Glycaemic Control by the Aqueous Bark Extract of *Spondias Pinnata* Against Alloxan Induced Diabetes Mellitus

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Introduction: Medicinal plant extracts have been used as a popular medicine for the treatment of diabetes mellitus since ages. The increasing trends in the use of plants as medicines globally, necessitate scientific investigations on novel antihyperglycaemic agents from medicinal plant extracts. The single administration of aqueous bark extract of *Spondias pinnata* (SPAq, Sinh. Emberella) showed remarkable efficacy in diabetic rats. Thus the present study aims to investigate the effect of repeated administration of SPAq on glycaemic control in normal and alloxan induced diabetic rats.

Methodology: Wistar rats were divided into five groups (n=6). Group 1 and 2 served as untreated normoglycaemic and alloxan induced diabetic rats (150 mg/kg i.p.) respectively. Group 3 and 4 were diabetic rats treated with the SPAq (1.00 g/kg) and glibenclamide (0.5mg/kg) for 30 days respectively. Group 5 was normoglycaemic rats treated with the same dose of SPAq. Oral glucose tolerance test was performed and body weights of animals were recorded at weekly intervals. Glycosylated haemoglobin percentage (%HbA_{1c}) was estimated on the 30th day.

Results: The SPAq and glibenclamide improved glucose tolerance by 41% and 53% ($p<0.05$) respectively in diabetic rats. The SPAq and glibenclamide reduced HbA_{1c} to 6.85±0.02% and 6.31±0.04% respectively ($p<0.05$). The decrease in HbA_{1c} in SPAq treated normoglycaemic rats was found to be statistically non –significant ($p<0.05$). In contrast the administration of SPAq to diabetic rats restored the bodyweights of animals compared to untreated diabetic rats.

Conclusion: The aqueous extract of *Spondias pinnata* improved the glycaemic control in alloxan induced diabetic rats.

Key words: *Spondias pinnata*, glycaemic control, diabetic rats

Introduction

Diabetes mellitus is one of five leading causes of death and debilitating disease in the world, presents enormous and increasingly important public health issues globally. The prevalence of diabetes mellitus in all age groups was estimated to be 2.8% in 2000 and the rate is expected to rise to 4.4% in 2030 (Ignacimuthu and Balamurugan 2011). In modern medicine no satisfactory therapy is available to cure diabetes mellitus (Ahmed et al. 2010). Current oral anti-diabetic agents, which include insulin releasers, insulin sensitizers and α-glucosidase inhibitors, have modest efficacy and limited modes of action. In addition, current antidiabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long term complications and low cost-effectiveness (Yi-Jou Hsu et al. 2009). Therefore, discovery and development of novel drugs for diabetes mellitus is still needed.

The use of plants as sources of remedies for the treatment of many diseases dates back to prehistory and people of all continents has this old tradition. According to a WHO estimate, majority of the population in developing countries depends on traditional and herbal medicine as their primary source of health care (Marquele-oliveria et al. 2008). As in many developing countries of the world, traditional medicine in particular the herbal medicine is part of the Sri Lankan culture. The rich Sri Lankan flora of medicinal plants contributes to this practice. About one hundred and twenty six plants belonging to fifty one families are used to treat diabetic patients in Sri Lanka and more than six hundred species are documented as antihyperglycaemic in traditional medicine presently (Ediriweera and Rathnasooriya 2009). However only a small portion of the antihyperglycaemic plants as well as formulations used in traditional medicine have been pharmacologically evaluated for their efficacy.

Spondias pinnata (Family: Anacardiaceae) is commonly known as Emberella. It is a small or moderate-sized, deciduous tree with a straight trunk. The bark of this tree is used for treating dysentery and diabetes mellitus .The juice of the leaves is used for ea-ache. The fruit is an antiscorbutic and the acidic and astringent pulp is used for bilious dyspepsia (Jayaweera 1980). The bark extract of this plant possess antimicrobial and antioxidative and hepatoprotective effects. (Keawsa-ard and Liawruangrath 2009, Rao and Jayaraju 2010, Daduang et al 2011). The single administration of aqueous bark extract of *Spondias pinnata* (SPAq, Sinh. Emberella) showed remarkable efficacy in diabetic rats. Thus the present study aims to investigate the effect of repeated administration of SPAq on glycaemic control in normal and alloxan induced diabetic rats.

Materials and Methods

Chemicals

Alloxan monohydrate, D-Glucose, Glibenclamide were purchased from Sigma- Aldrich Company (St Louise, MO, USA). Chemicals were of analytical grade and used without any purification. A Sanyo Gallenkamp (model SP65) spectrophotometer was used for spectrophotometric measurements.

Plant Material

The stem bark of S. *pinnata* was collected during May–June from the Southern region of Sri Lanka. The Botanical identity of all plants was determined by the descriptions given by Jayaweera and confirmed by comparing with the authentic samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen has been deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna Sri Lanka.

