Hypoglycemic and in Vitro Antioxidant Effects of Methanolic extract of Marsilea quadrifolia Plant

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ABSTRACT

Introduction: Oxidative stress induced by alloxan has been shown to damage pancreatic beta cell and produce hyperglycemia in rats. Hence the present investigation has designed to appraise antidiabetic and antioxidant principle of the methanolic extract of Marsilea quadrifolia (MEMQ). Methods: Hypoglycemic effect was evaluated in alloxan induced diabetic rat. The oral administration of plant extract at a dose of 300 mg/kg body weight was given to fasting glucose loaded rat with regard to normal control during 1 hr. study period and in alloxan induced (110 mg/kg body weight i.p.) diabetic rat in comparison with reference drug Metformin Hydrochloride (100 mg/kg) during 3 days study period. The antioxidant potential of MEMQ was checked by qualitative method and quantitatively through DPPH(1,1-diphenyl-2-picryl-hydrazyl) scavenging assay at 517 nm. Total phenolic content, total antioxidant capacity and reducing power activity was also assayed. Results: Considerable drop in elevated blood glucose level was observed in the alloxan induced diabetic (p<0.05 & p<0.001) rat. At a dose of 300 mg/kg the extract showed glucose level reduction of 47.57% in alloxan induced rat while 44.38% was found for Metformin after 3 days. Antioxidant activity using DPPH was found to increase in a concentration dependent manner with an IC50 value of 96.37 ± 3.62μg/ml higher than the standard one, IC50 16.59±0.59μg/ml. Total phenolic content was found 165.75 ± 0.961 mg/g in GAE and the total antioxidant capacity was equivalents of ascorbic acid (224.90 ± 1.42 mg/g). Conclusions: In all ways the extract showed significant antidiabetic and antioxidative potency. The present investigation suggests that MEMQ may be a potential source of natural antioxidant with good hypoglycemic activity.

Key words: Marsilea quadrifolia, Alloxan, Metformin Hydrochloride, Antioxidant activity.

INTRODUCTION

Free radicals cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins and contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, diabetes, cancer and AIDS[9]. Oxidative stress may have significant effect in the glucose transport protein (GLUT) or at insulin receptor[2]. Scavengers of oxidative stress may have an effect in dropping the increased serum glucose level in diabetes and may lessen the diabetes as well as reduce its secondary complications. For this reason, chemotherapy including antioxidants finds increased use in the treatment of such diseases. This has stimulated the production of patented antioxidant preparations based on various components of natural or synthetic origin. The use of natural antioxidants for the treatment and prophylaxis of free radical induced pathologies has certain advantages. Most of these agents produce no side effects, possess low toxicity, and effectively act upon the main factors damaging the vascular system. Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that are rich in antioxidant activity[3,4,5,6]. Diabetes is also the fourth-leading cause of death[7]. The diabetic population is rapidly increasing globally, particularly in the developing countries. South Asian region including Bangladesh is the most vulnerable focus. The current worldwide diabetic population is about 150 million and this will be doubled by 2025[8]. The estimated prevalence of diabetes in Bangladesh is around 4%, which is similar to the average prevalence in many
countries. But the prevalence of impaired glucose tolerance (IGT) here varies between 7.5-10% depending on urban and rural backgrounds [9]. A significant proportion of these patients obviously fall to get proper treatment and medication. Indigenous drugs since long, have been used for the treatment of diabetes [10]. Thousands of plants are known to have potential hypoglycemic effect. Bangladesh is abundant in antihyperglycemic plants. It is well established that diabetes is associated with low level of antioxidants and many plants show hypoglycemic property due to their antioxidant potential [11]. Marsilea quadrifolia (Marsileaceae), known as is an aquatic fern bearing 4 parted leaf resembling ‘4-leaf clover’ (Trifolium). Leaves floating in deep water or erect in shallow water or on land. Leaflets obtained, to ¾” long, glaucous, petioles to 8” long; Sporocarp (ferns) ellipsoid, to 3/16 long, dark brown, on stalks to ¾” long, attached to base of petioles. A juice made from the leaves is diuretic and febrifuge and also used to treat snakebite and applied to abscesses etc.[12]. The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant[12-13]. The plant contains an enzyme named Thiaminase[13]. The Petroleum ether, chloroform and ethyl acetate extract showed antibacterial, cytotoxic and antioxidant activity [14]. In the present study was aimed to evaluate the effect of Marsilea quadrifolia on blood sugar levels in alloxan treated rats and its antioxidant activity through various analytical methods.

