Original Article

ANTIDIABETIC AND ANALGESIC EFFECTS OF *GLYCOSMIS PENTAPHYLLA* (RETZ.) IN SWISS ALBINO MICE

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Abstract

Background and purposes: *Glycosmis pentaphylla* (Retz.) Correa, a medicinal plant is popularly used as herbal remedy for various ailments in Bangladesh. It was also reported that GP has both anti-hyperglycemic and analgesic effects and being widely used to reduce blood glucose and to alleviate pain for many years in this region though published literatures are scarce. The present study was designed to evaluate whether ethanolic extract of *Glycosmis pentaphylla* (GP) have anti-hyperglycemic and analgesic effects. A total of 60 Swiss Albino male mice of nine weeks (weight, 20-25g) were used for investigation. Of them, 40 were made diabetic by alloxan. They were investigated in two groups – a) 20 mice by oral glucose tolerance test (4 samples OGTT) – at 0, 30, 90 and 120 min; and b) 20 mice for a week-long antihyperglycemic activity on day 0, 1, 3 & 7. Both the groups were subdivided into four, each having 5 mice – i) the 'control' received only 0.5% methyl cellulose as vehicle; ii) 'Standard' received vehicle plus metformin; iii & iv) test 'DGP250' & 'DGP500' received vehicle plus GP extract with 250 & 500 mg /kg, respectively. For the analgesic activity, 20 mice were investigated in four subgroups, each having 5 mice and similar steps were adopted. Here, vehicle was used 1% Tween 80 and intra-peritoneal injection of Acetic acid for eliciting pain in all four subgroups. The 'standard' group got diclofenac sodium for comparison with the test groups 'GP250' and 'GP500'. In OGTT, Ethanolic extract of GP250 and GP500 reduced blood glucose at 90 min. But the levels of reduction were more significant at 120 min, 50.7% by GP250 and 66% by GP500 ($p < 0.001$). The reduction is almost comparable with that induced by metformin. Likewise, for a weeklong anti-hyperglycemic activity, the GP extracts were found as equally effective as metfomin, which was also dose dependent. In addition to antihyperglycemic effect, the ethanolic extract of GP showed significant analgesic effect that was also dose dependent. Our results indicate that GP extract has antihyperglycemic effect in both short and in weeklong duration, which is almost comparable to Metformin HCL, a known and widely used antihyperglycemic agent. The GP extract was also found to have an analgesic effect almost comparable to diclofenac sodium, a known analgesic drug. Further study is needed to confirm the anti-hyperglycemic and analgesic effect of GP including its side effects in long term use.

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Key words: *Glycosmis pentaphylla* (GP), Diabetes mellitus, antidiabetic and analgesic activity

Introduction

Diabetes is a global disease with a huge adverse impact on health and mortality particularly of cardiovascular disorders. Diabetes mellitus (DM) is major clinical disorder affecting nearly 10% of the populations all over the world.[1] In Bangladesh, the situation is most vulnerable; the number of people with diabetes will

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rise from 3.2 million in 2000 to 11.7 million by 2030.[2] Patient with diabetes have an increased risk of coronary heart disease, peripheral vascular disease, strokes and may account for more than 65% death among people with diabetes mellitus.[3,4] Multiple pathophysiologic mechanisms play a role in the risk of cardiovascular events in the metabolic syndrome including glucose intolerance, hyperglycemia, hypertension, dyslipidemia, atherosclerosis that are caused primarily by insulin resistance.[5,6] Traditional medicines are used to reduce blood glucose level as well as have beneficial effects on complication of diabetes.[7]

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort and analgesics relieve pain.[8] Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. Opiate analgesic such as morphine has strong addictive potential and other side effects including respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and several alterations of endocrine and autonomic nervous system while NSAIDs are well known for their ability to produce gastrointestinal bleeding, ulceration and other complications.[9] In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency.[10]