Preparation of the aqueous plant extract

The stem bark of *S. pinnata* was cut in to small pieces, dried at 40° C until a constant weight was reached, coarsely ground and an aqueous refluxed (4 hours) extract was prepared. A single dose of 1.00 g/kg was administered for 30 days orally to normal and diabetic test rats.

Animals

Healthy Wistar albino rats of 220±25 g body weight was used to carry out the experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (Temp 25: ± 2^{0}C and relative humidity: 55-65% and 12h/12h light and dark cycle). The rats were fed with a standard diet with free access to water before and during the experiment. Before the commencement of the experiment, the rats were randomized and allowed to acclimatize for a period of seven days under standard environmental conditions. Animals described as fasting were deprived of food and water for 10-12 hours adlibitum. All protocols used in this study were approved by the Ethics committee of the University of Ruhuna, Sri Lanka guided by the CIOMS international guiding principles of biomedical research involving animals (Anonymous 2005).

Experimental induction of diabetes mellitus

Diabetes was induced in 16 hours fasted rats by intraperitoneal administration of alloxan monohydrate dissolved in sterile saline at a dose of 150 mg/kg. The rats were maintained on 5% D-glucose solution for the next 24 hours. Rats were allowed to stabilize for three days and blood samples were drawn from tail vein on the third day to determine the blood glucose concentrations to confirm the development of diabetes mellitus. The rats with fasting blood glucose 9.71 mmol/L (equal to fasting serum glucose concentration of 11.10 mmol/L) were considered as hyperglycaemic and used for experiments.

Experimental design

Rats were randomly divided into five groups containing six animals in each group.

Group I : untreated healthy rats; received distilled water.

Group II : untreated diabetic rats; received distilled water.

Group III : normal rats; received the bark extract of S. *pinnata* (1.00g/kg).

Group IV : diabetic rats; received the bark extract of S. *pinnata* (1.00g/kg).

Group V : diabetic rats; received the standard drug glibenclamide (0.50 mg/kg)

The oral glucose tolerance test was performed at weekly intervals and blood glucose concentration was measured immediately by the glucose – oxidase method using an assay kit (Trinder 1969). The acute effect was evaluated over a four hour period using area under the OGTT curve. The body weights of animals were recorded at weakly intervals. The percentage of glycosylated haemoglobin (%HbA_{1C}) was estimated using a colourimetric enzyme assay kit (cation-exchange resin method) on the 30th day (Abraham 1978).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and the group means were compared by Dunnett's multiple comparison test. Values were considered statistically significant with $p < 0.05$.

Results

The percentage of improvement on glucose tolerance with of healthy untreated, diabetic untreated, heal thy and diabetic test rats for the dose of 1.00 g/kg is shown in table 1.

Group	Treatment	Percentage improvement on glucose tolerance(%)				
		1st day	7th day	14th day	21st day	28th day
3	Healthy rats +*Spondias pinnata*(1.00g/kg)	1.2	1.5	2.5	2.3	2.4
4	Diabetic rats +*Spondias pinnata*(1.00g/kg)	27.4**	27.8**	32.1**	38.8**	40.5**
5	Diabetic rats+glibenclamide(0.5g/kg)	39.3**	41.7**	48.2**	51.7**	53.3**

Table 1: Effect of aqueous extract of *Spondias pinnata* on glucose tolerance in healthy and diabetic test groups. The values are expressed as the percentage of improvement on glucose tolerance in each group compared to respective control groups (n=6). Data were analyzed by one way ANOVA followed by Dunnett's test. **P<0.01.

A significant improvement on glucose tolerance was found in diabetic test rats treated with the extract of *S. pinnata* (table 2). The improvement on glucose tolerance with the same dose in healthy treated rats was statistically non -significant ($p<0.05$). Further glibenclamide treated diabetic rats showed a noticeable improvement compared to diabetic untreated rats. The decrease in HbA_{1c} in SPAq treated healthy rats was found to be statistically non –significant ($p<0.05$).

Group	Treatment	Percentage reduction in HbA_{1c}
3	Healthy rats +*Spondias pinnata* (1.00g/kg)	1.19
4	Diabetic rats +*Spondias pinnata* (1.00g/kg)	23.46**
5	Diabetic rats+ glibenclamide	29.5**

Table 2: Effect of aqueous extract of *Spondias pinnata* on HbA_{1c} in healthy and diabetic test groups. The values are expressed as the percentage reduction in HbA1c in each group compared to respective control groups (n=6). Data were analyzed by one way ANOVA followed by Dunnett's test. **$p<0.01$.

The administration of *S. Pinnata* to diabetic rats restored the bodyweights of animals compared to diabetic untreated rats (Figure 1).