MATERIALS AND METHODS

Collection and Identification of plants

The aerial parts of Marsilea quadrifolia were collected from Ramna, Dhaka, Bangladesh in July 2011 and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 35441 has been deposited. The collected plant parts were separated from undesirable materials or plant parts. They were dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Extraction of Plant Material

The collected aerial parts of plant was cleared, dried under shade at room temperature and powdered. For the extraction of phytochemicals about 200 gm powder of the plant was taken in a glass jar and completely submerged with 600 ml ethanol. The container with its content was sealed by cotton plug and aluminum foil and keep for a period of 7 days accompanying occasional shaking and stirring. The extract was then filtered through filter paper (Double Rings filter paper 102, 11.0 cm). The filtrates was concentrated at 50°C under reduce pressure using vacuum pump rotary evaporator (STUART RF3022C, UK) to yield a solid residue 30gm.

Design of Experiment

The research work was carried out for about 4 weeks. In the first week various methods of in-vitro antioxidant screening methods and acute toxicity test were performed. And in the next 3 weeks diabetic condition was induced in the experimental rats and than treated with crude MEMQ for hypoglycemic activity study.

Experimental animal

Experiment was conducted on adult albino rats of either sex with the weights of 110-165 gm procured from International Centre for Diarrheal Disease Research Bangladesh (ICDDR,B). All rats were fed normal laboratory chow food containing 16% protein, 66% carbohydrate, 8% fats and water. All rats were housed at a (12:12) hr light and dark cycle at 25ºc and relative humidity (60-70) %.

Ethical Approval

The guidelines followed for animal experiment were accepted by the institutional animal ethical committee[15].

Experimental Design

Animals were alienated into five groups and for every group six animals were taken.

Group I (Normal control) rats served as positive control received physiological saline (0.9% NaCl; 5ml/kg.b.w.p.o).

Group II (Diabetic Control) intraperitoneally injected Normal saline treated Alloxan induced Diabetic rat.

Group III rats were received intraperitonially administration of Metformin Hydrochloride (100 mg/kg/day) at a period of 24 hr for 3 successive days and served as standard.

Group IV rats were received M. quadrifolia (300mg/kg/ day) orally at a hiatus of 24 hr for three consecutive days. Blood glucose was measured on 1st, 2nd and 3rd day.

Preparation of Alloxan solution

At first body weight of rats were measured. Then necessary amount of Alloxan was measured according to the body weight by following the dose of 110 mg of Alloxan per 1000 gm of body weight. Then calculated quantity of Alloxan was dissolved in 0.1 ml of sterile normal saline water.
Induction of alloxan

The rats were injected Alloxan monohydrate (Fluka, Germany), dissolved in sterile normal saline water at a dose of 110mg/kg body weights intraperitoneally once a day. Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin discharge; therefore the rats were treated with glucose solution orally. After few days rats with moderate diabetes having glycosuria and hyperglycemia that is blood glucose level go beyond normal level were chosen.

Preparation of dosage of active drug and plant extract

Metformin hydrochloride (a biguanide): Metformin hydrochloride was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form with sterilized water in such a concentration that each 0.1ml contained metformin hydrochloride according to the dose of 100 mg/kg/day, seeing as metformin is effective in such dose in case of humans.

M. quadrifolia: The crude extract obtained and was dissolved in Tween 80 and water to prepare the solution where each 1 ml contained M. quadrifolia according to the dose of 300 mg/kg/day. 1 ml of the tested solution was administered everyday during treatment to achieve required dose of respective agents.