Glycosmis pentaphylla is a small trees or shrubs belonging to family of Rutaceae, which grows to a height 5 m. It is widely distributed in tropical forest at low altitudes like India, South China, Thailand, Malaysia, Indonesia and Philippines Islands.[11] Arborine [2-benzyl-1-methyl-4-quinazolone] was isolated as the major compound from ethyl acetate soluble fraction of leaf extract of *Glycosmis pentaphylla*.[12] The root bark contains alkaloid skimmianine, g-fagarine, dictamine, arbone, 3-methoxycarbazone, glycone, glycozoline and glycozolicine and beta-sitosterol.[13] A paste prepared from leaves of GP and ginger can be used for treatment of eczema and other skin infection.[11] Extract of leaves of GP is used in fever, liver complains, cough and jaundice.[14] But still no scientific and methodical investigation has so far been reported in literature regarding its anti-diabetic and analgesic activity. Therefore as a part of our ongoing phytochemical and pharmacological investigations on local medicinal plants of Bangladesh, the present study has been designed to examine anti-diabetic and analgesic activity of ethanolic extracts of leaf of *Glycosmis pentaphylla*.

Methods and Materials

Plant materials

Fresh leafs of *Glycosmis pentaphylla*. (Vernicular name- tooth brush plant) was collected from Savar, Dhaka in September 2009 and plant authenticity was confirmed from the Bangladesh National Herbarium, Dhaka.

Preparation of ethanol extract

The collected leaves were washed and sun dried under shadow for several days. The powdered plant leaves were extracted with 95% ethanol at room temperature. The bottles were kept at room temperature for several 7-10 days with occasional shaking. The extract thus obtained was filtered through cotton and filter paper (Whatman Filter Paper No. 1). The filtrate was defatted with petroleum ether. Defatted liquor was allowed to evaporate using rotary evaporator at temperature 40-45°C. Finally, concentrated crude extract was obtained.

Drugs and chemicals

The active drug, Metformin hydrochloride and Diclofenac-Na were collected from Square Pharmaceuticals Ltd; Pabna Bangladesh. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India.

A total of 60 male Swiss Albino mice (weight, 20-25g) aged nine weeks were purchased from ICDDRB, Dhaka, Bangladesh and housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%) with water *ad. libitum*. The animals used in this study were cared according to guidelines of animal experiment. All laboratory investigations were done in the Pharmacy laboratory in Bangladesh University from July to December 2010.

These sixty mice were divided into three experimental groups, each group with 20 mice. Two groups (20 + 20 = 40) were studied for antihyperglycemic activity – one for short term and the other for a weeklong duration. Both of the two groups were made diabetic by alloxan (alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter). These alloxan induced two diabetic groups were investigated for a short term – a) by oral glucose tolerance test (n=20,

