Original Article

The Effect of Ethanol Extract of Guava Leaf (Psidium guajava Linn.) on Pancreatic β cells of Alloxan-induced Diabetic Rats

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ABSTRACT:

Background: *Diabetes mellitus is a metabolic disorder characterised by elevated blood glucose levels due to defects in insulin secretion, insulin action, or both, arising from genetic and environmental factors. Drug treatment of Diabetes is expensive and has many adverse effects. Herbal medicines have fewer side effects, less expensive and are well tolerated.*

Objectives: *The study was done to evaluate the effect of ethanol extract of Guava Leaf (Psidium guajava Linn.) on Pancreatic β cells of alloxan induced Diabetic Rats.*

Materials and methods: *The experiment was carried out in the department of Pharmacology, Dhaka Medical College, Dhaka, from July 2012 to June 2013. Forty healthy Long Evan Norwegian strains of rats were equally divided into 5 groups (A, B, C, D and E). Group B, C, D and E were made diabetic with injection Alloxan 120mg/kg body weight. Group A received Standard rat foods. Group C, D and E received guava leaves extracts respectively by oral administration. The whole pancreas from each animal were removed and histological examination was done.*

Conclusion: *The result showed that Ethanol Extract of Guava Leaf (Psidium guajava Linn.) has significant regeneration on pancreatic β cells.*

Key Words: Diabetes Mellitus, Ethanol extract, β cell, Alloxan, Guava Leaf

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Introduction:

The worldwide prevalence of Diabetes mellitus (DM) has risen dramatically over the past two decades. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in2030 (Wild S et al)[1]. Although the prevalence of both type 1 and type 2 DM is expected to rise more rapidly in future because of increasing obesity and reduced physical activities. At least 135 million people are suffering throughout the world, and the numbers are increasing in rural and poor populations throughout the world. DM is an important cause of morbidity and mortality in many countries in the world, including Bangladesh. It is one of the major causes of disability leading to loss of manpower, labour, and decreased productivity in all spheres of life (Salahuddin, 2006)[2].

Medicinal plants constitute an important natural wealth

of a country. They play a significant role in providing primary healthcare service to rural people. According to WHO, herbal medicine composed mainly of medicinal plants, are still curing diseases of an estimated 3.5 billion of the world's population as they can't afford Western pharmacological drugs. (Ghani, 2003)[3]

Medicinal herbs with anti-hyperglycemic activities are increasingly sought by diabetic patients and health care professionals. To date, over 400 traditional plant treatments for diabetes have been reported, although a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The WHO Expert Committee on Diabetes has recommended traditional medicinal herbs for further investigation. (Dey et al 2002)[4].

The common guava tree (Psidium guajava Linn.) is a member of Myrtaceae family, which is native to tropical and subtropical countries. Its fruit is commonly used as food and processed as juice and jam. Aside from these uses, Gutierrez et al. (2008)[5] have reviewed the potential pharmacological activities of the extract from fruit, leaf, bark or roots. The extract of whole plant of Psidium guajava excluding roots was reported to be acted as antibacterial, anti fungal, anti viral, hypoglycemic, diuretic and anti- inflammatory activities (Tripathi et al, 1981)[6]. A significant reduction of blood glucose level was observed in diabetic rats fed with fruit, stem bark, and leaves (Mukhtar et al. 2007)[7], (Rai et al., 2007)[8], (Shen, 2008)[9]. Research on extract of guava leaves has shown that, it has anti diabetic property and can reduce blood glucose level significantly (Oh et al., 2005)[10], (Ojowole, 2005)[11], Shen et al., 2008)9. Very little information is available of guava leaves extract on the protection of pancreatic β cell. So, there is a need to evaluate the effectiveness of guava leaves extract that can reduce the blood glucose level and increase the β cell function by repair and regeneration at the same time.

Materials and methods

The study has been performed in the department of Pharmacology at Dhaka Medical College, Dhaka, from July 2012 to June 2013.

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.

Ethanol extract was made in the Drug Research Laboratory Centre for Advanced Research of Science (CARS) of Dhaka University. 1 kg of fresh Psidium guajava leaves were collected, cleaned and shed dried for two weeks. Then the dried leaves were ground into powder using an electric grinder and the powder was stored in an airtight container in the laboratory. A total of 250 gm of the ground powder was weighed out and soaked in absolute ethanol (2L) with continuous shaking (40 rpm) at 25^{o}C for three days and filtered by Whatman filter paper. The extract was evaporated under vacuum rotator evaporator at 35 degrees to obtain the final deep green semisolid extract. A total of 30-gram extract was found in this way. The extract was preserved at 4^{0}C until preparation of suspension with distilled water for oral administration.

