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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 ß cells.

Introduction

It generally accepted that the sulphonyle ureas produce hypoglycemia in normal animals by stimulating the pancreatic ß 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 .

Many studies have shown that the pomegranate peel extract has wound healing properties(Chidambara, *et al*, 2004), possesses antioxidant activity (Chidambara *et al* , 2002), immunomodulatory activity (Gracious *et al*, 2001), antibacterial activity (Prashanth *et al*, 2001), gastroprotectective effect (Gharzouli *et al* ,1999), larvicidal activity (Morsy *et al*,1998), antifungal activity (Dutta *et al* ., 1998), antitumor action (Mavhjanav *et al* , (1997), antimicrobial effect (Navarro, *et al*,1996), antiviral activity (Zhang *et al*, 1995) and hypoglycemia effect (Nogueira and Pereira, 1984, 1986 a,b; Zofar and Singh 1990).

It was found that Punica grantum 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 grantum 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 to3gm powder pomegranate peel, left it for 10 minutes and filtered .The filterate was dried at 40-45C0 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 adlibitum. 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 fuchsine (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)

Nougueira 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 grantum 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 ß cell numbers, ß 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 ß cells relatively increased, while vacuolation still appeared in some cells (fig.10) . Punica granatum aqueous peel extract significantly lowered blood sugar and increased insulin

level (table1 & figs. 8, 9) and the number of ß cells increased (fig.10) Alloxan has shown to induce free radical production and cause tissue energy (Hallwell and Gutteridge, 1985). The pancrease is especially susceptible to the action of alloxan –induced free radical damage. Punica grantum aqueous peel extract possesses strong antioxidant property (Chidambara *et al*, 2002) can act as free radical scavenger and protect ß cells from damage .

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

The mechanism of the antidiabetic activity shown by the extract is protection of pancreas, stimulation of ß cells, increase number of ß 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 ß 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.

Groups / Parameters	Control	Diabetic	Diabetic treated with Pomegranate	Normal treated with Pomegranate
Body weight (gm)	145.83 ±4.92	123.33*↓ ±6.83	130 5±	140 ± 3.5
R BCs x10^6	5.35 ±0.66	2.25 *↓ ±2	5.5 ±0.5	7.5 x 10^6 ±1
W B Cs x 10^3	4.66 ±0.6	6.76 ±0.8	7 ±1	8 ± 2
Hb (gm %)	16.33 ±1	12.5*↓ ±0.8	16.5 ± 0.5	18 ±1
Glucose (mg/dl)	85 ±2	280*↑ ±5	120*↑ ±10	75*↓ ± 2.5
Insulin (µU/ml)	12.5 ±0.5	7.5*↓ ±0.8	12 ± 0.5	15 *↑ ±1

Number of rats in each group=6 P<0.05 compared with control group

Fig1: Showing the effect of treatment with 0.43g/kgBW Pomegranate(Punica granatum) peel aqueous extract for4 weeks in body weights in normal and diabetic rats

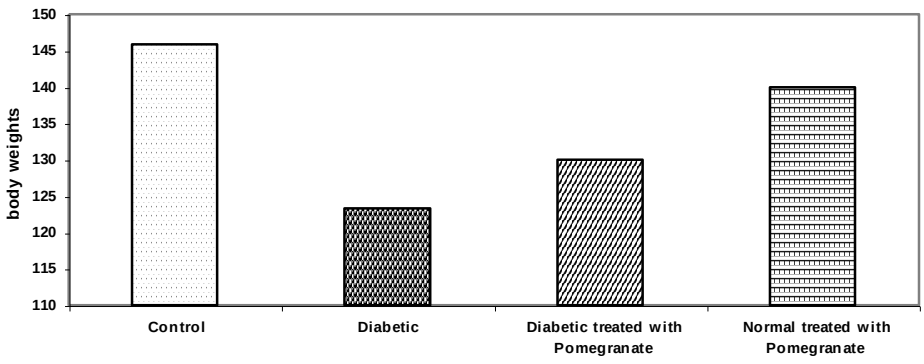


Fig 2: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in R BCs x 10^6 in normal and diabetic rats .

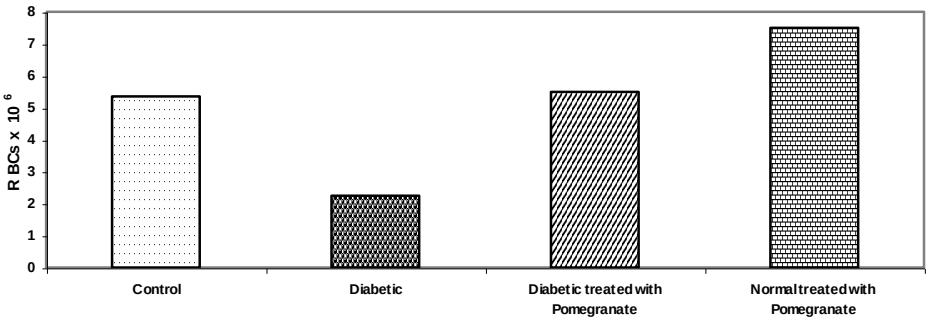


Fig 3: Showing the effect of treatment with 0.43g/kgBW Pomegranate(Punica granatum) peel aqueous extract for 4 weeks in W B Csx 10^3 in normal and diabetic rats

