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Evaluation of hypolipidemic, antidiabetic and anti-oxidant activity of *Eulophia herbacea* tubers

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Abstract

The tuber of *Eulophia herbacea* is widely used in the treatment of obesity in traditional medicine. Methanolic extract, aqueous extract and isolated glucomannan were tested for triton WR-1339 induced hyperlipidemia and alloxan-induced diabetes in rats. Blood samples were collected and analysed for lipid profile and blood glucose. Glucomannan was isolated and characterised. Anti-oxidant activity of test sample was determined by superoxide radical and lipid peroxidation assay and compared it with standards ascorbic acid. The findings showed that folk medicinal claim of *E. herbacea* tubers possess hypolipidemic, hypoglycemic and anti-oxidant effects.

Introduction

Plant *Eulophia herbacea* belongs to family orchidaceae is commonly known as kukkadkand or kutrikand (Anonymous, 2003). The genus *Eulophia* includes 200 species of orchids among these 26 species native to India (Bhattacharjee, 2001). It is terrestrial plant found on hill slopes in the area of Himalaya, Bengal, eastern part of India, and different parts of world. Several Indian species like *E. nuda, E. orcheata, E. herbaceae* posseses different ethnomedicinal claims. Traditionally, the tubers of plant are used for many purposes like treatment of tumors of scrofulous glands of neck, aphrodisiac, appetizer and to treat cardiac problems. This plant tubers used as salep (Khare, 2007), is white coloured flour obtained by milling of dried tubers of certain wild orchids (Dogan and Kayacier, 2004) and it is well known indigenous turkish drink. The preparation of salep contains 50-60% of glucomannan which lowers the blood cholesterol and blood glucose level (Tamer et al., 2006; Chua et al., 2010). Antidiabetic activity tuber extract of *E. epidendraea* in alloxan diabetic rats has been reported (Maridass et al., 2008). Traditionally the tubers of the plant are boiled in water and the infusion is drunk throughout the day for reducing fat (Personnel communication).

Due to several limitations of currently available drugs including side effects and failure of response after prolonged use, plant based medicines are gaining prominence in treatment of metabolic diseases like diabetes (Grover et al., 2002; Mukherjee et al., 2006).

In view of the fact that there is no systematic study on the hypolipidemic and hypoglycemic effect of *E. herbacea* tubers, therefore the present study focussed to evaluate the effect of this plant in triton WR-1339 induced hyperlipidemia, alloxan-induced diabetic and antioxidant activity.

Materials and Methods

Plant material: The plant material was collected from subtropical hilly area in Toranmal region, Nandurbar

District, Maharashtra, India, in the month of July and August 2012. The plant specimen of *E. herbacea* was authenticated by Mr. T. Chakra borty (voucher no. BANDEUH1) from Botanical Survey of India (BSI), Pune.

Chemicals: Triton WR 1339 was purchased Sigma chemicals (USA) and alloxan monohydrate from Himedia, India. Glibenclamide was received from Alembic Ltd India as gift sample. Atorvastatin was purchased from Biochem pharmaceuticals industries ltd, Mumbai. The biochemical kits were from Span diagnostic Ltd, Transasia bio medicals Ltd., & Agappe diagnostic kit. The other chemicals required are purchased from Loba chemie laboratory, Mumbai.

Preparation of extract: 200 g of powdered tubers of *E. herbacea* was extracted with methanol by using soxhlet apparatus. All the extracts were filtered & concentrated under reduced pressure in rotary flash vacuum evaporator to collect methanolic extract (ME). The dried extracts are then collected and preserved in desiccators. The fresh crude drug powdered was extracted by cold maceration for seven days. Several daily shakings or stirring are given at room temperature to get aqueous extract (AQE). After seven days, extract was filtered and the marc was again kept for maceration with same solvent for complete extraction. After filtration the aqueous extract were combined and concentrated by using rotary vacuum evaporator, dried and stored in refrigerator.