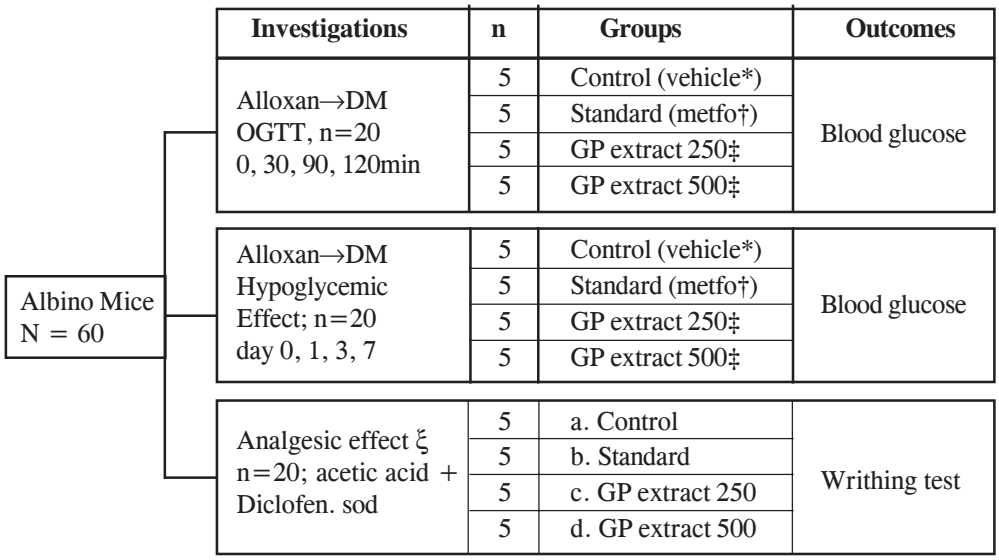

Fig 1. *Study design*

* - Vehicle 0.5% methyl cellulose given to all 4 groups, † - Vehicle + metformin HCL
‡ - vehicle + ethanolic extract of Glycosmis Pentaphylla): Dose given orally (GP: 250 and 500 mg/kg),
ξ – a. Control: vehicle 1%Tween 80 at 0min + 0.7% Acetic acid (intraperitoneal) at 30min; 'writhing' contraction test every 5 min interval for 10 minutes after Acetic Acid injection
b. Standard: Diclofenac Sodium at 15min, 'writhing' test as control
c &d. GP250 and GP500 given at 15 min, 'writhing' test as control;

4 samples OGTT) – at 0, 30, 90 and 120 min; and b) for a week-long antihyperglycemic activity (n=20) on day 0, 1, 3 & 7. Both the groups were subdivided into four, each having 5 mice – i) the 'control' received only 0.5% methyl cellulose as vehicle; ii) 'Standard' received vehicle plus metformin; iii & iv) test 'DGP250' & 'DGP500' received vehicle plus GP extract with 250 & 500 mg /kg, respectively. All blood samples were taken from tail-vein for estimation of blood glucose by Glucometer, a reflectance photometer. For the analgesic activity, 20 mice were investigated in four subgroups, each having 5 mice and similar steps were adopted as for the antihyperglycemic effect. Here, vehicle was used 1% Tween 80 and intra-peritoneal injection of Acetic acid for eliciting pain in all four subgroups. The 'standard' group got diclofenac sodium for comparison with the test groups 'GP250' and 'GP500'.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed using Swiss albino mice. All mice were divided into four groups and each group comprised of five mice. Groups were Diabetic Control (DC groups receiving vehicle 0.5% methyl cellulose), Diabetic Standard group (DS mice received Metformin HCl, 100 mg/kg), DGP-250 and DGP-500 (mice received ethanolic extracts 250 mg/kg and 500 mg/Kg dissolved in 0.5% methyl cellulose, respectively). After fasting 16 hours, diabetes was induced to all groups by intra-peritoneal injection (IP) of alloxan monohydrate (100 mg/kg) dissolved in saline (0.75mg/0.3ml). After 72 hours, blood glucose levels were measured from tail-vein by Glucometer. Blood glucose level higher than 11.5 mmol/l was considered as diabetic.

Anti hyperglycemic test

Short term test: One ml (50mg/ml) of glucose solution in a dose of 2 gm/kg body weight was administered to all groups by gastric tube. Simultaneously, one ml of 0.5% methyl cellulose for the control (DC) and 1 ml (2.5mg/ml of 0.5% methyl cellulose) of standard drug metformin and one ml of ethanolic extract for group 250 (6.25mg/ml) and for group 500 (12.5mg/ml) were administered orally to respective groups. The blood glucose content was measured after 30 mins, 90 mins and 120 mins.

Weeklong test: Similarly one ml of methyl cellulose (0.5%), standard drug metformin and extracts (250 mg/kg and 500 mg/kg) were administered once daily at 8 AM for seven days to respective mice groups. Blood glucose was measured before drug administration on 1st, 3rd and 7th day.

