the saponins. The product was filtered and dried at 100°C for constant weight.[17]

In Vivo Study

To ascertain the in vivo efficacy of extract, subacute hypoglycemic study was carried out in male Wistar albino rats (150–170 g) with no prior drug treatment. The animals used in the present study were 8–10 weeks old. The animal experimental protocol was approved by the Institutional Animal Ethical Committee (No. JCDMCOP/IAEC/06/17/39). The animals were handled as per the guidance of the Committee for the Purpose of Control and Supervision of Experimental on animals, New Delhi, India. Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution (60 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5. The negative control rats were injected
with the same concentration of citrate buffer only. STZ injected rats were allowed to drink 5% glucose solution overnight to overcome initial drug-induced hypoglycemic mortality.

The rats were fasted for 3 h and randomly divided into six groups (n = 6) as, Group 1: Non-diabetic served as normal control received vehicle (0.1 M citrate buffer, pH 4.5, 10 ml/kg, P.O); Group 2: T1DM rats (diabetic control) received vehicle (0.1 M citrate buffer, pH 4.5, 10 ml/kg, P.O); Group 3: T1DM rats treated with pure glibenclamide (GLB) (10 mg/kg b.w., P.O.); Group 4: Diabetic rats treated with low dose (LD) of C. album methanolic extract (200 mg/Kg b.w., P.O.); Group 5: Diabetic rats treated with a mild dose of C. album methanolic extract (350 mg/Kg b.w., P.O.); and Group 6: Diabetic rats treated with high dose (HD) of C. album methanolic extract (500 mg/Kg b.w., P.O.). After randomization into various groups and before initiation of the experiment, the rats were acclimatized for 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Biochemical analysis was carried out by collecting blood samples from retro-orbital plexus.

To estimate the acute effect, blood glucose observations were carried out at 0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h, 18 h, and 24 h. Further, to investigate the chronic effect, blood glucose, and plasma insulin levels were measured at the end of the study. Glucometer (903G, Accu-Chek Active Glucometer, Roche Diabetes Care India Pvt. Ltd., Mumbai, India) and enzyme-linked immunosorbent assay (Immuno Concept India Private Limited, Delhi, India) method were used for the determination of blood glucose and plasma insulin levels, respectively. Blood samples were collected after overnight fasting of rats and estimated for fasting blood sugar levels on the 3rd day of STZ administration (for model confirmation) and at the end of study after treatment (i.e., 28th day). Body weight measurement was done on a weekly basis to determine the effect of treatments. At the end of treatment, various biochemical parameters were examined such as triglyceride, cholesterol, insulin, low-density lipoproteins (LDL) L, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT). Oral glucose tolerance test (OGTT) at the end of 28 days’ study was carried out to evaluate insulin resistance level after 6 h fasting. Zero min blood sample was taken from the rats of different groups. The animals were treated 30 min before the glucose load (2 g/kg). Six more samples were taken 30 min before glucose loading followed by 15, 30, 60, 120, and 180 min post glucose loading.

For histological examinations, animals were sacrificed by cervical dislocation. The whole liver and pancreas were removed, placed in 10% formalin solution and immediately processed by the paraffin technique. Sections (5 μm thick) were cut and stained by hematoxylin and eosin.

### Statistical Analysis

Statistical analysis was carried out using the GraphPad Prism software (GraphPad Software, Inc., CA, USA). The data were analyzed using one-way ANOVA followed by post hoc Tukey test. *P < 0.05* was considered as significant, and the tested groups were compared with the normal group. Descriptive variables are presented as a mean ± standard error of the mean.

### RESULTS AND DISCUSSION

The present study was designed to investigate the antidiabetic effects of C. album extract in STZ-induced diabetic rats. To induce T1DM, STZ (2-Deoxy-2-[(methylnitrosoamino)-carbonyl]-D-glucopyranose) (60 mg/kg, i.p.) was injected into the experimental rat models. After 72 h of STZ injection, the confirmation of DM in rats was done by the determination of elevated fasting blood glucose level. Symptoms of diabetes Type I were clearly observed within 2–4 days following single intraperitoneal injection of STZ. STZ selectively destruct the insulin-secreting pancreatic β-cells and leads to poor glucose uptake by peripheral tissues.

The yield of extract from the powdered root material was 12.02% w/w. The phytochemical screening of methanolic extract was carried out using reported phytochemical tests, which indicated the presence of proteins, alkaloids, saponin glycosides, amino acids, and flavonoids. Carbohydrates, glycosides, sterols, and tannins were absent. The total phenolic and flavonoids content of the extract were found to be 233.6 mg tannic acid equivalents/g of extract and 89.41 mg quercetin equivalents/g of extract, respectively, while the total saponins content was found to be 3.1 mg saponins/100 g of powder mass. The results of various phytochemical tests are shown as shown in Table 1.

| Table 1: Results of test for purity of C. album root methanolic extract |
|-----------------------------|-----------------------------|
| **Tests for phytocomstituents** | **Results** |
| Carbohydrate | − |
| Monosaccharides | − |
| Proteins | − |
| Amino acids | + |
| Steroids | − |
| Glycosides | − |
| Saponins | + |
| Alkaloids | − |
| Tannins | − |
| Flavonoids | + |