Isolation of glucomannan: The young tubers of *E. herbacea* were separated, rinsed with water and cut into small pieces. The plant tissue was then heated in boiling ethanol (EtOH) for 1 hour to inactivate enzymes and subsequently extracted with water for 15 hours. The polysaccharide was precipitated by the addition of EtOH (4 vol). The yield of isolated glucomannan (IGM) was calculated. The resulting product was then tested by Molisch test as confirmatory test of polysachharides (Buchala et al., 1974). The UV absorption spectrum of IGM were recorded in distilled water in the range of 200-400 nm on Shimadzu UV 2401 spectrophotometer at 1 cm path length. CHO analysis was done on Thermo -Finnegan CHNO elemental analyzer.

Animals: Twelve weeks old male Wistar rats weighing 145-170 g were obtained from registered vendors. The animals were housed in separate cages containing four animals per cage and were maintained under control conditions of 22 ± 2°C, with 12 hours light/dark cycle. Food and water were provided *ad libitum* till the day prior to the study. The ethical clearance was obtained from the Institutional Animal Ethical Committee (Approval no. 651/02/C/CPSCEA).

Triton-induced hyperlipidemia: In this model, overnight fasted male rats were randomly divided into nine groups of six rats in each group such that the difference in the weights of rats within and between groups does not exceed ± 20% of the average weights of the rats. All the rats except normal control group were injected with triton WR-1339 (Sigma, USA) at a dose of 400 mg/kg to achieve the hyperlipidemia in animal models, while normal control group was injected with normal saline (NS) of the same volume. The triton WR-1339 was dissolved in NS to a final concentration of 4%. Twelve hours following the triton WR-1339 injecting, other groups of animals were treated with ME, AQE and IGM at the dose of 200 and 400 mg/kg body weight orally by gastric intubation. Simultaneously, the positive control group was given atorvastatin at a dose of 10 mg/kg intragastric administration. The serum samples were separated from the rat blood samples, collected from the retro-orbital plexus six days after triton WR-1339 injection (Patil et al., 2004). Later animals were sacrificed and liver was removed, cleaned and washed in ice-cold normal saline for anti-lipid peroxidation activity.

Histopathological examination: Rat liver was obtained from Triton model, and it was perfused with ice cold 0.15 M KCl solutions via portal vein. Each liver sample was then stored in the formalin solution. A small portion from liver tissue was fixed in 10% formalin in phosphate buffer (pH 7.0) for 24 hours at room temperature for histopathology. Tissues were stained with haematoxylin & eosin and observed under light microscope (Umbare et al., 2009).

Alloxan-induced diabetes: The animals were fasted for 12 hours prior to the induction of diabetes as described by Joy and Kuttan (1999) with slight modification. Alloxan freshly prepared in 0.5%Tween 80 was administered intraperitoneally (i.p.) at single dose of 150 mg/kg. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of alloxan. Rats with blood glucose level of above 200 mg/dL were considered to be diabetic and used for the studies.

Experimental design: The rats were randomized into seven groups comprising of six animals in each groups as given below. ME and AQE (200 and 400 mg/kg)/ glibenclamide (GLB) was administered orally using an intra-gastric tube once daily for 10 days (Merina et al., 2010). Group I served as normal control received an intraperitonial administration of normal saline. Group II served as diabetic control group received alloxan in the dose of 150 mg/kg by intraperitonial route. Remaining groups i.e. III, IV, V, VI and VII received ME 200, ME 400, AQE 200, AQE 400 mg/kg and glibenclamide (0.5 mg/kg) (Chika and Bella, 2010) respectively. On the 11th day blood samples were collected to estimate biochemical parameters. Serum glucose was estimated by enzymatic glucose oxidase

method. Percent reduction in serum glucose was calculated with respect to the initial level.

Biochemical estimation: Serum TC (Demacker et al., 1980), total TG **(**Foster and Dunn, 1973**)**, LDL, VLDL (Friedwald et al., 1972) and HDL (Assmann et al., 1983) were estimated using standard enzymatic kits (Span diagnostic Ltd., India; Transasia Biomedicals Ltd., India) spectrometrically. Atherogenic index was calculated by using reported formula (Maruthappan and Shree, 2010).