Hypoglycemic activity

Glucose tolerance test

A glucose tolerance test is the administration of glucose to determine how quickly it is cleared from the blood. The rat were tested in a fasting state (having no food or drink except water for at least 10 hours but not greater than 16 hours). An initial blood sugar was drawn and then the rat were fed glucose. The rat then had their blood tested again 30 minutes, 1 hour, 2 hours and 3 hours after drinking the high glucose drink [16].

Blood sugar assessment

Fasting blood glucose level was evaluated in normal and diabetic rats from the tail vein by strip technique (Bioland Glucometer, Germany). At first it is done just prior to extract administration of first day then it is continued for 3 days just one hour after the administration of plant extract.

In vitro antioxidant activity screening

Qualitative assay: A suitable diluted stock solution was spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted [17].

Quantitative assay

The amount of phenolic compounds

Phenols react with phosphomolybdic acid in Folin-ciocalteau reagent in alkaline medium and produce a blue colored complex (molybdenum blue) that can be anticipated colorimetrically at 650 nm. The total phenolic content of MEMQ was determined using Folin-ciocalteau reagent[18] method. The content of total phenolics in the MEMQ was calculated from regression equation of the calibration curve (y = 0.0138x+0.1275, R² = 0.9881) and is expressed as galic acid equivalents (GAE).

Determination of total antioxidant capacity

The antioxidant activity of the MEMQ was evaluated by the phosphomolybdenu method according to the procedure of Prieto [19]. The antioxidant activity is expressed as the number of equivalents of ascorbic acid using the following equation:

\[
C = \frac{(c \times V)}{m}
\]

Where, C = total antioxidant activity of plant extract in Ascorbic acid (mg/ml), c = Concentration of Ascorbic acid established from the calibration curve (mg/ml), V = volume of extract (ml), m = weight of pure plant extract(g).

Free radical scavenging activity measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH)

Based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, the free radical scavenging activity of MEMQ extract, was determined by the method described by Braca [20]. The percentage inhibition activity was calculated from:

\[
\% \text{Inhibition} = \left( \frac{(A_0 - A_i)}{A_0} \right) \times 100
\]

Where, \(A_0\) is the absorbance of the control and \(A_i\) is the absorbance of the extract/ standard. IC 50 value was calculated from the equation of line obtained by plotting a graph of concentration (μg mL⁻¹) versus % inhibition.

Reducing power assay

The reducing power of MEMQ was determined according to the method described by Oyaizu [21]. Increased absorbance of the reaction mixture indicated increased reducing power.
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Quantitative assay
Total Phenolic content

Total phenolic content of extract of *M. quadrifolia* was found 165.75 ± 0.961 mg/g in GAE and the result is shown in Table 3.

Total antioxidant capacity

Table 3 showed Total antioxidant capacity of methanolic extract of *M. quadrifolia* as the number of equivalents of ascorbic acid (224.90 ± 1.42 mg/g).

DPPH radical scavenging assay

The percentage (%) scavenging of DPPH radical was found to be concentration dependent. The results of DPPH scavenging activity with IC<sub>50</sub> value of the experimental extract and the standard ascorbic acid are given in Table 4.

Reducing power assay

For the measurement of the reductive capacity, we investigated the Fe<sup>3+</sup> to Fe<sup>2+</sup> transformation in the presence of extract. Like the antioxidant activity, the reducing power of *M. quadrifolia* amplified with increasing concentration of the sample. Figure 1 exhibits the reductive ability of MEMQ compared with ascorbic acid.

Acute toxicity Test

The extract was safe up to a dose of 1600 mg/kg body weight. Behavior of the animals was closely observed for
the first 3 h then at an interval of every 4 h during the next 48 h. The extract did not cause mortality on rats during 48 h observation or any behavioral change.

