Antidiabetic effect of an aqueous extract of Pomegranate (Punica granatum L.) peels in normal and alloxan diabetic rats

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Abstract
Hypoglycaemic drugs are either too expensive or have undesirable side effects including hematological, coma and disturbances of liver and kidney. Limiting of diabetes without any side effects is still a challenge to the medical system. This leads to exert effort to search for effective, safer and less cost antidiabetic plants.

This investigation aims to evaluate the role of Punica granatum powder peels extract in its human therapeutic dose on beta cell numbers blood glucose and plasma insulin levels in normal and alloxan diabetic rats for 4-weeks of treatment.

The treatment revealed that pomegranate aqueous extract significant decreased blood glucose and increased insulin levels in normal and diabetic treated rats. Pancreas showed increased number of beta cells in normal and treated diabetic rats.

In conclusion pomegranate peel aqueous extract can reduce blood sugar through regeneration of β cells.

Introduction
It generally accepted that the sulphonyle ureas produce hypoglycemia in normal animals by stimulating the pancreatic β cells to release more insulin. These drugs, however, do not decrease blood glucose in alloxan diabetic animals (Goth, 1985). In addition, the exogenous administration of insulin is well known to produce hypoglycaemia in both normal and alloxan induced subjects (Larner, 1985). This leads to increasing demand for natural products with potent antidiabetic and less side effect.

Various parts of Pomegranate (Punica granatum L.) have been used for various medicinal purposes.


It was found that Punica granatum peels contain tannins, anthocynins, flavonoids, pectins (Nozire and Serpil, 1993), three estrogen compounds luteolin, quercetin and kaempferol (Van-Elswijk et al, 2004).

This investigation aims to evaluate the role of Punica granatum powder peels extract in its human therapeutic dose on beta cell numbers, blood glucose and plasma insulin levels in normal and alloxan diabetic rats for 4-weeks of treatment.

Material and methods
Plant material
200ml boiling distilled water were added to 3gm powder pomegranate peel, left it for 10 minutes and filtered. The filtrate was dried at 40-45°C in the incubator.

Animals
Male albino rats weighing 120±10gm were obtained from the observed first generation for a period of time. The animals were caged and provided with food and water ad libitum. Injection of alloxan...
monohydrate (Sigma USA), in a dose of 120mg /kg B.wt. dissolved in acetate buffer immediately prepared before usage. Seven days after injection of alloxan blood glucose levels of all surviving rats were determined. Only rats with glucose levels above 200mg/ml were considered diabetic and employed for the assay.

**Experimental design**

24 adult male albino rats weighing (110-130gm) were divided into four groups 6 rats each. Group I, represented control, group II diabetic rats, group III diabetic rats treated with 0.43g of P. granatum peel aqueous extract and group IV treated with 0.43g of P. granatum peel extract, that dose equivalent to human therapeutic dose (Paget and Barnes, 1964) daily for 4 weeks.

**Blood sampling**

Blood samples were collected from retro-orbital vein in two separate tubes , one tube with EDTA for determination of hematological parameter. Red blood cells (RBCs) and (WBCs) were counted in Hemocytometer. The second tube containing potassium oxalate and sodium fluoride for estimation of glucose (Trinder, 1969)and insulin was estimated by kits obtained from IMX Abbott Labs/IL/USA. Fresh pancreas samples were collected in formal saline,stained in H&E Some section of pancreas were stained by modified aldehyde fuchsia (M.A.F.) (Kiernan, 1999).

**Statistical analysis**

All data obtained were analyzed using student 't' test according to (Sendecor and Coebram,1969)

**Results and Discussion**

Preliminary studies showed that P. granatum rind extract possessed significant blood sugar lowering activity. (Nogueira and Pereira,1984, 1986a,b&Zafar and Singh,1990)

Nogueira and Pereira, 1986b attributed, the antihyperglycaemic action of the peel extract of P. granatum, to the inhibitory intestinal absorption of glucose in rats .

This investigation aims to evaluate the role of Punica granatum powder peel aqueous extract in its human therapeutic dose on beta cell numbers, blood glucose and plasma insulin levels in alloxan diabetic rats for 4-weeks of treatment As well as to illustrate the mechanism of hypoglycaemic effect of the extract

There was a significant weight loss in diabetic rats (table1), (fig.1), while in diabetic group treated with P. granatum aqueous peel extract ,an improvement in their body weights was observed. The ability of pomegranate rind extract to prevent body weight loss seems to be due to its antidiabetic activity. Pomegranate peel extract treated normal rats resulted in non-significant increase in body weight after4-weeks.

The red blood cells(table1,fig.2) and haemoglobin decreased significantly in diabetic rats (table1,fig.4). WBCs showed non-significant change at 30 days by alloxan treatment (table1,fig.3) Diabetic rats treated with pomegranate peel extract displayed non-significant alternation in the red blood cells , haemoglobin and white blood cells from their corresponding control .In rats treated with pomegranate peel extract ,the red blood cells and haemoglobin increased significantly as the result of pomegranate peel haemostatic property (Machado et al, 2002) while WBCs displayed non-significant variation comparable to control for 4-weeks.