Experiment:

A total number of 40 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 a normal rat diet and given water ad libitum.

The rats were divided into 5 (A, B, C, D and E) groups. Each consists of 8 rats.

The rats in group B, C, D and E were made diabetic with intra peritoneal injection Alloxan 120mg/kg body weight after an overnight fast on day 1. Diabetes was confirmed on day 4 after Alloxan injection by determining the blood glucose level.

Group A served as the normal control group (negative control), while group B served as the diabetic control (positive control).

Group C, D and E received 0.25g/kg/day, 0.50/kg/day and 1g/kg/day of guava leaves extracts respectively by

oral route. The extract administration lasted for 10 days. The whole pancreas from each animal was removed after sacrificing the animal. The pancreas was collected in 10% formalin solution and immediately processed by paraffin technique. Sections of 5µ thickness were cut and stained by the Gomori staining technique for histological examination. The percentage of necrotic β cells were counted using the following formula:

Percentage of necrosedβ cells=(No of necrosed β cell)/(-No of total β cells) x 100%

Data were subjected to One Way ANOVA to compare the difference among treatments and followed by a Duncan multiple rang test (DMRT).

Result:

The effects of ethanol extract of Psidium guajava L on the reduction of number of pancreatic β cells (%) are shown in table 1.

Table 1: the percentage of pancreatic β cells necrosis of control and diabetic rats treated with ethanol extract of guava leaves for 10 days

Treatment group	% of necrosed pancreatic β cell (mean ± SD)
Group A (negative control, n=8)	14.89±0.85
Group B (positive control, n=8)	48.25±2.25
Group C (0.25g/kg/day, n=8)	40.45±4.35
Group D (0.50g/day, n=8)	38.93±3.63
Group E (1g/kg/day, n=8)	30.05±3.62

Mean values in the same column show significant difference ($P<0.05$)

The result indicated that there was a significant ($P<0.05$) reduction in the percentage of necrosis of pancreatic β cells in guava leaf extracts treated rats (Group C, D and E) when compared with untreated rats (Group B). The percentage of necrosis was still significantly more than that obtained for normal rats (group A). Groups C and D showed close similarity.

Discussion:

The present study was carried out to evaluate the effect of ethanol extract of guava leaf (Psidium guajava Linn.) on pancreatic β cells of alloxan induced diabetic Rats.

In the present study, diabetes (necrosis of pancreas) was induced by Alloxan. The dose and route of administration of alloxan monohydrate was selected from Andrade et al. (2000)[12] and Kim et al (2006)[13] 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)[14]. In this study, intra peritoneal administration of single dose of Alloxan (120mg/kg), increased blood glucose level significantly.

The doses of Psidium guajava leaves (0.25g/kg/day, 0.50g/kg/day, 1g/kg/day body weight), and the duration and equation of the study was selected in keeping confirmatory with dose and duration used in research work by Amalia et al. (2010)[15], Yesmin (2010)[16], Banu et al (2012)[17], Deguchi Y, Miyazaki K (2010)[18], Mukhtar et al (2006)[7]

The leaves of guava are rich in flavonoid, in particular, quercetin. Quercetin is the main flavonoid in guava leaves that contributes 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)[16].

In study it was found that flavonoids act directly on pancreatic beta cell leading to activation of the cAMP/P-KA signaling cascade to exert an insulinotropic effect (Liu et al 2006)[19].

Puchchakayala (et al 2012)[20] revealed that the blood glucose lowering activity of flavonoid compounds: Boswellic acid, Ellagic acid, Quercetin and Rutin may be by stimulating beta cells to release more insulin. The pronounced activity of these compounds could be because of enhanced peripheral glucose utilization of skeletal muscle in addition of that beta cell stimulation.

In this study, it is shown that, guava leaf extract may have some chemical compounds that can regenerate the pancreatic β cells that was necrosed previously by alloxan. Signs of regeneration of β cells, potentiation of insulin secretion from surviving pancreatic β bells and reducing the blood glucose level have been reported after consumption of some plant extracts (Shanmugasundaram et al. 1990)[21]; (Yadav et a.l, 2008)[22].

Conclusion:

The results of this study indicate that the ethanol extract of Psidium guajava Linn. leaves have significant effects

on regenerating pancreatic β cells in Alloxan-induced diabetic rats. A higher dose of the extract has a greater restorative effect than a lower dose. Further investigation with a higher dose for a long period of time may show clearer features of these findings.

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