Atherogenic index = (Total cholesterol – HDL) / HDL

Total phenolic content: Total phenolic content of methanolic extracts of *E. herbacea* L. tubers was measured by Folin-Ciocalteau reagent method. In this, phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce complex (molybdenum blue), which was measured at 760 nm using UV spectrophotometer. Various concentrations (20-100 µg/mL) of gallic acid were prepared in triplicate. 1.5 mL of Folin-Ciocalteau reagent was added to volumetric flasks containing standard solutions and test solution. After 5 min., 4 mL of 7%sodium carbonate solution was added to volumetric flask containing standard and test solution. Final volume was made up to 10 mL by using distilled water. Blank determination was done by using methanol in place of test or standard solutions. The solutions were mixed thoroughly and absorbance was measured at 760 nm against a reagent blank after incubation for 90 min. at room temperature (Mukherjee et al., 2006; Kaur and Kapoor, 2002).

Total flavonoid content: Total flavonoids content was determined by the Aluminium chloride colorimetric assay. An aliquot (l mL) of standard solution of rutin (20, 40, 60, 80, 100 µg/mL) was added to 10 mL volumetric flask containing 4 mL distilled water into it. Then added 0.3 mL of 5% $NaNO_2$, after 5 min, 0.3 mL of 10% $AlCl_3$ was added. At sixth min, 2 mL 1M NaOH was added, and the total volume was made up to 10 mL with distilled water. Same dilutions were prepared with the test solution. Blank determination was done by using methanol in place of test or standard solutions. Mixed well and take absorbance at 520 nm against blank (Liu et al., 2005).

Antioxidant activity

Estimation of superoxide free radical scavenging activity by riboflavin-light-NBT system: 200 µL EDTA, 100 µL NBT, 50 µL riboflavin, 2.5 mL phosphate buffer pH 8.0 and 200 µL extracts dissolved in methanol were mixed in test tube. Reaction was started by illuminating the reaction mixture for 15 min. After illumination the absorbance was read at 590 nm in micro titer plate ELIZA reader (BIO-Tek Power wave™ XS, Model-96 well micro plate). Same procedure was followed for control by using methanol in place of samples. Ascorbic acid was used as positive control. IC_{50} values for each extracts and standards were calculated and expressed as µg/mL (Bafna and Mishra, 2005).

Estimation of anti-lipid peroxidation (ALP) by using liver homogenate (in vivo): Rat liver homogenate 10% was prepared according to the procedure described elsewhere (Tripathi and Sing, 2001). The mixtures containing 50 µL of homogenate (10%), 100 µL of 0.15 M KCl and 50 µL of different concentrations (20-100 µL) extracts of *E. herbacea* were prepared. Lipid peroxidation was initiated by adding 10 µL of 1M ferric chloride ($FeCl_3$). The reaction mixture was incubated for 30 min at 37°C. After incubation, the reaction was stopped by adding 200 µL of ice-cold 0.25 N HCl containing 15% trichloroacetic acid (TCA) and 0.38% thiobarbituric acid (TBA), and 20 µL of 0.05% butylated hydroxyl toluene (BHT). These reaction mixtures were heated for 60 min at 80°C, cooled and centrifuged at 5,000 rpm for 15 min. The absorbance of supernatant was measured at 532 nm using microplate reader (BIO–Tek Power wave™ XS, Model-96 well plate) against blank, which contained all reagents except liver homogenate and drug. Identical experiments were performed to determine the normal (without drug and $FeCl_3$) and induced (without drug) lipid peroxidation level in the tissue. The percentage of lipid peroxidation effect (%ALP) was calculated by following formula.

$$\%ALP = \frac{FeCl_3 \text{ O.D.} - \text{Sample O.D.}}{FeCl_3 \text{ O.D.} - \text{Normal O.D.}} \times 100$$

Stastical analysis: Data was expressed as mean ± SEM. Statistical analysis was carried out with one-way ANOVA followed by Dunnet's multiple comparison test using graph pad prism software. A level of significance of $p<0.05$ was considered as statistically significant.