Acetic acid-induced writhing test for analgesic activity

Analgesic activity of ethanolic extract was studied by acetic acid-induced writhing test in mice. Mice were divided into four groups (each group comprises five mice). Control group mice received vehicles (1% Tween 80 in water), Standard Group received Diclofenac-Na 10 mg/kg body weight , Test Group I and Test Group II were received 250 and 500 mg/kg b. wt. of ethanolic extract of GP. Test samples and vehicle were administered orally 30 mins before intra-peritoneal administration of 0.7% acetic acid and Diclofenac-Na (0.25mg/ml) was administered intra-peritoneally 15 mins before injection of acetic acid. After 5 mins interval, mice were observed for specific contraction of body referred to as "writhing" for next 10 mins.[15]

Phytochemical screening

The freshly prepared leaf extract of GP was qualitatively tested for presence of chemical constituents. Phytochemical screening of GP extract was performed using following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent, Flavonoids with use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's, Fehling's and Benedict's reagent; proteins and amino acids with Millon's, Biuret, and xanthoprotein test. Saponin was tested using hemolysis method. These tests were identified by characteristic color changes using standard procedures Nayak and Pareira.[16]

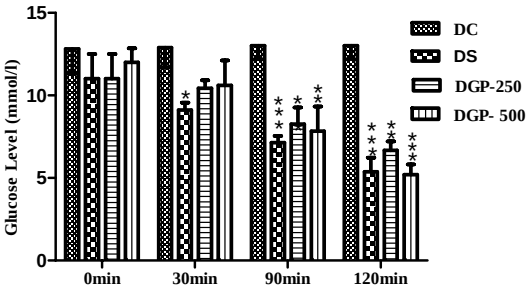

Fig 2. *Glucose tolarence effect of Glycosmis pentaphylla extract in diabetic mice. Values were expressed in Mean ±SEM. Each group comprises 5 mice. Control group received 0.5% Methyl cellulose and standard group received Metformin 100 mg/kg. *p < 0.05, **p < 0.01, and ***p < 0.001 indicate compared with diabetic control.*

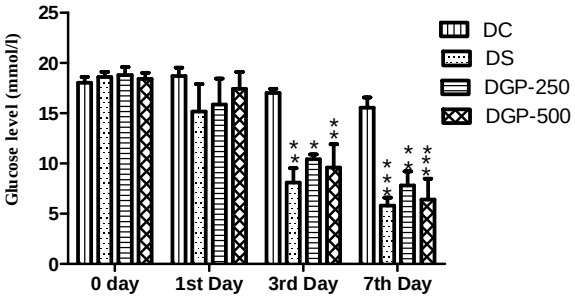

Fig 3. *Antihyperglycemic effect of Glycosmis pentaphylla extract in diabetic mice. Values were expressed in Mean ± SEM. Each group comprises 5 mice. Control group received 0.5% Methyl cellulose and standard group received Metformin 100 mg/kg body weight mice.*p < 0.05, **p < 0.01, and***p < 0.001 indicate compared with diabetic control.*

Statistical analysis

The results were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by using ANOVA followed by Tukey's test using Graph pad Prism Software version 5.03 (Graph Pad Software, San Diego, CA, USA, www.graphpad.com). P values < 0.05 were considered as statistically significant.

Results

Glucose tolerance effect of ethanolic extract

After oral administration of glucose, blood glucose levels were significantly higher in mice and results shown in Figure 2. In diabetic control peak blood glucose concentration was observed after 30 mins and remained high after next hour. Mice treated with extract in Group DGP-250 and Group DGP-500 showed a significant decrease in blood glucose concentration 50.7% and 66% respectively, at 120 mins compared with diabetic control mice.

