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Anti-hyperglycaemic potential of *Psidium guajava* raw fruit peel

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***Background & objectives*: This study was undertaken to evaluate the glycaemic potential of aqueous extract of *Psidium guajava* unripe fruit peel on blood glucose level (BGL) of normal and streptozotocin induced mild and severely diabetic rats as an extension of our previous work carried out on *Psidium guajava* ripe fruit peel.**

***Methods*: The aqueous extract of *P. guajava* unripe fruits was prepared. Male 6-8 wk old albino Wistar rats were selected for the experiments. Diabetes was induced by streptozotocin infection. Blood glucose levels were measured by glucose oxidase method. Antihyperglycaemic activity of the extract was assessed in mild and severely diabetic rats.**

***Results*: The maximum fall of 21.2 per cent ($P<0.01$) and 26.9 per cent ($P<0.01$) after 3 h of glucose administration during glucose tolerance test (GTT) was observed in BGL from a dose of 400 mg/kg, identified as the most effective dose, in normal and mild diabetic rats respectively. In severely diabetic rats the maximum fall of 20.8 and 17.5 per cent in fasting blood glucose (FBG) and post prandial glucose (PPG) levels, and 50 per cent ($P<0.01$) in urine sugar levels was observed with the same dose. Haemoglobin level increased by 5.2 per cent ($P<0.05$) and body weight by 2.5 per cent ($P<0.05$) after 21 days treatment.**

***Interpretation & conclusions*: Normal, mild and severely diabetic rat models had shown hypoglycaemic as well as antidiabetic effect of the unripe guava fruit peel aqueous extract. Further studies need to be done to characterize the active components of the peel.**

Key words Anti diabetic - glucose tolerance test – hypoglycaemic - *Psidium guajava* - raw fruit peel

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate[1] emerging as a major urban health problem in India. The prevalence of type 2 diabetes has been steadily increasing in urban areas from 2.1 per cent reported in early 1970[2] to 12.1 per cent in 2000[3]. The WHO has revised its estimate of the persons with diabetes in India which was 31.7 million in 2000 and this number is likely to increase to 79.4 million in 2030[4]. In natural system of medicine many plants have been claimed, to be useful in the treatment of diabetes mellitus[5-8].

Psidium guajava Linn (Family: Myrtaceae) is a semi deciduous tropical tree commonly known as guava or '*Amrood*' in north India and is widely grown

throughout India for its fruits. A high percentage of vitamin C, carotene, vit B_1, B_2, B_6, free sugars (glucose, fructose and sucrose) has been reported to be present in these fruits[9]. Guava fruits are known to be a source of antioxidant[10]. Although the fruit of *P. guajava* is known to contain free sugars[9] yet the fruit juice showed hypoglycaemic effect in alloxan treated mice and diabetic volunteers[11]. Several flavonoids, terpenoids and their glycosides[11-13], *etc*. have been reported in guava fruits and these compounds have been shown to possess antidiabetic properties[14-16]. *P. guajava* leaves have been reported to have hypoglycaemic effect[17], on blood glucose level (BGL) of normal and streptozotocin (STZ) induced mild diabetic as well as severely diabetic rats during glucose tolerance test (GTT) and post parandial glucose (PPG) studies respectively. We have earlier shown that the ripe fruit peel aqueous extract exhibited hyperglycaemic effect[18] and the observed effect was correlated with its low concentration of Mg[6].

Higher concentration of Mg presents in *P. guajava* unripe fruit peel aqueous extract[6] prompted us to conduct the present investigation in order to evaluate its glycaemic potential. Thus, the present study was carried out to evaluate the effect of *P. guajava* unripe fruit peel aqueous extract on BGL of normal, mild and severely diabetic rat models.

Material & Methods

Plant material: Unripe fruits of *P. guajava* collected from the guava's garden Khushrobagh, Allahabad, India, were authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, India. A voucher specimen was submitted. The raw guavas were peeled off and the thin greenish peel of the unripe fruits was cut into small pieces. The pieces were mechanically crushed and continuously extracted for 48 h with hot water. The extract was filtered and concentrated in rotatory evaporator at 35 ± 5°C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield: 13.4%, w/w).