+: Present, −: Absent. C. album: Chenopodium album
A rapid decline in fasting blood glucose level was observed in *C. album* root extract treated diabetic rats. At the end of the 12 h experiment, a maximum percent decrease in fasting blood glucose (139.5 ± 4.8 mg/dl, *P* < 0.01) was observed in animals treated with HD of the extract when compared to the control group. In case of animals treated with a low and mild dosage of the extract, the reduction in glucose level after 12 h was slower (148.3 ± 1.5 mg/dl and 144.2 ± 4.1 mg/dl, respectively, *P* < 0.01). The results were compared with the standard drug (GLB) at the dose of 10 mg/Kg. The animals treated with GLB showed a significant reduction in blood glucose level (148.7 ± 5.9 mg/dl, *P* < 0.01) for 6 h [Figure 1]. GLB is a second generation of hypoglycemic sulfonylureas. The primary effect of this drug is inhibition of ATP-sensitive potassium channels, which leads to depolarization of the cells and insulin secretion.[20] It is hypothesized that *C. album* extract possesses similar physiological action in the pancreatic cells.

STZ induced a significant decrease in plasma insulin level as compared to healthy control animals. The treatment of diabetic rats with GLB (10 mg/kg) and HD of *C. album* extract significantly normalized insulin level. Administration of the *C. album* extract to the animals showed elevation in the circulating insulin levels, being significant at the HD as compared to diabetic control group [Figure 2]. This insulin enhancing action might be the major factor triggering decrease in the basal glucose level and hence suggesting that the antihyperglycemic effect of the extract due to its insulin-releasing stimulatory effect.

The weight of normal control animals was stable during 28 days of the study. The weight of untreated diabetic and diabetic animals treated with LD of the extract was decreased constantly throughout the 28 days’ study. This decrease in the body weight might be due to the reduced food intake during the treatment. The loss of body weight in normal control and untreated diabetic rats might be due to the increased muscle wasting[21] and catabolism of tissue proteins.[22] However, this decrease in the body weight was not significant when compared with the normal control group (*P* < 0.01). The results revealed that mild and HD of *C. album* extract had a positive effect on body weight of rats as compared to diabetic control [Figure 3]. The ability of *C. album* extract to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia, which was an indication of proper glucose utilization, its protective effect in muscle wasting and controlling protein turnover and/or improvement in DM associated disorders.

In STZ-induced diabetic animal models, increase in blood glucose levels is also accompanied by the disturbances in the lipid profile characterized by an increase in plasma cholesterol,
triglycerides, and LDL level. The marked hyperlipidemia in untreated diabetic control rats characterizes the unbalanced lipid profile in the diabetic state. However, treatment with the *C. album* extract normalized plasma lipid status, which was presumably mediated by control of lipid metabolism. STZ-induced diabetic rats showed a significant increase in plasma cholesterol and triglyceride level as compared to the healthy control rats [Figure 4]. However, GLB (10 mg/kg) treatment and HD *C. album* extract have significantly decreased cholesterol, triglyceride, and LDL level as compared to diabetic rats [Figure 4].

Liver plays a crucial role in glucose and lipid homeostasis. This key function of liver makes it vulnerable to affect in diseases associated with metabolic disorders. Several biochemical tests are useful in the evaluation of hepatic dysfunction in the diabetic state. The most common biomarkers that indicate the status of hepatic functioning include the enzymatic activities of SGPT and SGOT. Activities of SGPT and SGOT enzymes were non-significantly increased in the STZ-treated group as compared to the healthy control group. The treatment with GLB (10 mg/kg) and all the doses of *C. album* extract showed a decreased pattern of these liver enzymatic activities as compared to the diabetes group [Figure 5]. Consistent with the fact, the increase in SGOT and SGPT levels were found in the STZ-induced diabetic animals, whereas all the doses of *C. album* extract showed a decreased pattern of these liver enzymatic activities as compared to the diabetes group. Further, histopathological data confirm that T1DM leads to pathological alterations in the hepatic and pancreatic tissue while the treatment with *C. album* extract improves the histopathological alterations.

In OGTT, the animals treated with a different dose of *C. album* extract showed a significant reduction in blood glucose levels from 30 min onward (*P* < 0.001). The extract significantly reduced the blood glucose level in glucose loaded animals at 30, 60, 120, and 180 min. The effect of different dose of the extract on fasting blood glucose levels at the end of 28 days’ study in diabetic male Wistar albino rat models is presented in Figure 6.

Liver of normal rats showed a normal hepatic architecture with no sign of sinusoidal dilation and cytotoxicity. The hepatic sinusoids are clearly visible between the hepatocytes. However, liver of diabetic rats showed periportal necrosis of the hepatocytes and exhibit cytotoxicity focal necrosis of the hepatocytes pylotonic nuclei of the hepatocytes were also present. Rats treated with *C. album* extract showed, hepatic lobules appeared more or less like control. This hepatoprotective effect was comparable to that of the standard drug, GLB [Figure 7]. Histology of the normal pancreas of rats showed that the exocrine component of the pancreas consisted of closely packed acini. The interlobular duct was surrounded with supporting tissues. The endocrine tissue of the pancreas and islets of Langerhans were scattered...
In the present study, oral administration of methanolic extract of *C. album* roots to STZ induced male Wistar albino rat models for 4 weeks showed antihyperglycemic activity and normalized biochemical parameters such as lipid profile: Total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol; serum enzymes such as alanine aminotransferase and aspartate aminotransferase when compared to STZ induced diabetic control rats by the mechanism(s) which appear to be similar to that of GLB, which involve insulin sensitization effect. The results suggest that the methanolic extract of *C. album* roots is effective in the prevention of experimentally induced diabetes substantiate its traditional claim.

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