Study of the effect of *Garcinia pedunculata* fruit pulp on normal and alloxan induced diabetic mice

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Abstract

Garcinia pedunculata is an evergreen tree used in conventional therapy for a number of diseases including diabetes mellitus. The present investigation was designed to evaluate the hypoglycemic effect of the matured ripe fruit pulps of Garcinia pedunculata in normal and alloxan induced diabetic albino mice. Effect of Garcinia pedunculata extract on body weight in normal and experimental diabetic mice was also investigated in this study. Test groups of mice were made diabetic with intra-peritoneal injection of alloxan.

The mice were administered orally with the ethanolic extract of Garcinia pedunculata with a single dose of 500 mg/kg body weight from a period of day 1 to 14 days. The doses of plant extracts applied caused no acute toxicity or any behavioral changes in study animals. The levels of blood glucose were examined in all four experimental groups. The oral administration of ethanol extract for 14 days exhibited reduction in the blood glucose level compared to the diabetic control. The results supported the claim made by different traditional practitioners that aqueous extract of Garcinia pedunculata fruit pulps are effective for diabetic treatment.

Keywords: *Garcinia pedunculata*, diabetes mellitus, alloxan, hypoglycemic effect, ethanol extract.

Introduction

Diabetes mellitus is a primary disorder of carbohydrate metabolism which affects a large number of people throughout the world. Diabetes is generated due to relative lack or complete absence of insulin and insulin resistance[5]. Diabetes mellitus is a complex, serious chronic condition which is characterized by high level of blood glucose and the excretion of excess amount of glucose in the urine[9].

On clinical grounds diabetes is subdivided into Type-1 or insulin dependent diabetes milletus (IDDM) and Type-2 or non-insulin dependent diabetes milletus (NIDDM). It has been established that diabetes is associated with various long term damage, dysfunction and eventually the failure of organs, especially the liver, eyes, nerves, kidneys, heart and blood vessels. WHO projected that diabetes will be the 7th leading cause of death in 2030[6]. Therefore, management of diabetes mellitus is of prime concern in the current scenario.

Diabetes is fortunately one of the most preventable diseases. The pathogenesis of diabetes is managed by insulin and oral administration of various hypoglycemic drugs. There are various allopathic medicines currently available in the market as approved and prescribed by physicians to treat diabetic patients[4]. However, these drugs have many drawbacks. Many of the oral antidibetic agents have a large number of serious adverse effects. Management of diabetes is still becoming a big challenge for the pharmaceutical world. Therefore, the search for more safe antidiabetic agents has continued to be an important area of investigation. Many researchers are now trying to find and develop new medicines for diabetes mellitus that are safe[2].

Majority of the North East Indian population lives in rural areas. There is a high prevalence of Type 2 diabetes among the adults in Assam. Due to limitations and adverse effects of synthetic drugs, there is a clear need for development of indigenous, inexpensive herbal medicines[12]. Plant therapy were used for a long time as an alternative medicine to control metabolic disease like diabetes. Plants are always one of the most important sources of medicines. In nature there are a large number of plant species which are useful for the welfare of man and animals. A large number of plant species are still remained unexplored.

So there is an urgent necessity to explore them and to conduct systematic phamacognostic and pharmacological investigations to ascertain their therapeutic properties. According to the WHO, more than 80% of the world's population relies on traditional herbal medicine. There are many plants present in nature which possess marked antidiabetic activity[1]. There has been an increasing demand of herbal antidiabetic medicines due to their low cost, easy availability, effectiveness and lesser side effect.

Garcinia pedunculata is a large evergreen tree. This tree is most commonly found in North East India. In Assam it is locally and popularly known as Borthekera. *Garcinia pedunculata* is a tree growing around 20 metres tall with fluted trunk and spreading branches. This tree is distributed mostly near riverbank. Fruits are reported as edible and provide food supplementary. Ripe fruit is about 8-12 cm in diameter, globose and golden yellow colour. The mature ripe fruits are eaten by cooking.

Usually fruits are sliced down into small pieces, sun-dried and preserved for further uses. Fruits are found to be sour tested. Sun-dried fruits can be preserved for a long time and are used as foods. The processed dried fruit pieces of *Garcinia pedunculata* is known as "Suthi" in Assamese.