Animal care and maintenance: Experiments were performed with 6-8 wk old, healthy, male albino Wistar rats, of body weight 150-200 g. Rats obtained from National Institute of Communicable Diseases (NICD), Delhi, India, were housed under standard environmental conditions (25 ± 2º C temperature, 50 ± 5% humidity with a 12:12 h dark and light cycle) and maintained with free access of water and a standard laboratory diet (carbohydrates; 30%, proteins; 22%, lipids; 12% and vitamins; 3%) *ad libitum*. The study was approved by the Institutional Ethical Committee.

Induction of diabetes in rats: Diabetes was induced by a single intraperitonial (ip) injection of freshly prepared streptozotocin (45 mg/kg wt) in 0.1 M citrate buffer (*p*H 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, mild and severely diabetic rats of type 2[5,6] were selected for the study: Mild diabetic: FBG 120-250 mg/dl and PPG >350 mg/dl, Severely diabetic: FBG >250 mg/dl and PPG >350 mg/dl.

Estimation: BGL was estimated by glucose oxidase method[19] using standard kit of Ranbaxy laboratories limited, New Delhi, India. Haemoglobin was estimated by cyanmethaemoglobin method[20] and urine sugar was detected by reagent based Uristic from Bayer Diagnostics, India.

Experimental design: Initial screening of the extract for the glycaemic activity was done with a range of variable doses in normal and mild diabetic rats by conducting GTT studies. The most effective dose identified in case of normal and mild diabetic cases used for assessing antidiabetic effect in severely diabetic models based on FBG and PPG, urine sugar (US) and body weight (bw)[5,6].

Assessment of hypoglycaemic activity by GTT in normal healthy rats: Twenty four normal rats fasted overnight were divided in to four groups of six rats each and their FBG levels were checked initially. Group I served as control received vehicle (distilled water only) and animals of groups II, III and IV received variable single oral doses of 300, 400 and 500 mg/kg respectively, of extract powder suspended in distilled water. The blood samples were collected again from the tail vein of these groups and blood glucose levels were estimated after 120 min of treatment. The BGL value at 120 min was treated as ˙0˙ h value for GTT. The animals were then orally administrated with 4 g/kg of glucose and their glucose tolerance was studied at 1 h interval for another 3 h, considered as 1, 2 and 3 h values.

Assessment of antihyperglycaemic activity by GTT in mild diabetic rats: Group V (n=6) received 250 mg/kg of the reference drug tolbutamide (synthetic hypoglycaemic agent). The blood samples were collected from the tail vein and blood glucose levels were estimated after 120 min. The BGL value at 120 min was treated as ˙0˙ h value for GTT. The animals were then orally administrated with 2 g/kg of glucose

and their glucose tolerance was studied at 1 h interval for another 3 h, considered as 1, 2 and 3 h values.

Assessment of antihyperglycaemic activity in severely diabetic rats: Long term study of 21 days was conducted in severely diabetic rats. Eighteen severely diabetic rats were divided in to three groups of six rats each. One group served as diabetic control received vehicle (distilled water only), whereas other two groups were treated with 400 mg/kg of extract and 250 mg/kg of tolbutamide respectively, once a day up to 21 days. Blood and urine samples were collected initially and then weekly up to 21 days. The levels of blood glucose, haemoglobin, urine sugar and body weight were checked regularly. A parallel study on normal control was also carried out.

Median LD_{50} experiment: This experiment was carried out on normal healthy rats to check the margin of safety. The behaviour of the treated rats appeared normal. No toxic effect was observed at dose up to 10 and 15 times the effective dose (400 mg/kg) of the extract. The rats were observed for gross behavioural, neurologic, autonomic and toxic effects continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h.

*Statistical analysis***:** Statistical analysis was performed using two-way analysis of variance (ANOVA), using statistical package PRISM 3.0 version. The significance of differences between and within various groups was determined. Differences were considered to be significant when $P<0.05$.

Results

A constant decrease in BGL during GTT in normal healthy rats was seen after a single oral administration of variable doses of 300, 400 and 500 mg/kg bw of raw fruit peel aqueous extract of *Psidium guajava*. The maximum fall (19.6 and 20.5%) was observed with the three doses at 3 h of glucose administration (Table I). Since the highest fall of 21.2 per cent was associated with the dose of 400 mg/kg, this dose was identified as the most effective dose.

