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Effects of Aqueous Extract of Guava (*Psidium guavaja linn*) Leaves on Blood Glucose Level in Alloxan Induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is an important cause of morbidity and mortality in many countries including Bangladesh. Herbal medicines have several advantages such as fewer side effects, less expensive and well accepted. **Objectives:** The study was done to evaluate the effects of aqueous extract of *Psidium guajava linn* leaves on blood glucose level of alloxan induced diabetic rats.

Material and Methods: The experiment was carried out in the department of Pharmacology of Dhaka Medical College, Dhaka from July 2012 to June 2013. Thirty-two healthy Long Evan Norwegian strains of rats were equally divided into four groups (A, B, C and D). group A received standard rat food. Group B was given aqueous extract of *Psidium guajava* leaves 100mg/kg/day. Diabetes was introduced by administration of Alloxan 120mg/kg body weight in group C and D.

Conclusion: The result showed that aqueous extract of *Psidium guajava* leaves has no effects on blood glucose level in normal rats but can reduce blood glucose level in alloxan induced diabetic rats which needs further investigation.

Keywords: Diabetes mellitus, *Psidium guajava*, Alloxan, Rats, Blood glucose

Introduction

Diabetes mellitus is a metabolic disorder resulting in raised blood glucose level (hyperglycemia) from defect in insulin secretion, insulin action or both that arises from genetic as well as environmental factors. It is defined by documenting blood glucose in fasting state(≥7.0mmol/L) or 2 hours after an oral standard glucose drink (≥11.0mmol/L) [1]. The chronic hyperglycemia is associate with long term damage, dysfunction and failure of various organs, specially the eyes, kidneys liver heart and blood vessel [2]. Herbal medicines have several advantages such as fewer side effects, better patient tolerance, relatively less expensive and well accepted due to long history of use. The most important cause is that herbal medicine provides rational means for the treatment of many diseases that are obstinate and incurable in other system of medicine [3]. Guava tree is basically from Meso American area. It can also find in tropical and subtropical areas. Guava tree is member of myrtaceae family, all the parts of the tree widely use in curing many health problems. A lot of work on pharmacological researches has been done to demonstrate the use of extract from guava leaves which provided that guava

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leaves extracts are such a useful medicine, widely used by doctors and pharmacists. WHO also says that plants would be the best source of obtaining different types of medicines and drugs. These natural products are widely used by human with its effective results. Extraction from guava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C [4]. The leaves of guava are rich in flavonoid, in particular, quercetin. Quercetin is the main flavonoid in guava leaves contribute to its anti hyperglycemic effects. Guava leaves also has anti oxidant properties which is attributed to the poly phenol found in the leaves [5] .It has also been demonstrated that flavonoids of guava leaves can act as insulin secretagogues or insulin mimetics, probably by influencing pleotropic mechanism, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression the rate-limiting enzymes involving in carbohydrate metabolism pathway. In study it was found that flavonoids were act directly on pancreatic beta cell leading to activation of the cAMP/PKA signaling cascade to exert an insulinotropic effects [6]. With the back ground information, in this study, attempt has been made to evaluate the effect of *Psidium guajava Linn* extract in alloxan induced diabetic rats. Blood glucose level has been estimated to the extent of pancreatic damage.

Materials and Methods

The study has been performed in the department of Pharmacology at Dhaka Medical College, Dhaka from July 2012 to June 2013. A total number of 32 healthy Long Evan Norwegian strain weighing between 140 – 150 gm and age between 8-10 weeks which were purchased from Bangladesh Centre for Scientific and Industrial Research (BCSIR) lab were used for the study. The rats were allowed to live at room temperature with 12 hours of light and 12 hours dark schedule. They were fed normal rat diet and given water *ad libitum*. The rats were divided into 4 (A, B, C, D) groups. Each consists of 8 rats.

Collection and Preparation of Plant Materials

Psidium guajava leaves were collected from local garden. The plant was authenticated as *Psidium guajava* (guava) leaves by Bangladesh National Herbarium, Mirpur, Dhaka. DABC association number 38570. Aqueous extract was made in the Drug Research Laboratory Centre for Advanced Research of science (CARS) of Dhaka University. 1 kg of fresh *Psidium guavaja* leaves were collected, cleaned and shed dried for two weeks. Then the dried leaves were grounded into powder using electric grinder and the powder was stored in the air tight container in the laboratory. A total of 250 gm of the ground powder was weighed out and soaked in 1000ml of distilled water with continuous shaking (40rmp) at 25°C for three days and filtrated by Watman filter paper. The extract was evaporated under vacuum rotator evaporator at 35-degree temperature to obtain final deep green semisolid extract. A total of 32-gram extract was found in this way.