This plant contains many natural substances which can promote health. This plant has very high medicinal value. Fruits of this plant are found to be used as astringent, cardiotonic, cooling, antiscorbutic and emollient. Ripe fruit pulps of *Garcinia pedunculata* are used as antidiarrhoeic, antidysentric, in flatulence and in dyspepsia. *Garcinia pedunculata* is one of the plants that has long been used in the traditional herbal medicine against diabetes mellitus among rural community of peoples in Assam[11]. Thus the present study was designed to investigate the hypoglycemic effect of the ethanol extract of *Garcinia pedunculata* ripe fruit pulps in normal and alloxan induced diabetic mice.

Material and Methods

Plant Material: The plant was identified and authenticated by the Department of Botany, Gauhati University. After proper authentication, fresh matured ripe fruits of *Garcinia pedunculata* were collected from rural village area of Nalbari district, Assam, located in between 26 °N and 27 °N latitude and 91 °E and 91°47′ E longitude during summer.

Preparation of ethanol extract of *Garcinia pedunculata*: The matured ripe fruits of *Garcinia pedunculata* were collected locally. The fruit samples were peeled off and cut into small pieces and were dried under shade. Dried fruit pulps were soaked for 2 days in 500 mL of ethanol in a round-bottom flask with occasional shaking and stirring. The resultant mixture was magnetically stirred for further 24 hours at room temperature. The mixture was passed through cotton and then filtered through Whatmann filter paper. Then the excess of solvent of the filtrate was removed using rotary flash evaporator. The obtained crude ethanol extract was stored in airtight container in refrigerator for further studies. The yield of ethanolic extract (w/w from dried starting material) was 6.48 %.

Animal used: Healthy adult white laboratory mice of either sex with average weight of 25-30g were used as the experimental animals for the study. The selected animals were housed under normal environmental conditions in plastic cages of 18 cm × 12 cm size. They were allowed free access to standard dry pellet diet and water. The mice were kept in the laboratory as per the guidelines. The experimental protocol was approved by the Ethical Committee of Animal Research of Gauhati University.

Chemicals: All chemicals, reagents and solvents used were of analytical grade. Alloxan monohydrate was purchased from Sigma Aldrich.

Induction of experimental diabetes with Alloxan monohydrate: The induction of diabetes was done by using Alloxan monohydrate. Alloxan monohydrate freshly dissolving in distilled water was injected intraperitoneally at a single dose of 180 mg/kg body weight to mice. Hyperglycermia was confirmed by measuring the blood glucose level after 7 days of injection. Mice with diabetes indicated by hyperglycemia were considered for antidiabetic study. The mice that did not show elevated blood glucose were rejected from our investigation.

Experimental designs

The mice were divided into four groups and each group was consisting of six mice.

Group I: Normal control mice received only normal diet and water.

Group ll: Normal mice treated with a single dose of ethanol extract of *Garcinia pedunculata* orally at a dose of 500 mg/ kg body weight daily one time for 14 consecutive days.

Group III: Mice of this group were alloxan-induced diabetic model and served as diabetic controls without any therapy.

Group IV: Diabetic mice were treated with ethanol extract of *Garcinia pedunculata* orally at a dose of 500 mg/ kg body weight daily one time for 14 consecutive days.

Oral administration to mice: The plant extracts were dissolved in minimum volume of water and administered orally to the mice by using syringe with the help of polythene tubing fixed to the tip of a long needle.

Estimation of Blood Glucose: The blood glucose levels of mice were estimated by using one touch commercial glucometer and glucose test strip. In this experiment GLUCOCARD™ 01 SENSOR blood glucose monitoring glucometer was used.

Statistical analysis: All the values of results were expressed as mean values ± standard error of mean (SEM). The significances of the differences between the means of the tests and control studies were established by t-test and ANOVA; *P* values <0.05 were considered significant.

Results and Discussion

Preliminary phytochemical screening: Preliminary phytochemical screening of crude extract of the ripe fruit of *Garcinia pedunculata* was done for their chemical constituents by utilizing standard conventional protocols. The results of phytochemical studies on ethanol extracts are given in table 1.