Results

Preliminary phytochemical investigation on methanolic and aqueous extract of *E. herbacea* revealed the carbohydrates, proteins, amino acids, flavonoids, tannins, phenolic, mucilage, steroid and vitamins. Further HPLC analysis of the methanol extract confirmed the presence of vitamins. The yield of glucomannan was found to be 4.3% w/w and showed positive test of molisch reagent for the presence of polysaccharides. ëmax of isolated glucomannan was found to be 292 nm. It is compare with UV spectra of standard glucose and mannose. In CHO analysis carbon, hydrogen and oxygen was present in isolated glucomannan. The UV absorption spectrum of hydrolysate was found to be 292.6 nm. LC-MS analysis of hydrolysate of isolated glucomannan

was also carried out for identification of glucose and mannose; it confirmed the presence of glucomannan in *E. herbacea* tubers.

Liquid chromatography-mass spectroscopy (LC-MS) analysis of plant extracts is becoming a very valuable tool to detect the presence of certain constituents before aiming a large-scale purification and to minimize replicative phytochemical studies (Alali and El-Allali, 2005). A liquid chromatography spectrum of hydrolysate of isolated glucomannan shows two prominent peaks of glucose and mannose. The molecular ion peak shows m/z ratio 215.1 and 377.1 suggest monomer and dimer chain of glucose and mannose. MS spectra of molecular ion peak m/z 215.1 shows highest peak at 179 i.e. (glucose mol. weight. 180.2 g/mol).

In present study, hyperlipidemia was induced in the rats by injecting triton at dose of 400 mg/kg i.p. It is evident from results that the serum levels of total cholesterol (TC) 242.9 ± 10.7, triglycerides (TG) 203.6 ± 18.2, very low density lipoproteins (VLDL) 40.7 ± 3.6 and low density lipoproteins (LDL) 159.4 ± 13.5 were significantly increased ($p<0.001$) whereas serum high density lipoproteins (HDL) 42.8 ± 2.7 level was significantly reduced ($p<0.001$) in triton WR-1339 induced hyperlipidemic rats compared to normal control group. If we seen a relation between LDL, and VLDL with HDL it was exactly opposite. HDL was good lipoprotein for our body as their level decreases the chances of heart disease increases. Since HDL has been responsible for the transport of cholesterol from peripheric circulation to the liver where it is metabolized (Ricardo, 2001). It was further evident that methanol extract (ME), aqueous extract (AQE) and isolated glucomannan (400 mg/kg body weight) treated groups significantly reduced the levels total cholesterol (TC) 172.4 ± 5.3, 123.1 ± 8.6 and 168.2 ± 7.0, triglycerides (TG) 123.5 ± 6.9, 92.5 ± 7.1 and 82.7 ± 2.8, VLDL 24.7 ± 1.4, 18.5 ± 1.4 and 18.9 ± 2.7 and LDL 74.5 ± 7.4, 48.5 ± 8.6 and 92.2 ± 5.8 whereas significantly increased HDL 72.1 ± 1.4, 66.8 ± 3.7 and 59.6 ± 2.2 respectively in dose-dependent manner. These observations indicate that significant reduction in the TC, TG, LDL, VLDL and increase in HDL by ME, AQE and isolated gluco-mannan is comparable to reference compound atorvas-tatin (Table I).

In microscopical examination, physical appearance of extract group remains unaltered as compare to control group. Microscopial examination of liver section of normal group shows hepatic lobule and portal tract. Between portal tract and central vein lies the liver parenchyma radiating hepatic cells. At some places hemorrhages with inflammatory cell infiltrate seen. liver section of control group shows the same results, but there was large area of hemorrhages with inflammatory cell infiltrate and at some places necrosis is seen. Large plaques are prominently seen made up of RBCs and connective tissues. In ME and AQE 200, 400 mg/kg plaque formation, shows small area of hemorrhage and less of necrosis. Glucomannan 200 and 400 mg/kg treated rat liver shows occasionally plaques. Liver section of atorvastatin showing the same results but less amount of hemorrhages as compare control group.