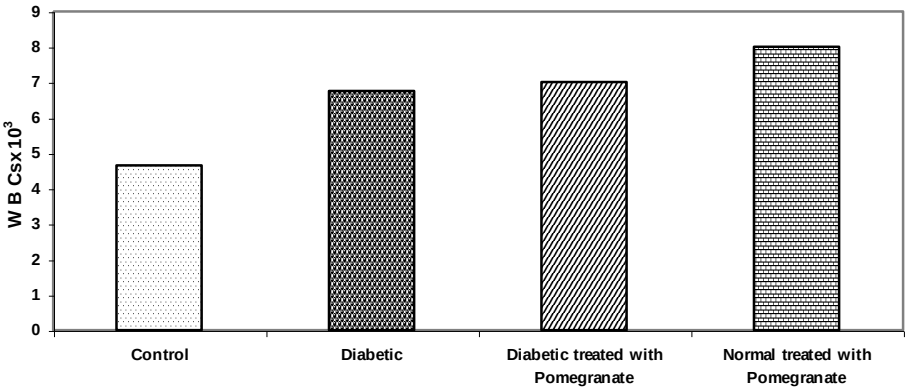


Fig 4: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in Hb (gm %) in normal and diabetic rats .

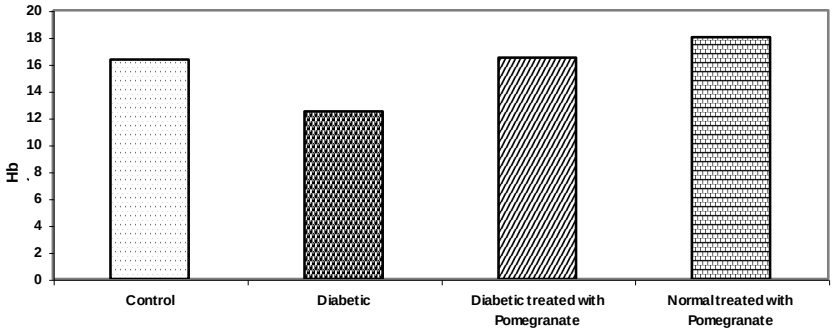


Fig 5: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in Glucose (mg/dl) in normal and diabetic rats

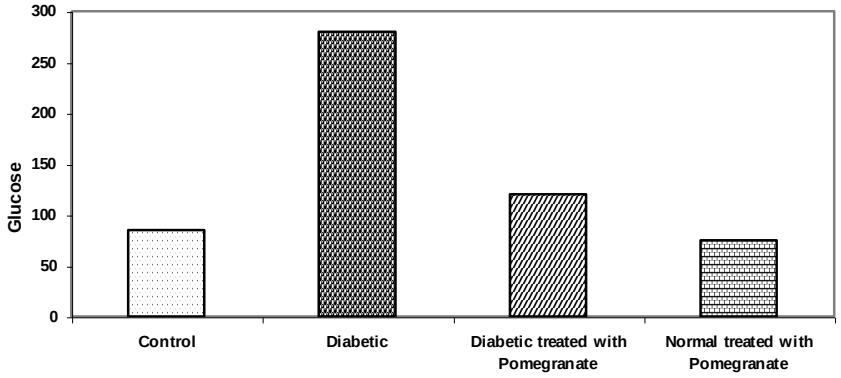
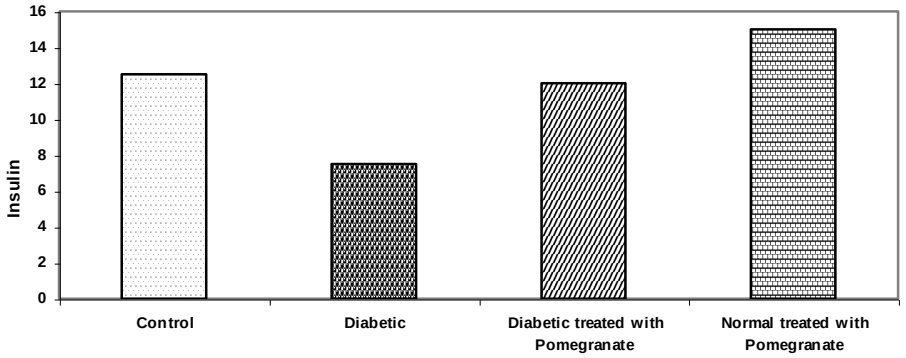


Fig 6: Showing the effect of treatment with 0.43g/kgBW Pomegranate (Punica granatum) peel aqueous extract for 4 weeks in Insulin (µU/ml) in normal and diabetic rats



Legend of figure

Fig.7: Control rat pancreas showing islet of langerhans containing α , ß, and δ cells. ß 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 ß cell numbers, ß cell vacuolization and necrosis in some surviving ß cells M.A.F. x 400.