Toxicity studies: Acute toxicity studies were conducted for the ethanol extract of *Garcinia pedunculata* fruit in order to select a suitable dose for evaluation of antidiabetic activity. The mice were treated with the extract at doses of 100, 500 and 1000 mg/kg body weight orally. The mice were observed for 24 hours for signs of toxicity. Even at this high dose also, there were no gross behavioural changes of the animals.

Effect on body weight: Weight loss is generally one of the major complications in diabetes. In our experiment also it has been observed that weights of the diabetic mice were reduced. It arises due to impairment in insulin action which is caused by alloxan toxicity.Control untreated mice were found to be stable in their body weight during the experiment

period. A decrease in body weight was observed in all alloxan treated diabetic mice compared to the normal control group. All the animals in diabetic control group (Gr III) showed a loss in body weight (from 30.48 g to 21.72 g) and it was persistently observed till the end of experiment i.e. 21 days (Table 2).

Table 1
Phytochemical present in ethanol extract of *Garcinia pedunculata*

Phytochemical	Ethanol extract
Steroids	-
Glycosides	+
Alkaloids	-
Flavonoids	+
Anthraquinones	-
Terpenoids	-
Reducing sugar	+
Saponins	+
Tannins	+
Phenolic compounds	+

Key: + = Present; - = Absent

In group IV mice, the body weight was initially reduced (from 28.98 g to 22.88 g) after alloxan monohydrate treatment. But it has been observed that the average body weights in the group IV were slightly increased (22.88 g to 23.17 g) as compared to those of the diabetic control mice by the treatment of *Garcinia pedunculata* extract after 14 days of treatment. The treatment *Garcinia pedunculata* extract slightly prohibited the reduction in body weight in the diabetic mice showing slight gain in weight. Alloxan mediated body weight loss was slightly reversed by the *Garcinia pedunculata* extract.

Effect on blood glucose level: The blood glucose levels were estimated on various days starting form day 0^{th} up to 14^{th} day. The effect of extracts on blood glucose levels in normal and alloxan induced diabetic mice are reported in table 3. The blood glucose levels were found to be increased gradually as the time increased post administration of alloxan in mice. The results showed that treatment with single dose of alloxan at a dose of 180 mg/kg body weight after 7 days caused significant increase ($p < 0.05$) in blood glucose levels of mice. A marked rise in fasting blood glucose level was observed in diabetic control compared to normal control mice.

Oral administration of single dose of ethanol extract of *Garcinia pedunculata* (500 mg/kg body weight) was given upto 14^{th} day. The mean blood glucose level decreased from 114 mg/dl to 104 mg/dl of non-diabetic normal mice in group II treated with ethanol extract. The extract produced 8.77% reduction of blood glucose level in normal mice. A decrease in blood glucose level was observed in group IV diabetic mice from an initial level of 302 mg/dl to 259 mg/dl after 14 days of extract treatment. The *Garcinia pedunculata* extract produced 14.24% reduction of blood glucose level in diabetic mice.

Comparison of the mean values of blood glucose levels in the extract treated and untreated (control) groups of alloxan-induced diabetic mice have suggested some favorable antidiabetic effects of *Garcinia pedunculata.* Extract of *Garcinia pedunculata* produced a small amount of reduction in the blood glucose in both normal and diabetic mice. The results of the present study have shown a mild hypoglycemic effect of ripe fruit of *Garcinia pedunculata* when administered to alloxan induced diabetic mice.

Diabetes mellitus is one of the major global diseases found in all nations of the world. Diabetes creates specific long-term complications affecting the retina, heart, kidney and nervous system. Effective and proper blood glucose control are essential for preventing diabetic complications in diabetic patients.

Table 2
Effect of ethanol extract of ripe fruit of *Garcinia pedunculata* on body weight (g) in normal and alloxan-induced diabetic mice.