Table I

Effect of methanolic extract, aqueous extract and glucomannan in Triton-WR 1339-induced hyperlipidemic rats on lipid profile

Groups	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	Atherogenic index
Normal	151.9 (8.4)	90.2 (8.1)	62.3 (1.0)	76.2 (8.0)	33.8 (1.6)	1.4 (0.1)
Control	242.9 (10.7)	203.6 (18.2)	42.8 (2.7)	159.4 (13.5)	40.7 (3.6)	4.4 (0.6)
Methanolic extract (200 mg/kg)	181.2 (9.3)^c	124.4 (8.8)^c	60.7 (2.5)^b	85.0 (5.1)^c	24.9 (1.8)^c	1.7 (0.1)^c
Methanolic extract (400 mg/kg)	172.4 (5.3)^c	123.5 (6.9)^c	72.1 (1.4)^c	74.5 (7.4)^c	24.7 (1.4)^c	2.0 (0.1)^c
Aqueous extract (200 mg/kg)	191.2 (3.8)^c	110.0 (12.3)^c	56.3 (5.8)^a	100.9 (3.8)^c	22.0 (2.5)^c	1.7 (0.3)^c
Aqueous extract (400 mg/kg)	123.1 (8.6)^c	92.5 (7.1)^c	66.8 (3.7)^c	48.5 (8.6)^c	18.5 (1.4)^c	1.7 (0.2)^c
Glucomannan (200 mg/kg)	176.6 (12.3)^c	102.8 (5.1)^c	53.0 (1.7)	93.4 (9.5)^c	20. 5 (1.0)^c	1.7 (0.1)^c
Glucomannan (400 mg/kg)	168.2 (7.0)^b	82.7 (2.8)^c	59.6 (2.2)^b	92.2 (5.8)^c	18.9 (2.7)^c	1.6 (0.1)^c
Atorvastatin (4 mg/kg)	180.3 (7.2)^c	95.8 (4.8)^c	68.4 (4.0)^c	77.0 (8.4)^c	34.8 (1.3)^c	1.4 (0.1)^c

Data were expressed as mean (SEM), n = 5. The data were analyzed using one-way ANOVA followed by Dunnet's multiple comparison test $^a p<0.05$, $^b p<0.01$, and $^c p<0.001$ compared to the hyperglycemic control group

Table II

Effect of methanolic and aqueous extracts in alloxan-induced hyperglycemic rats on blood glucose and lipid profile

Groups	Blood glucose (mg/dL)	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL
Normal	122.9 (4.9)	121.5 (3.8)	102.0 (1.4)	65.4 (2.4)	42.8 (3.3)	21.7 (0.9)
Control	173.5 (7.8)	217.2 (5.6)	249.0 (10.5)	41.2 (1.3)	126.3 (4.6)	49.7 (2.2)
Methanolic extract (200 mg/kg)	101.5 (2.9)[b]	144.5 (9.2)[b]	161.9 (3.1)[b]	61.7 (2.6)[b]	56.8 (9.6)[b]	32.2 (0.7)[b]
Methanolic extract (400 mg/kg)	99.9 (1.5)[b]	127.9 (5.5)[b]	130.2 (12.5)[b]	70.5 (1.8)[b]	25.0 (4.4)[b]	26.2 (2.6)[b]
Aqueous extract (200 mg/kg)	117.7 (5.5)[b]	154.7 (2.4)[b]	139.2 (3.5)[b]	51.3 (1.1)[a]	63.9 (2.1)[b]	27.8 (0.7)[b]
Aqueous extract (400 mg/kg)	105.7 (3.3)[b]	131.3 (1.8)[b]	117.8 (3.4)[b]	63.0 (0.9)[b]	56.4 (1.0)[b]	23.6 (0.7)[b]
Glibenclamide (0.5 mg/kg)	116.1 (2.0)[b]	137.0 (4.2)[b]	112.8 (7.3)[b]	66.8 (2.5)[b]	47.7 (4.9)[b]	22.6 (1.5)[b]

Data were expressed as mean (SEM), n = 5. The data were analyzed using one-way ANOVA followed by Dunnet's multiple comparison test [a]p<0.01, and [b]p<0.001 compared to the hyperglycemic control group

