Journal of Applied Pharmaceutical Science
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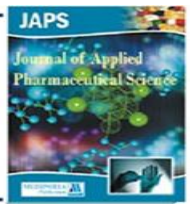

ISSN: 2231-3354
Received on: 24-08-2011
Revised on: 28-07-2011
Accepted on: 31-08-2011

Antioxidant and antihyperglycemic activities of methanolic extract of *Glinus oppositifolius* leaves

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ABSTRACT

The present study was carried out to investigate the antioxidant and antidiabetic potential of methanolic extract of *Glinus oppositifolius* leaves. The antioxidant potential was examined by DPPH radical scavenging assay, nitric oxide (NO) scavenging assay, determination of total flavonoid content and total antioxidant capacity assay. Antihyperglycemic activity was studied by oral glucose tolerance test (OGTT) in normal mice and in alloxan-induced diabetic mice. In DPPH radical scavenging assay the IC_{50} value of the extract was found to be greater than 1000 µg/ml (ascorbic acid, IC_{50} = 14.45 µg/ml) while it was 269 µg/ml in nitric oxide (NO) scavenging assay (quercetin IC_{50} = 15.24 µg/ml). The amount of total flavonoid was 25 .46 mg/g and total antioxidant activity was 79.48 mg/g equivalent to Quercetin and Ascorbic acid respectively. A significant effect (p<0.05) on oral glucose tolerance was noted at the dose of 200 mg/kg and 400 mg/kg body weight in mice. At the same doses, the extract showed significant (p<0.05) reduction of blood glucose level in Alloxan-induced diabetic mice compared to the standard drug Metformin. Overall, the results of the present study indicate that the methanolic extract of *Glinus oppositifolius* leaves possess moderate antioxidant activity and significant antihyperglycemic activity.

Key words: *Glinus oppositifolius*, antioxidant, antihyperglycemic, alloxan, oral glucose tolerance.

INTRODUCTION

Glinus oppositifolius (Linn.) DC. (Family: Molluginaceae) is a branched herb containing linear to obovate, opposite leaves and greenish flowers growing all over Bangladesh. It is also known as *Molugo oppositifolia* Linn. In Bangladesh the plant is called 'Gima'. Dried and powdered stems with leaves of the herb are used for treating jaundice and abdominal pain (Burkhill., 1985) . A decoction of fine powder of the aerial parts of the plant is used in the treatment of malaria (Diallo et al., 1999). A maceration of pounded plant material with oil or water is used in the treatment of wound (Debes., 1998) . *G. oppositifolius* are used by the traditional healers for treating joint pain, inflammation, diarrhoea, intestinal parasites, fever boils and skin disorders (Diallo et al., 1999, Diallo., 2000). Ethanolic extract of the plant has been reported to depress central nervous activity (Ghani., 2003) .The leaves contain spergulagenic, spergulagenin A and a trihydroxy ketone (Ghani., 2003). A bioactive pectic polysaccharide isolated from *G. oppositifolius* is found to possess immunomodulating property (Inngerdingen et al., 2005). As a part of our ongoing research on medicinal plants (Hasan et al., 2009 and 2010, Zohera et al., 2010, Mazumder et al., 2008) an attempt has been taken to evaluate the antioxidant and antihyperglycemic potential of the methanolic extract of *G. oppositifolius* leaves.

MATERIALS AND METHODS

Collection and preparation of plant material

The whole plant was collected from a village near Aricha, Manikganj, Bangladesh and was identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka, Bangladesh. A voucher specimen of the plant has been deposited (Accession No.: 32528) in the herbarium for further reference. The leaves of the plants were washed, dried under shed and then, dried in an oven at reduced temperature (<50^{0}C). The powdered plant materials were submerged into methanol in an air-tight flat bottom container for seven days, with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent which were collected and then evaporated to get the extract that was used in the experimental works.

Chemicals and drugs

1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany). Ammonium molybdate was purchased from Merck, Mumbai, India. The standard drug, Metformin hydrochloride was the generous gift sample from Square Pharmaceuticals Ltd., Pabna, Bangladesh. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Experimental animals

The *in vivo* experiments were carried out on Swiss albino mice of 3-4 weeks age, weighing 20-25 g. They were obtained from International Centre for Diarrheal Disease and research, Bangladesh (ICDDR,B). The animals were given standard mice feed and water and were kept in the laboratory environment for seven days for acclimatization. All mice were maintained in groups of five at 24 ± 1°C with light/dark cycle of 12 hours. They were starved overnight but allowed fresh water before experimentation.

Phytochemical screening

The methanolic extract of *G. oppositifolius* was qualitatively tested for detecting carbohydrates, saponins, flavonoids, tannins, alkaloids, reducing sugars, gums and steroids following the standard procedures (Ghani, 2003) .