Groups	Treatment	Mean body weight in gram			
		Initial	0^{th} day	7^{th} day	14^{th} day
Group I. (Untreated normal control mice)	Without any therapy	29.76 ± 0.64	28.59 ± 0.17	27.70 ± 0.28	27.96 ± 0.39
Group II. (Plant extract treated normal mice)	plant extract	28.68 ± 0.30	29.06 ± 0.09	29.24 ± 0.35	29.95 ± 0.10*
Group III. (Diabetic control mice)	Alloxan only	30.48 ± 0.22	23.64 ± 0.27	22.47 ± 0.18	21.72 ± 0.16
Group IV. (Plant extract treated diabetic mice)	Alloxan + plant extract	28.98 ± 0.14	22.88± 0.15*	22.45 ± 0.24	23.17 ± 0.11*

Results were expressed as mean values ± standard error of mean (Mean ± SEM) for 6 mice in each group (n=6); *$p < 0.05$ Vs Control.

Table 3
Effect of ethanol extract of ripe fruit pulp of *Garcinia pedunculata* on blood glucose level of normal and alloxan-induced diabetic mice.

Groups	Treatment	Blood glucose level (mg/dl)		
		0^{th} day	7^{th} day	14^{th} day
Group I. (Untreated Normal Control mice)	Without any therapy	106 ± 3.40	108 ± 3.57	112 ± 2.79
Group II. (plant Extract treated Normal mice)	plant extract	114 ± 1.83	110 ± 3.24*	104 ± 3.16
Group III. (Diabetic control mice)	Alloxan only	296 ± 2.38	302 ± 4.04	314 ± 5.44
Group IV. (plant Extract treated Diabetic mice)	Alloxan + plant extract	302 ± 3.11*	277 ± 3.16	259 ± 2.90*

Results were expressed as mean values ± standard error of mean (Mean ± SEM) for 6 mice in each group (n=6); *p < 0.05 Vs Control.

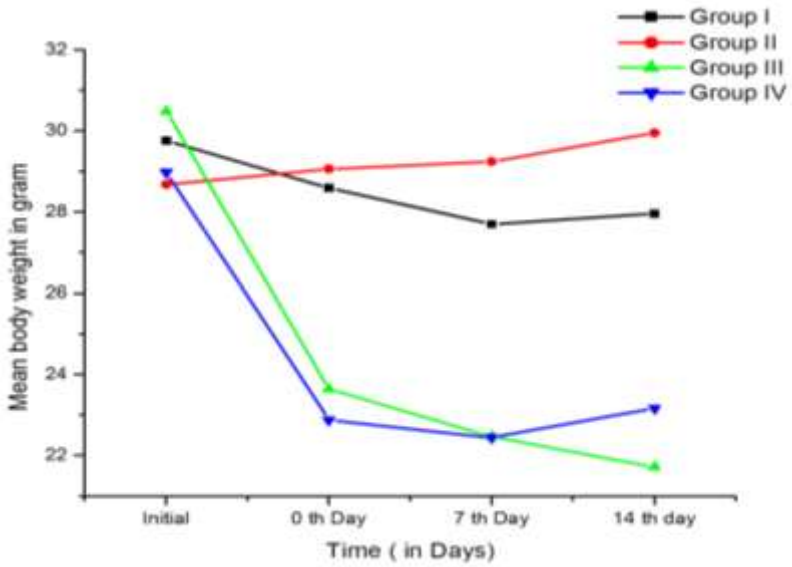

Fig. 1: Effect of ethanol extract of ripe fruit of *Garcinia pedunculata* on body weight (g) in normal and alloxan-induced diabetic mice.