Anti hyperglycemic effect of ethanolic extract in diabetic mice hypoglycemic test was performed and

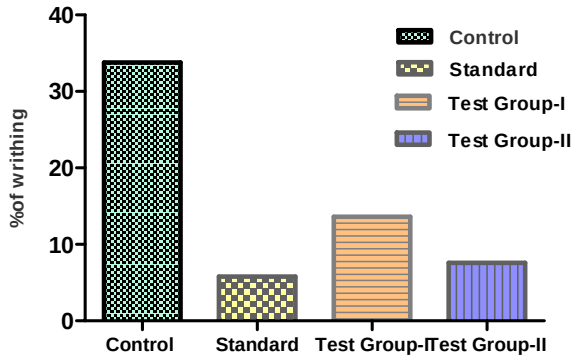

Fig 4. *Analgesic effect of ethanolic extract of Glycosmis pentaphylla. Values were expressed in Mean ± SEM. Control group mice received vehicles (1% Tween 80 in water), Standard Group received Diclofenac-Na 10 mg/kg body weight, Test Group I and Test Group II were received 250 and 500 mg/kg b. wt. of ethanolic extract of GP, respectively.*

compared with diabetic control (DC group). After 7 days of treatment with extract glucose level were significantly lowered 48.1% and 63.10% in Group DGP-250 and DGP-500, respectively (figure 3). Hypoglycemic effect was found dose dependent.

Analgesic activity of ethanolic extract

Analgesic effect of extract was observed by acetic acid-induced writhing in mice showed in Figure 4. Analgesic effect of Test Group-II exhibit highest (75.51%) writhing response than Test Group-I. Result showed dose dependent antinociception inhibitory effect against chemical induced pain in mice. However, reference drug Diclofenac-Na was more potent than plant extract at all dose levels (Figure 4).

Phytochemical screening test result of ethanolic extract

Chemical constituents were identified by characteristic color changes. The screening results were as follows: Alkaloids + ve; Carbohydrates - ve; Proteins and amino acids + ve; Phenols + ve; Flavonoids + ve; Saponin + ve and Tannins + ve. Where + ve and – ve indicates presence and absence of compounds.

Discussion

Diabetes mellitus is characterized by chronic hyperglycemia together with disturbance in glucose and lipid metabolism resulting from a deficient insulin secretion, insulin action or both. Traditional medicinal plants are used throughout the world for diabetic management. The study of such medicine might offer a new dimension for the future. In the light of literature on *Glycosmis pentaphylla* made an attempt for study antidiabetic and analgesic effects of GP leaf extract in mice. This test can be used to diagnose prediabetes and diabetes. Glucose lowering effects were found after oral administration of ethanolic extract in mice as shown in Figure 2 and figure 3. This may be due to the presence of hypoglycemic flavonoids, triterpines or saponin glycosides that required further investigation. The extract may effective for controlling diabetes by various mechanisms which finally lead to improvement of carbohydrate metabolizing enzymes towards re-establishment of normal blood glucose level. Induction of diabetes with alloxan was associated with decrease in hepatic glycogen, which could be attributed to decrease in the availability of active form of enzyme glycogen synthetase probably because of low levels of insulin.[17,18] Treatment with plant extract might increase level of enzyme to control level indicating an over-all increase in glucose influx. The exact mechanism of action needs further investigation.

Glycosmis pentaphylla has not been subjected to pharmacological investigations so far analgesic screening to provide scientific justification to its traditional claim in various pains. Therefore, present study has shown to establish remarkable analgesic potential of GP (figure 4). Acetic acid-induced writhing model represent pain sensation by triggering localized inflammatory response. The Prostaglandins mainly prostacyclin and prostaglandin-E have been reported to be responsible for pain sensation by exciting A-fibres. Activities in A-fibres cause a sensation of sharp well localized pain. Any agent that lowers the number of writhing will demonstrate analgesia preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition.[19] The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and prostaglandin pathway.[20] Flavonoids being powerful antioxidants are reported to play a role in analgesic activity by targeting prostaglandins.[21] Overall analgesic action of GP is assumed to be due to inhibition of prostaglandin synthesis.

Conclusions

Our results indicate that GP extract has antihyperglycemic effect in both short and in weeklong

duration, which is almost comparable to Metformin HCL, a known and widely used antihyperglycemic agent. The GP extract was also found to have an analgesic effect almost comparable to diclofenac sodium, a known analgesic drug. Further study is needed to confirm the anti-hyperglycemic and analgesic effect of GP including its side effects in long term use.

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