(Table1, fig.5) displayed significant elevation in plasma glucose and significant reduction in insulin level in diabetic rats (table1,fig.6). Pancreas showed reduction in β cell numbers, β cells necrosis and intracellular vacuolation (fig8) compared to control (fig.7). Diabetic rats treated with 0.43g/KgB.W. of Punica granatum aqueous peel extract for 4 weeks displayed significantly lowered blood sugar level (table1, fig 5) and augmentation in insulin level(table 1& fig.6) .The number of β cells relatively increased, while vacuolation still appeared in some cells (fig.10) . Punica granatum aqueous peel extract significantly lowered blood sugar and increased insulin
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level (table1 & figs. 8, 9) and the number of β cells increased (fig.10) Alloxan has shown to induce free radical production and cause tissue energy (Hallwell and Gutteridge, 1985). The pancreas is especially susceptible to the action of alloxan –induced free radical damage. Punica granatum aqueous peel extract possesses strong antioxidant property (Chidambara et al, 2002) can act as free radical scavenger and protect β cells from damage.

Plant extract treated normal rats revealed significant reduction in blood sugar and elevation in insulin level. The number of β cells increased.

The mechanism of the antidiabetic activity shown by the extract is protection of pancreas, stimulation of β cells, increase number of β cells and subsequent release of insulin. Also it may increase insulin receptor.

In conclusion pomegranate peel aqueous extract can reduce blood sugar through regeneration of β cells.

### Table (1): Showing the effect of treatment with 0.43g/kgBW Pomegranate(Punica granatum) peel aqueous extract for 4 weeks in body weights, some haematological parameters, blood glucose and insulin in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic treated with Pomegranate</th>
<th>Normal treated with Pomegranate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>145.83 ± 4.92</td>
<td>123.33*↓ ± 6.83</td>
<td>130 ± 5</td>
<td>140 ± 3.5</td>
</tr>
<tr>
<td>R BCs x 10^6</td>
<td>5.35 ± 0.66</td>
<td>2.25 *↓ ± 2</td>
<td>5.5 ± 0.5</td>
<td>7.5 x 10^6 ± 1</td>
</tr>
<tr>
<td>W BCs x 10^3</td>
<td>4.66 ± 0.6</td>
<td>6.76 ± 0.8</td>
<td>7 ± 1</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Hb (gm %)</td>
<td>16.33 ± 1</td>
<td>12.5*↓ ± 0.8</td>
<td>16.5 ± 0.5</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>85 ± 2</td>
<td>280*↑ ± 5</td>
<td>120*↑ ± 10</td>
<td>75*↓ ± 2.5</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>12.5 ± 0.5</td>
<td>7.5*↓ ± 0.8</td>
<td>12 ± 0.5</td>
<td>15 *↑ ± 1</td>
</tr>
</tbody>
</table>

Number of rats in each group=6  P<0.05 compared with control group
Fig 2: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in RBCs x 10^6 in normal and diabetic rats.

Fig 3: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in WBCs x 10^3 in normal and diabetic rats.

Fig 4: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in Hb (gm %) in normal and diabetic rats.
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Legend of figure

**Fig. 7:** Control rat pancreas showing islet of langerhans containing α, β, and δ cells. β Cells are the most abundant cells. M. A.F. x 400.

**Fig. 7a:** Control rat pancreas M. A.F. x 1000.

**Fig. 8:** Diabetic rat pancreas showing, reduction in the pancreatic β cell numbers, β cell vacuolization and necrosis in some surviving β cells M.A.F. x 400.

**Fig. 8a:** Diabetic rat pancreas M.A.F. x1000

**Fig 9:** Pancreas of normal rats treated with Punica granatum peel extract showing regeneration of β cells M. A.F. x 400.

**Fig. 9a:** Pancreas of normal rats treated with Punica granatum peel extract M.A.F. x1000

**Fig. 10:** Pancreas of diabetic rats treated with Punica granatum peel extract showing regeneration of β cells, while vacuolation still appeared in some cells M.A.F. x400

**Fig. 10a:** Pancreas of diabetic rats treated with Punica granatum peel extract M.A.F. x1000
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References

5. Goth, M. D. (1985): Medical Pharmacology, 9th Ed. Mosby. Saint Louis p.471. In contrast to the oral antidiabetic agents, the exogenous administration of insulin is well known to produce hypoglycaemia in both normal and alloxan induced subjects.
التأثير المعبلج لمرض السكر بالمستخلص المائي لقشر الرمان في الجرذان المصاب بهم

مرض السكر المستحدث بالألوكزان

أيناس على مهدي خليل
 الهيئة القومية للرقابه والبحوث الدوائية

تظهر الأدوية المعالجة لمرض السكر أعراضًا جانبية غير مرغوب فيها تضمن
تغير صورة الدم والغليوبه وأضطرابات في وظائف الكبد والكلى بالأضافة إلى ارتفاع تكلفتها ومواجهة الطب مشكلة كيفية الفضاء على مرض السكر بدون أعراض جانبية,
مما تطلب بذل الجهد للبحث عن نباتات تعالج مرض السكر أكثر فاعليًا وأمانًا وأقل تكلفه.

يهدف هذا البحث تقييم فاعليّة المستخلص المائي لقشرة الرمان بجرعته العلاجية
على عدد خلايا بيتا وسكر الدم والأنسولين في الجرذان المصاب بهم مرض السكر
وأخرى غير المصاب لمدة أربعة أسابيع.

أظهرت هذه المعاملة إخفاقًا في مستوى الجلوكوز وأرتفاعًا في مستوى
الأنسولين وأظهر البنكرياس زيادة عدد خلايا بيتا في الجرذان المصاب بهم وغير المصاب
بمرض السكر.

ويستنتج هذا البحث أن المستخلص المائي لقشر الرمان يؤدي إلى ذيادة عدد
خلايا بيتا وبالتالي إلى خفض مستوى السكر في الدم.