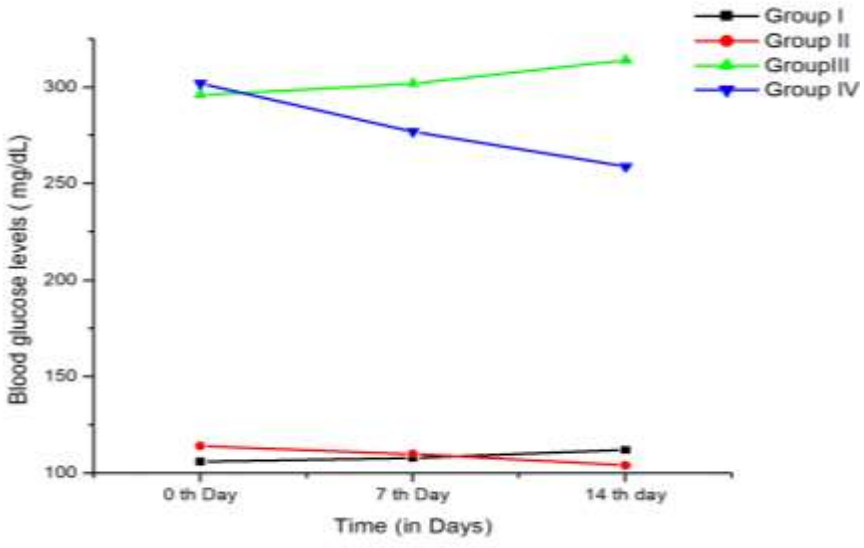

Fig. 2: Effect of ethanol extract of ripe fruit pulp of *Garcinia pedunculata* on blood glucose level of normal and alloxan-induced diabetic mice.

Apart from insulin, different types of blood glucose - lowering drugs including insulin sensitizers, insulin secretagogues, glucosidase inhibitors etc. have been developed[3]. Most of the glucose-lowering drugs have many side effects including risk for hypoglycemia, rare lactic acidosis, abdominal discomfort, headache, dizziness, weight gain, permanent neurological deficit, mild anemia, liver injury or liver toxicity etc.

Several research scholars have reported flavonoids, sterols, tannins, phenolic compounds, glycosides, saponins, alkaloids and polyphenols as bioactive antidiabetic principles. The results of the phytochemical screening carried out on the ethanol extract of *Garcinia pedunculata* fruit have revealed the presence of glycosides, flavonoids, reducing sugar, saponins, tannins and phenolic compounds. Saponin, an abundant secondary metabolite present in the *Entada phaseoloides* seed was reported to have dramatically reduced fasted blood glucose levels in type 2 diabetic[14]. Flavonoids are the largest group of naturally obtained aromatic compounds which are isolated from plants.

Alloxan monohydrate is a cytotoxic compound. As it has been widely accepted that alloxan monohydrate selectively destroys the insulin secreting beta-cells of pancreas, hence it is used to induce diabetes in laboratory animals. Alloxan causes destruction of ß-cells of the islets of langherhans resulting in reduction of synthesis of insulin. Alloxan-induced diabetes generally produces all the main characteristics of diabetes including polyphagia, polydipsia, polyuria, weight loss and hyperglycemia.

Various experimental studies have clearly shown that diabetes is associated with increased formation of different free radicals and decrease in antioxidant potential. Oxidative stress is increased in diabetes. Hyperglycemia is also associated with the generation of reactive oxygen species (ROS) and consequent excessive oxidative stress which ultimately leads to several diabetic complications[10]. Through several mechanisms, antioxidants counter the action of free radicals. Fruit of *Garcinia pedunculata* has strong antioxident activity[7].

Antioxident activity of *Garcinia pedunculata* fruit is experimentally established. It can help diabetic patients through its antioxident activity and can protect β cells from oxidative stress. Thus, the widely available *G. pedunculata* is a health benefit fruit which might be helpful in preventing or slowing the progress of oxidative stress of diabetic patients and could be used as an easy accessible source of natural antioxidant. *Garcinia pedunculata* fruit extract was also reported to have hepatoprotective activity[8]. It can protect the liver damage in diabetic patients.

The results of this study using extracts of the *Garcinia pedunculata* fruit have shown that it has some potentiality as antidiabetic medication. The percentage increase of blood glucose levels in the untreated groups appeared to be higher than that in the extract treated group.

From the present experimental results, it can be suggested that *Garcinia pedunculata* fruit are useful as an antidiabetic remedy and its complications. The present investigation indicates that the fruit extract of this plant should be studied further.

Conclusion

The result depicted that ethanol extract of ripe fruit pulps of *Garcinia pedunculata* has mild hypoglycemic and antihyperglycemic effect. The observation and studies are only preliminary and based on the present findings under our experiment conditions, antidiabetic activity of the plant extract can further be studied in diabetic rats. The plant extract was found to be completely safe for further biological activity studies as no lethality was observed upto 1000 mg/kg per oral dose in mice.

However, further additional investigations are required to carry out the separation, purification and identification of the bioactive components of *Garcinia pedunculata* extract and to elucidate the proper mechanism of hypoglycemic effect of the extract.

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