**DISCUSSION**

**Hypoglycemic activity**

Present study indicates that MEMQ (300 mg/Kg b. wt) significantly decreased serum glucose level in hyperglycemic rats. Alloxan is the most frequently employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is escalating evidence that alloxan caused diabetes by rapid exhaustion of a cell, by DNA alkylation and gathering of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a fall in insulin release there by a drastic diminution in plasma insulin concentration leading to stable hyperglycemic states[22]. It induces diabetes by dose dependent destruction of β-cells of islets of langhans[23-24]. So, in the present study alloxan was chosen to create diabetic condition in rat and significant hyperglycemia was achieved within 48 hours after alloxan (110 g/kg b.w. i.p) injection. The results obtained (Table 1) showed that after a single administration of glucose 200 mg/kg in rat, there was a significant reduction (p < 0.05) of fasting blood glucose level during the 3 h study period. The research on Antidiabetic activity in alloxanised rats, administration of MEMQ of 300 mg/kg body weight administered for 3 days was able to correct this anomaly significantly (p<0.05 & p<0.001). Significant reduction of blood glucose was observed from the 3rd day of the study. The comparable effect of the experimental extract with Metformin HCl may suggest similar mode of action since alloxan permanently destroys the pancreatic β cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effect. On the progression of treatment with methanolic extract of *M. quadrifolia* (300 mg/kg body weight) produced maximum reduction to 6.83±0.29 mmol/L on 3rd day whereas reduction to 5.53±0.27 mmol/L was found for metformin on 3rd day (Table 2). These observations suggest that the experimental extract might acquire insulin like effect on peripheral tissues either by promoting glucose consumption metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of β cells [25].

**In vitro antioxidant activity**

**Total phenol content**

Phenolic compounds are commonly found in both edible and nonedible plants and they have been reported to have copious biological effects, including antioxidant activity. The antioxidant property of phenolic compounds is due to their redox properties, which can play a vital role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [26]. In this current study MEMQ possessed phenolic compound 165.75±0.961 mg/g in GAE.

**Total antioxidant capacity**

Total antioxidant capacity of the crude extract, expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The result revealed the reducing power of the crude extract. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [27]. Many plants contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids, tannins and thus can be utilized to scavenge the excess free radicals from the human body[28].

**DPPH radical scavenging activity**

DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1,
1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. From the results, it may be postulated that the plant extracts have hydrogen donors thus scavenging the free radical DPPH. Based on the data obtained from this experiment, DPPH radical scavenging activity of MEMQ \((IC_{50} 96.37\pm3.62 \mu g/ml)\) was drastically higher than the standard one \((IC_{50} 16.59\pm0.59 \mu g/ml)\).

**Reducing power assay**

The reducing capacity of a compound may furnish as a valuable indicator of its potential antioxidant power [29]. At the concentrations the extract tested the reducing power of the investigated plant was increasing along with the standard ascorbic acid. The present data on the reducing power of the studied plant extract recommended that it is likely to contribute the antioxidant activity.

**Acute toxicity Test**

The absence of mortality and signs of toxicity up to 5 times the maximum effective dose, with MEMQ proves for that the plant has wide safety margin.

**CONCLUSION**

Our results support the view that some herbal medicinal plants did not inhibit glucose diffusion using in vitro model glucose absorption. In particular, their phenolic compounds and antioxidant activities may be useful for meal planning in type2 diabetes. They could contribute to sustain plasma antioxidant level because antioxidants present in the plants and herbs prevent the development of vascular diseases seen in type 2 diabetes. The preliminary investigation on the antidiabetic efficacy of MEMQ will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. The antioxidant activities of medicinal plants may be due to the occurrence of phenolic compounds, containing the hydroxyl groups that confer the hydrogen donating ability. The present inquiry suggests that medicinal plants which acquire good antioxidant potential are the best supplements for the diseases coupled with oxidative stress. But we still don’t know which chemical components are exactly responsible with the aforementioned effects so to find out the lead compounds liable for aforesaid activities from the above plant are in progress.

**REFERENCE**


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