Experiment Design

The experiment was divided into two parts: experiment 1 and experiment 2.

Experiment 1

This part of the experiment was carried out to demonstrate the effect of aqueous extract of *Psidium guajava* A (Guava) leaves on blood glucose level in experimental non diabetic rats. It was comprised of 16 rats which were divided into 2 group, each having 8rats. Groups were leveled as group A and Group B. all the rats were fasted over night before the collection of blood.

Group A (Control group)- was given standard rat food for 14 days. Fasting blood glucose was estimated on day 1 and day 15 of the experiment.

Group B: was given aqueous extract of *Psidium guajava* leaves 150mg/kg/day orally along with standard rat food for 14 days. Fasting blood glucose level was estimated on day 1 and day 15 of the experiment.

Experiment 2

It was comprised of 16 rats which were divided into 2 groups each having 8 rats. Groups were labeled as group C and Group D. all these rats were fasted overnight before collection of blood. Group C (diabetic control group): was given alloxan 120mg/kg intraperitonially for induction of diabetes on day 1. After alloxan injection rats were given standard food. Fasting blood glucose level were estimated on day 1(before alloxan), on day 4 and day 15 of the experiment. Group D: was given alloxan 120mg/kg intraperitonially on day 1. After alloxan injection rats were given standard rat food. Then after 3 days aqueous extract of *Psidium guajava* leaves 150mg/kg was given along with rat food for 10 days. Fasting blood glucose level was estimated on day 1(before alloxan), on day 4 and on day 15 of the experiment.

Observation and Result

Effects of AEPGL (aqueous extract of *Psidium guajava* leaves) on blood glucose level in non diabetic rats: In group A: the blood glucose levels (Mean± SD) were 5.80± 0.52 and 5.80± 0.45 on day 1 and day 15 respectively. percentage change was 2.30. the results are shown in table 1. In group B, the blood glucose levels (Mean± SD) were 5.50± 0.46 and 5.60± 0.39 on day 1 and day 15 respectively. percentage change was 2.18. the results are shown in table 1.

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Table 1: showing the Effects of AEPGL (aqueous extract of *Psidium guajava* leaves) on blood glucose level in non diabetic rats

Group	FBG (mmol/L) on day 1 (Mean± SD)	FBG (mmol/L) on day 15 (Mean± SD)	Percent change
A(n=8)	5.80± 0.52	5.80± 0.45	2.3
B(n=8)	5.50± 0.46	5.60± 0.39^{ns}	2.18

Percentage change on day 15 from day 1. Comparison of FBG level on day 15 with control (group A) done by unpaired student's 't' test, Ns= not significant.

Group A- standard rat food and water were given. Group B- AEPGL (aqueous extract of *Psidium guajava* leaves) at 150mg/kg/day and standard rat food and water were given. Effects of Alloxan on blood glucose level of group C and D rats on day 4: In group C, the blood glucose levels (Mean± SD) were 5.50± 0.64 and 15.00± 3.35 on day 1 and day 4 respectively. percentage change was 173.30. the results are shown in table 2. In group D, the blood glucose levels (Mean± SD) were 5.80± 0.63 and 15.05± 3.45 on day 1 and day 4 respectively. percentage change was 163.60. the results are shown in table 2.

Table 2: Showing the Effects of alloxan on blood glucose level in group C and D

Group	FBG (mmol/L) on day 1 (Mean± SD)	FBG (mmol/L) on day 4 (Mean± SD)	Percent change
C(n=8)	5.50± 0.64	15.00± 3.35	173.3
D(n=8)	5.80± 0.63	15.05± 3.45^{ns}	163.6

Percentage change on day 4 from day 1. Comparison of FBG level on day 4 with control (group C) done by unpaired student's 't' test.

Ns= not significant

Group C and D were given alloxan 120mg/kg single I/P injection on day 1.