In alloxan-induced diabetic rat, the rise in blood glucose level reached its peak value (174.0 ± 7.8) and then remained stable throughout the study period. Treatment with ME and AQE (400 mg/kg body weight) of *E. herbacea* produces significant (p<0.001) reduction in blood glucose level 99.9 ± 1.5 and 105.7 ± 2.9 respectively. In diabetic rats there was significant (p<0.001) increase in total cholesterol 217.2 ± 5.4 and triglyceride level 249 ± 10.5 while decrease in HDL level 41.2 ± 1.3. We found that after administration of ME and AQE of *E. herbacea* there was significant (p<0.001) decrease in cholesterol 127.9 ± 5.5 and 131.3 ± 1.8; triglyceride level 130.2 ± 12.5 and 117.8 ± 3.4 and increase in HDL level 70.5 ± 1.8 and 63.0 ± 0.9 respectively. Also the similar effects were observed in other parameters like LDL and VLDL (Table II).

The total phenolic and flavonoid content in *E. herbacea* tubers were found to be 92.5 ± 0.5 and 55.4 ± 2.5 µg/g expressed as µg of gallic acid equivalent (GAE) and rutin (RE) equivalent respectively.

The extracts were found to be an efficient scavenger of super oxide radical generated in riboflavin-light-NBT system *in vitro* and their activity were compared to that of standard antioxidant ascorbic acid. The IC_{50} values of ME, AQE and IGM were found to be 11.7, 14.3 and 13.6. The IC_{50} value of ME, AQE and IGM was found to be lower than the standard ascorbic acid (36.0), which suggests that *E. herbacea* tubers has significant antioxidant potential than standard ascorbic acid. The capacity to inhibit lipid peroxidation in rat liver by ME, AQE and IGM was in dose dependent manner. The IC_{50} values of AA, ME, AQE and IGM were found to be 118.8, 128.0, 153.2 & 120.1 respectively (Figure 1).

Discussion

The present study demonstrate for the first time reports on hypolipidemic, hypoglycemic and antioxidant effects of extracts and glucomannan (Tamer et al., 2006) from the tubers of *E. herbacea*, as this plant contains carbohydrates as major chemical constituents.

HPLC chromatogram of water soluble vitamins showed the presence of vitamin C, pyridoxine (B_6) and thiamine (B_1). Among the B group of water-soluble vitamins, both thiamine (B_1) and pyridoxine (B_6) are important. They play different specific and vital functions in metabolism, and their lack or excess produces specific diseases (Ekinci and Kadakal, 2005). The concentration of thiamine and pyridoxine was found to be 52.7 and 7.5 µg/mL respectively. Thus, it can be concluded that these species of tubers can contribute significantly to the nutrient requirements of human and should be used as a source of nutrients.

Different species of *Eulophia* are reported for important biological activities like *E. campestris* for Aphrodisiac (Khare, 2007), *E. Dabia* for tonic appetizer (Bhattacharjee, 2001), *E. nuda* for anthelminitics (Nadkarni, 1991). The present investigation is thus concentrated on screening of *E. herbacea* tubers for hypolipidemic, hypoglycemic and antioxidant activity.

Triton WR-1339 has been widely used an investigative tool for studying lipid metabolism and for investigating metabolic interrelationship between plasma lipoproteins (Adeneye et al., 2010). In addition, triton physically increases VLDL level by rendering them refractive to the metabolic action of plasma and tissue lipolytic enzymes, thereby preventing or delaying their plasma clearance (Banerjee et al., 2006). Thus, the