In order to verify the highest fall observed with the dose of 400 mg/kg, the same GTT study was performed with all the graded doses on mild diabetic rats along with standard drug tolbutamide (250 mg/kg bw). The maximum fall of 26.9 per cent was seen with 400 mg/kg bw dose after 3 h of glucose administration. The dose of 250 mg/kg bw of tolbutamide produced a

Table I. Effect of graded doses of *P. guajava* unripe fruits peel aqueous extract on blood glucose level during GTT in normal rats

Experimental groups	Treatment (mg/kg bw)	Pre treatment FBG (mg/dl)	Blood glucose levels (mg/dl) Post-treatment (h)			
			0	1	2	3
Control	Distilled water	73.5 ± 5.7	73.1 ± 3.6	108.4 ± 5.1	103.5 ± 4.3	94.2 ± 4.2
Treated 1	300	74.7 ± 4.1	73.4 ± 3.7	90.2 ± 4.8	84.0 ± 4.2	75.7 ± 3.8
Treated 2	400	75.5 ± 4.5	75.1 ± 4.1	88.3 ± 3.1**	81.8 ± 5.1	74.2 ± 3.7**
Treated 3	500	73.2 ± 4.7	71.7 ± 4.6	89.6 ± 5.1*	82.3 ± 4.9	75.4 ± 4.4**

*P**<0.05; ***<0.01 compared with control. Values are mean ± SD of 6 rats.
FBG, fasting blood glucose; GTT, glucose tolerance test

Table II. Effect of graded doses of *P. guajava* unripe fruits peel aqueous extract on blood glucose level during GTT in mild diabetic rats

Experimental Groups	Treatment (mg/kg bw)	Pre treatment FBG (mg/dl)	Blood glucose levels (mg/dl) Post treatment (h)			
			0	1	2	3
Control	Distilled water	144.7 ± 5.7	144.6 ± 3.6	395.1 ± 5.1	321.7 ± 4.3	270.5 ± 4.2
Treated 1	300	152.6 ± 4.1	142.8 ± 3.7	319.9 ± 4.8	245.8 ± 4.2	209.3 ± 3.8
Treated 2	400	150.3 ± 4.5	140.2 ± 4.1	309.1 ± 3.1	238.5 ± 5.1**	197.5 ± 3.7
Treated 3	500	146.7 ± 4.7	141.1 ± 4.6	311.5 ± 5.1	239.8 ± 4.9 *	201.1 ± 4.4
Treated 4	Tolbutamide	151.9 ± 4.2	138.5 ± 3.9	301.2 ± 4.4*	228.7 ± 4.9 *	195.9 ± 4.2**

*P**<0.05; ***<0.01compared with control
FBG, fasting blood glucose. Values are mean ± SD of 6 rats

Table III. Effect of graded doses of *P. guajava* unripe fruit peel aqueous extract on glycaemic indices and body weight in severe diabetic (SD) rats

Experimental rats	Treatment (Aq. extract)	Pre-treatment levels	Post-treatment levels		
			7 days	14 days	21 days
*FBG (*mg/dl)					
Normal (control)	Distilled water	85.7 ± 4.6	85.9 ± 4.1	90.8 ± 4.9	85.4 ± 3.4
SD (control)	Distilled water	315.4 ± 6.9	318.1 ± 7.2	321.7 ± 6.5	326.9 ± 6.8
SD (treated)	400 mg/kg	322.7 ± 7.1	311.4 ± 7.3*	290.2 ± 6.5*	255.7 ± 6.8*
PPG (mg/dl)					
Normal (control)	Distilled water	152.7 ± 5.1	154.6 ± 4.6	152.1 ± 4.8	152.4 ± 4.3
SD (control)	Distilled water	429.6 ± 7.3	434.2 ± 7.6	438.7 ± 7.1	445.9 ± 8.2
SD (treated)	400 mg/kg	425.3 ± 7.4	407.1 ± 7.9*	398.5 ± 8.3*	350.7 ± 7.5*
Haemoglobin (g/dl)					
Normal (control)	Distilled water	12.7 ± 1.2	12.6 ± 1.5	12.7 ± 1.9	12.8 ± 1.8
SD (control)	Distilled water	9.3 ± 1.7	9.1 ± 1.4	9.1 ± 5.2	8.6 ± 5.5
SD (treated)	400 mg/kg	9.6 ± 1.5	9.6 ± 1.9*	9.8 ± 1.4*	10.1 ± 1.7*
Urine sugar					
Normal (control)	Distilled water	NIL	NIL	NIL	NIL
SD (control)	Distilled water	+++	+++	++++	++++
SD (treated)	400 mg/kg	+++	+++	++**	++**
Body weight (g)					
Normal (control)	Distilled water	210 ± 5.0	210 ± 5.0	210 ± 5.0	210 ± 5.0
SD (control)	Distilled water	195 ± 5.0	195 ± 5.0	190 ± 5.0	190 ± 5.0
SD (treated)	400 mg/kg	200 ± 5.0	200 ± 5.0	205 ± 5.0*	205 ± 5.0*