Antioxidant activity test

DPPH radical scavenging capacity assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was studied. 200µl of plant extract or standard of different concentration were taken in different test tubes and 2 ml of reagent solution (0.004 gm of DPPH in 100 ml ethanol) was added to each test tube. The test tubes were incubated for 30 minutes to complete the reaction. The absorbances of the solutions were measured at 517nm using a spectrophotometer (Shimadzu, UV-1601PC) against blank (Braca et al., 2002). The percentage (%) inhibition activity was calculated from the equation: $[(A_0 – A_1)/A_0] \times 100$. Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard. Then percentage inhibitions were plotted against log concentration and from the graph IC_{50} was calculated.

Nitric oxide scavenging capacity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm. Four ml of plant extract or standard solution of different concentration were taken in different test tubes and 1.0 ml of Sodium nitroprusside, (5 mM) solution was added into the test tubes. Then they were incubated for 2 h at 30^{o}C to complete the reaction. Two ml solution was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) and the absorbances of the solutions were measured at 550 nm using a spectrophotometer against blank (Alisi et al., 2008) . Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: $[(A_0 – A_1)/A_0] \times 100$. Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard. IC_{50} was calculated by linear regression method.

Determination of flavonoid content

Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al., 2002). One ml of sample (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1M potassium acetate and 5.6 ml of distilled water. Then it was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The flavonoid content was then calculated.

Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto et al., 1999) .0.3 ml extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28m M sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solutions were incubated at 95^{o}C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Antihyperglycemic activity

Oral glucose tolerance test (OGTT)

Mice were kept in overnight fasting to estimate a baseline blood glucose level (0 minutes). Without delay, a glucose solution (2 gm/kg body weight) was administered by gavage (Chaturvedi et al., 2004). Mice were divided into four groups each containing 5

mice. Group I and II received the vehicle (1% Tween 80 in water, 0.4 ml) and Metformin (60 mg/kg b.w.) respectively while Group III and IV received plant extract at 200 mg/kg and 400 mg/kg b.w. per orally. Blood glucose level was measured by one touch glucometer (Accu Check) at 0, 30, 60, and 120 min after the administration of extract.

Activity in alloxan-induced diabetes

Diabetes was induced by intraperitoneal injection of Alloxan to mice (60 mg/kg.b.w.). After 72 h, animals showing plasma sugar level more than 8 mmol/dl were considered diabetic. The diabetic animals were stabilized for five days and the next day experiment was started. Group I served as normal control (1% Tween 80 in distilled water, 5ml/kg.b.w.p.o), Group II served as diabetic control, Group III received Metformin (60 mg/kg b.w. p.o) as standard drug and Group IV received the plant extract (200 mg/kg.b.w. p.o). The blood glucose level was then tested by using glucometer by taking blood sample from the tail vein at 0, 4, 8 and 24 h after drug and plant extract administration (Kasiviswanath et al., 2005).

Statistical analysis

Statistical analysis for animal experiments was carried out using one-way ANOVA followed by Dunnett's multiple comparison test using SPSS 17. The results obtained were compared with the control group. p values <0.05, <0.001 were considered to be statistically significant.

RESULTS

The plant extract gave positive reaction for carbohydrates, alkaloids, tannins, flavonoids and saponins. Steroids, gums and reducing sugar were absent in the plant extract. In DPPH radical scavenging assay, the IC_{50} value of the extract was >1000 µg/ml while the IC_{50} value for the reference ascorbic acid was 14.45µg/ml (Figure 1).

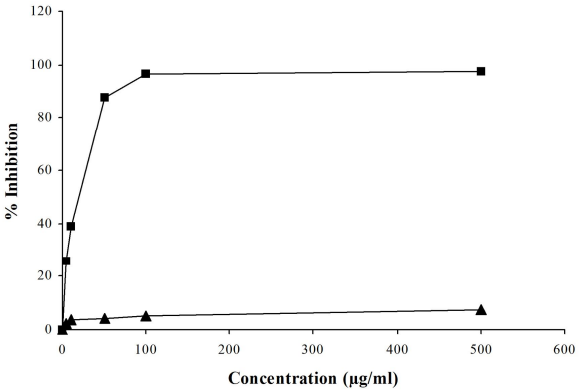

Fig 1: DPPH radical scavenging activity of the methanolic extract of *Glinus oppositifolius*(─▲─) and ascorbic acid (─■─).

The molecule of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. Figure 2 shows the comparative NO scavenging activity of the extract. The IC_{50} value of the extract was 269µg/ml whereas the standard Ascorbic acid showed an IC50 value of 5.47µg/ml.

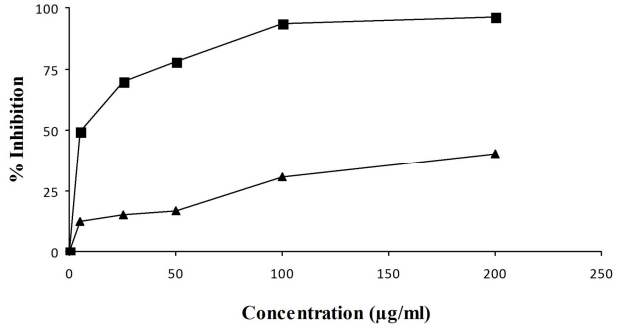

Fig 2: NO scavenging activity of the methanolic extract of *Glinus oppositifolius*(─▲─) and ascorbic acid (─■─).