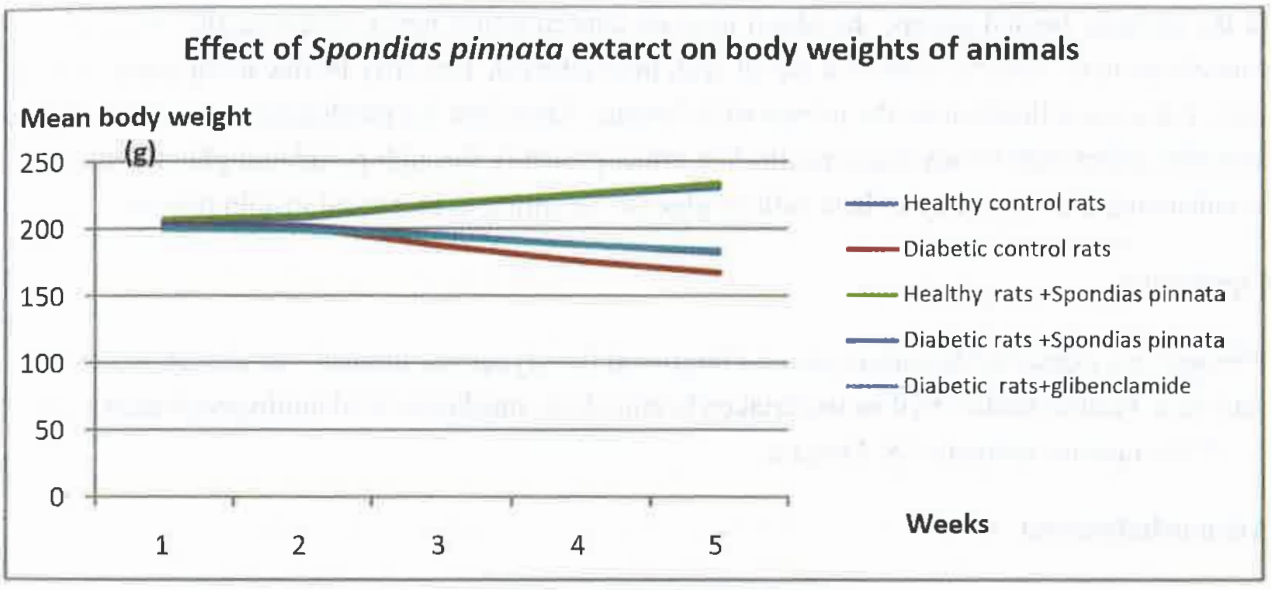

Fig 1: Effect of *Spondias pinnata* on body weights of animals (n=6)

Discussion

Medicinal plants selected for the screening were based on popularity, documented effectiveness and applicability among medical practitioners in Sri Lanka. The traditional usages of medicinal plants are generally and most commonly in the form of their aqueous extracts. Thus plant extracts were prepared in the traditional way in which they are prepared for human consumption and they are assumed to be relevant medicinally and nutritionally. The minimum effective dose of *S. Pinnata* which is approximately equal to human therapeutic dose was administered to healthy and diabetic test rats. The overall improvement on glucose tolerance with the plant extract over a period was evaluated using the total area under oral glucose tolerance curve which has been followed by many authors (Girolomi et al. 2010, Oguanobi et al. 2012, Koffuor et al. 2011).

Alloxan is widely used as a diabetogenic agent in experimental animals and it induces diabetes by destroying the insulin producing beta cells of the islets of Langerhans in the pancreas (Szkudelski 2009). In addition alloxan induced rat model has been used to study the antidiabetic effects of several plant products (Etuk 2010). In uncontrolled or poorly controlled diabetes mellitus there is increased glycosylation of a number of proteins including haemoglobin. The excess glucose present in blood reacts with haemoglobin leads to increase the percentage of glycosylated haemoglobin. In the present study diabetic rats showed higher levels of HbA_{1c} compared to those in normal rats. The administration of aqueous extract for 30 days prevented a significant elevation in glycosylated haemoglobin compared to diabetic rats. This is substantiating its potential in long term glycemic control of diabetes mellitus.

The alloxan induced diabetic rats showed decrease in bodyweight throughout the experimental period. The treatment with aqueous extract of *S. Pinnata* restored the body weight in diabetic rats indicating the possible role of the extract in restoration of protein metabolism.

However the improvement on glucose tolerance, reduction in % of HbA_{1c} and the restoration in body weights of animals were statistically non- significant in healthy treated rats.

In the diabetic treated group, he blood glucose concentration never exceeded the blood glucose concentration of diabetic untreated rats at each time interval. This may be due to the supportive action of glucose utilization by the extract of *S.Pinnata*. Therefore the mechanism behind this glucose lowering effect may involves an insulin-like effect probably through peripheral glucose utilization or enhancing the sensitivity of beta cells to glucose, resulting in increased insulin release.

Conclusion

The aqueous extract of *Spondias pinnata* improved the glycaemic control in alloxan induced diabetic rats. Further studies will be undertaken to elucidate mechanisms of antihyperglycaemic activity of the aqueous extract of *S. Pinnata.*

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