*P<0.05, **<0.01 compared to pre-treatment levels.

Values are mean ± SD of 6 rats. FBG, fasting blood glucose; PPG, post pranadial glucose; +, 25 per cent sugar

fall of 28.9 per cent in mild diabetic rats after 2 h of glucose administration. Moreover, the doses of 300 and 500 mg/kg bw produced a fall of 22.6 and 25.6 per cent on BGL of mild diabetic rats at 3 h (Table II). The dose of 400 mg/kg of aqueous extract of *P. guajava* appeared to be most effective dose as it produced significant hypoglycaemic effect. Hence, this dose was evaluated in severe hyperglycaemic rats for diabetes management.

Rats were treated with optimum effective dose of 400 mg/kg of aqueous extract once a day in noon for 21 days. The administration of extract produced marked antihyperglycaemic effect in treated diabetic rats at the end of the treatment and a significant reduction of 20.8 per cent in FBG and 17.5 per cent in PPG levels was observed by comparing with their initial values. (The fall in tolbutamide treated rats was 23.1 per cent in FBG and 20.6 per cent in PPG levels and was comparable with the result of the most effective dose) (Table III)). A fall of 50 per cent in urine sugar and an increase of 2.5 per cent in bw were observed in the extract treated severely diabetic group. This dose of extract produced potential improvement of 5.2 per cent in haemoglobin level also at the end of experiment (Table III).

Experiment on normal healthy rats showed that the behaviour of the treated rats appeared normal. No toxic effect was reported at doses up to 10 and 15 times of the effective dose of the aqueous extract and there was no death in any of the groups.

Discussion

Mortality is elevated in patients with type 2 diabetes mellitus generally due to metabolic abnormalities[21,22]. Prevalence of unrecognized diabetes mellitus and impaired glucose tolerance has mostly been followed by acute strokes[23].

The present study revealed that the *P. guajava* unripe fruit peel aqueous extract had marked hypoglycaemic as well as antihyperglycaemic effect in normal and STZ induced mild diabetic rats respectively. The maximum fall during GTT in BGL was found with the dose of 400 mg/kg bw. Such a phenomenon of less hypoglycaemic response at higher dose is not uncommon with indigenous plants and has already been observed in *Annona squamosa*[24], *Trichosanthes dioica*[25,26], and *Ficus bengalensis*[27]. Hence, the dose of 400 mg/kg was identified as the most effective dose in both the

cases of normal as well as mild diabetic rats. This dose had approximately the same effect as that of synthetic drug tolbutamide. Thus, the effectiveness of the aqueous extract of guava unripe fruit peel in mild diabetic rats was almost comparable with the synthetic drug tolbutamide.

In case of severely diabetic rats, the daily treatment with 400 mg/kg of extract for 21 days brought FBG significantly down and prevented the bw loss. The fall in urine sugar levels and improvement in Hb after long term treatment indcated its antidiabetic effect.

In conclusion, this study provided a new therapeutic avenue against human diabetes and diabetes related complications. Further characterizations of active components of *P. guajava* unripe fruit peel for diabetes and lipid management are warranted.

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