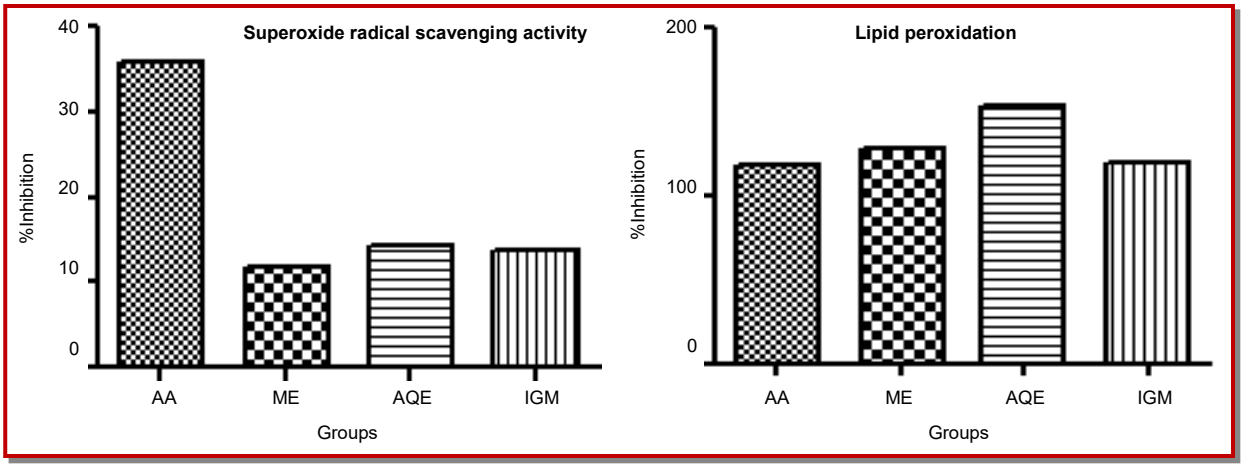

Figure 1: Percent inhibition of AA, ME, AQE and IGM against superoxide radical and lipid peroxidation

overall effect of triton WR-1339 is accelerated hepatic triglyceride and cholesterol biosynthesis which result in elevated plasma triglyceride, total cholesterol, LDL and VLDL while causing the reverse in the plasma level of HDL. All the extracts of *E. herbacea* show the activity in dose dependent manner. Present studies reveal that tubers of *E. herbacea* can be used as effective anti-hyperlipidemic at higher dose. Equally literature has supported that hypolipidemic effect of glucomannan (Chua et al., 2010). Thus extracts and glucomannan could reduce the risk of cardiovascular disease.

Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. Insulin plays an important role in the metabolism of lipids and protein. It is well established that alloxan administration to experimental rats selectively causes pancreatic cell-membrane disruption and ultimately cytotoxicity after its intracellular accumulation (Lu et al., 2009). In the present study, serum total cholesterol, triglycerides were significantly decreases in extracts treated diabetic rats as compared to untreated diabetic rats. All lipids parameters were tested and found to be improved after the treatment of ME and AQE of *E. herbacea.* Additionally it clearly proves that glucomannan obtained from *E. herbacea* have more marked hypolipidemic and antioxidant activity than other extracts.

Antioxidant activity was confirmed by *in vitro* and *ex vivo* models. Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as by products. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans (Jain and Agrawal, 2008). The cytotoxic potency of oxidized LDL has been linked to its content of lipid peroxidation products and inhibiting LDL oxidation should, therefore, limit the cytotoxicity of LDL in arterial walls. The cellular content of anti-oxidants is an important determinant of cellular injury from oxidized LDL (Minhajuddin et al., 2005). Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free-radical terminators (Cao et al., 1997). Flavonoids in many plants have a wide range of actions. Superoxide anion is an important primary free radical since the biological system converts it into more reactive OH radical and singlet oxygen. The study observed significant free-radical scavenging activity of *E. herbacea* extract against a wide variety of free radicals in a concentration-dependent manner among the tested doses which was comparable with standard antioxidants. The scavenging potential of *E. herbacea* extract might be due to the presence of phenolics and antioxidant vitamins (Chidambaram and Carani Venkatraman, 2010). In the present study, frequent consumption of these plants exhibited important role in prevention of many diseases caused by free radical damage like cardiovascular diseases and cancers. In biological systems, lipid peroxidation generates a number of degradation products such as malondialdehyde and is found to be important cause of cell membrane damage. The antioxidant activity of plant was also due to the presence of ascorbic acid.

Conclusion

E. herbacea shows hypoglycemic and hypolipidemic effects.

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Conflict of Interest

Authors declare no conflict of interest

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