Effects of AEPGL (aqueous extract of *Psidium guajava* leaves) on blood glucose level in diabetic rats

In group C, the blood glucose levels (Mean± SD) were 5.50± 0.64 and 16.00± 2.85 on day 1 and day 15 respectively. percentage change was 193.20. the results are shown in table 3. In group D, the blood glucose levels (Mean± SD) were 5.80± 0.63 and 8.05± 2.05 on day 1 and day 15 respectively. percentage change was 43.10. the results are shown in table 3.

Table 3: Showing the Effects of AEPGL (aqueous extract of *Psidium guajava* leaves) on blood glucose level in diabetic rats

Group	FBG (mmol/L) on day 1 (Mean± SD)	FBG (mmol/L) on day 15 (Mean± SD)	Percent change
C(n=8)	5.50± 0.64	16.00± 2.85	173.3
D(n=8)	5.80± 0.63	8.05± 2.05***	+43.10.

Percentage change on day 4 from day 1. Comparison of FBG level on day 4 with control (group C) done by unpaired student's 't' test

***= significant.

Group C- standard rat diet and water were given Group D- AEPGL (aqueous extract of *Psidium guajava* leaves), 150mg/kg/day and standard rat food with water were given.

Discussion

The present study was carried out to evaluate the effect of aqueous extract of *Psidium guajava* leaves on experimentally induced diabetic rats. The blood glucose lowering effect of aqueous extract of *Psidium guajava* leaves was tested in non diabetic and experimentally induced diabetic rats. The aqueous extract of *Psidium guajava* leaves was given for 14 days in non diabetic rats and for 10 days in alloxan induced diabetic rats. In the present study, diabetes was induced by alloxan. The dose and route of administration of alloxan monohydrate was selected from Andrade et al. (2000) [7] and Kim et al (2006) [8] respectively. The blood glucose levels in rats were measured 72 hours after administration of Alloxan which was done according to experiment of Etuk et al (2010) [9]. In this study, intra peritoneal administration of single dose of alloxan (120mg/kg), increased blood glucose level significantly. Similar observations were reported by number of researchers. Ghosh et al (2010) [10] observed the condition of diabetes after 24 hours of intravenous injection of sterile, freshly prepared 1% alloxan monohydrate solution at a dose of 40 mg/kg in albino rats. Jeloder et al (2005) [11] in their experiment observed the effects of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of diabetic rats, by inducing diabetes in 20 out of 25 adult male albino rats by intraperitoneal injection of 185 mg/kg of alloxan. In present study, the rise of blood glucose level in experimental rats was also very high. The dose of *Psidium guajava* leaves (150mg/kg body weight, and the duration of the study was selected in keeping confirmatory with dose and duration used in research work by Yesmin (2010) [5], Banu et al (2012) [12], Deguchi Y, Miyazaki K (2010) [13], Mukhtar et al (2006) [14]. The leaves of guava are rich in flavonoid, in particular, quercetin. Quercetin is the main flavonoid in guava leaves contribute to its anti hyperglycemic effects. Guava leaves also has anti oxidant properties which is attributed to the poly phenol found in the leaves (Yesmin, 2010) [5]. Flavonoid compounds act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance (Bhathena et al 2002) [15]. The (-)- epicatechin gallate, myricetin, quercetin, apigenin, (-) epigallocatechin demonstrated a marked reduction in glucose absorption, when compared with control, by competitive inhibition of sodium dependent glucose transporter (Shimiju et al 2000) [16]. It has also been demonstrated that flavonoid compounds of guava leaves can act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanism, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/

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or expression the rate limiting enzymes involving in carbohydrate metabolism pathway. In study it was found that flavonoids act directly on pancreatic beta cell leading to activation of the cAMP/PKA signaling cascade to exert an insulinotropic effect (Liu et al 2006) [6]. It was observed that aqueous extract of *Psidium guajava* leaves has glucose lowering effect in alloxan induced diabetic rats but no effect on blood glucose level on non diabetic rats. The result suggested that the aqueous extract of *Psidium guajava* leaves may be useful anti diabetic agent in treatment of diabetes mellitus. It is suggested to measure plasma insulin level, haemoglobin A1C, liver glycogen level, lipid hydro peroxidation level and free radicals in the tissue after treatment with aqueous extract of *Psidium guajava* leaves. Despite all these limitations, interpretations of the results obtained in this study was made carefully and cautiously.

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