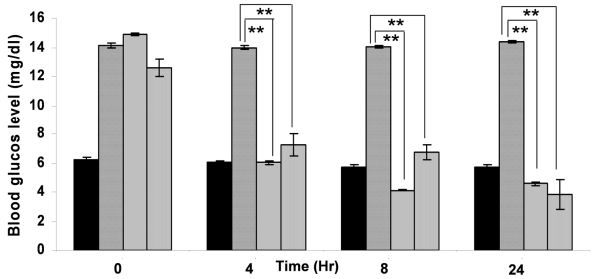

Fig 3: Effect of the methanolic extract of *Glinus oppositifolius* on fasting blood glucose level in normal control and alloxan induced diabetic mice. Values are presented as mean ± SEM, (n = 5); * p < 0.05, ** p < 0.001, Dunnett's test as compared to control. ■ Group I; ▒ Group II; ▒ Group III; ▒ Group IV. Group I served as Normal control (1% Tween 80 in distilled water, 5ml/kg body weight p.o), Group II served as diabetic control, Group III received Metformin (60 mg/kg body weight p.o) as standard drug and Group IV received the plant extract at the dose of 200 mg/kg body weight p.o.

Table 1: Effect of *G. oppositifolius* on normal mice in oral glucose tolerance test.

Group	Treatment	Dose (p.o.)	Blood glucose level			
			0 min	30 min	60 min	90 min
Group I	Vehicle (1% Tween 80 in water)	0.4 ml/mouse	6.84±0.233	10.66±0.760	12.38±0.356	10.58±0.498
Group II	Metformin	60 mg/kg b.w.	6.96±0.282	5.88±0.331**	5.54±0.507**	5.28±0.494**
Group III	*Glinus oppositifolius*	200 mg/kg b.w.	6.76±0.282	8.62±0.707*	7.66±0.626**	7.20±0.548**
Group IV		400 mg/kg b.w.	7.36±0.301	8.38±0.439*	7.64±0.399**	6.38±0.498**

Values are presented as mean ± SEM, (n = 5); * p < 0.05, ** p < 0.001, Dunnett's test as compared to control.

Flavonoid content was calculated from the regression equation of the calibration curve (y = 0.009x - 0.036). The total flavonoid content was found to be 25.46 mg/g plant extract equivalent to Quercetin. The total antioxidant capacity of the methanolic extract was determined from the calibration curve established by ascorbic acid at 695 nm. The regression line was y = 0.005x - 0.028. The value was found to be 79.48 mg/g plant extract equivalent to ascorbic acid. The result of oral glucose tolerance test is shown in Table 1. Both doses (200 mg/kg and 400 mg/kg b.w.) of the extract showed a dose dependent glucose level lowering

activity when compared with the vehicle. The results were found statistically significant ($p<0.05$-0.001). The blood glucose levels were significantly higher in diabetic mice. Alloxan induced diabetic mice with simultaneous administration of *G. oppositifolius* extract showed a significant decrease in blood glucose level compared with the diabetic control group and the results are shown in Figure 3. The results revealed that the short term treatment with crude methanolic extract of *G. oppositifolius* showed significant antihyperglycemic activity.

DISCUSSION

The results of phytochemical screening revealed the presence of polyphenolic compounds such as tannins and flavonoids which have been reported to have multiple biological effects, including antioxidant activity (Vinson et al., 1995). Antioxidant activity of the methanolic extract of *G. oppositifolius* may be attributed to the flavonoid content. The results also showed mild DPPH radical scavenging activity and moderate NO radical scavenging activity of the extract which may help to arrest the chain of reactions initiated by excess generation of $NO^{\cdot}$ that are detrimental to the human health (Hasan et al., 2009) . This finding correlates well with a recent study (Kumar et al., 2009) which showed that the methanolic extract of *G. oppositifolius* possesses NO scavenging activity. The oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's main source of energy. OGTT can be used to diagnose pre diabetes and diabetes. OGTT is most commonly done to check for diabetes that occurs with pregnancy (gestational diabetes). The methanolic extract of *G. oppositifolius* showed significant ability to reduce the elevated glucose level in normal mice compared to the standard drug Metformin. Alloxan causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas (Ahmed et al., 1998). Studies have shown that alloxan is selectively toxic to pancreatic beta cells, causing cell necrosis (Oberley et al., 1988). Alloxan administration produced elevated level of lipid peroxidation, hydroperooxides and conjugated diene that is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage (Sabu et al., 2004). The results confirm the antioxidant activity of the extract which might have also contributed to the antidiabetic activity by means of scavenging the oxygen free radicals (Krishnakumar et al., 1999). The findings of the present study indicate a significant short term anti-diabetic effect of the methanolic extract of *G. oppositifolius* which may be supported by the antioxidant activity of the plant. Further studies can be continued to evaluate the chronic antidiabetic effect of the plant extract for a better understanding of antidiabetic effect of the plant which might contribute to the isolation of new lead compounds responsible for such antidiabetic and antioxidant effect.

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