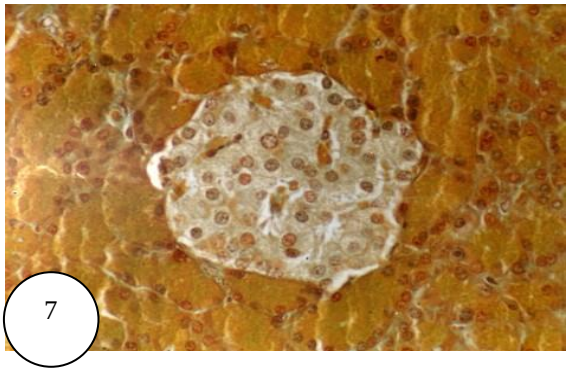
Fig.8a: Diabetic rat pancreas M.A.F. x1000

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

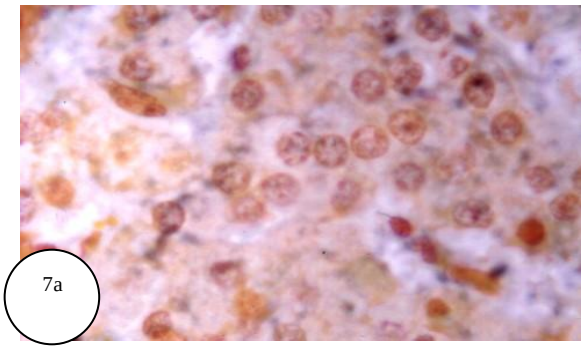
Fig.9a: Pancreas of normal rats treated with Punica granatum peel extract M.A.F. x1000

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

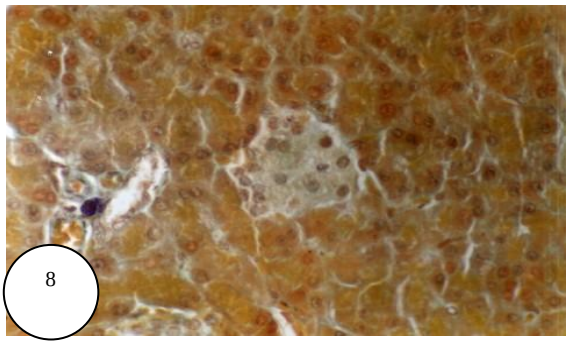
Fig.10a: Pancreas of diabetic rats treated with Punica granatum peel extract M.A.F. x1000



M.A.F X 400



M.A.F X 1000



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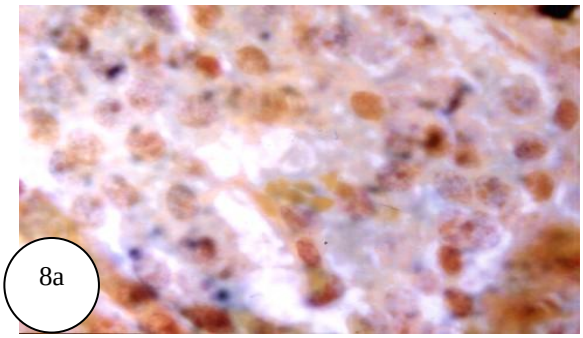

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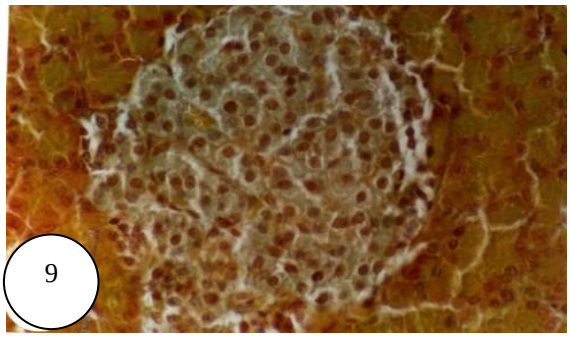

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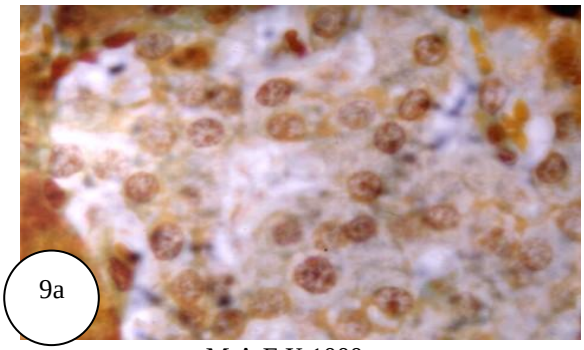

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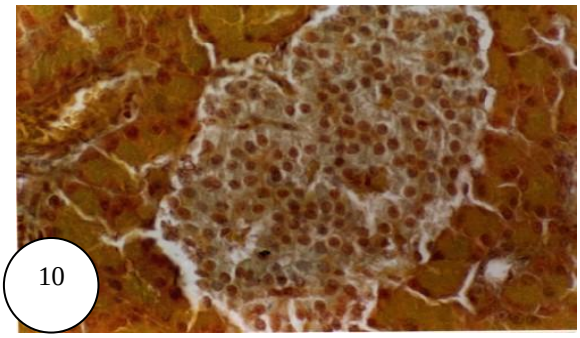

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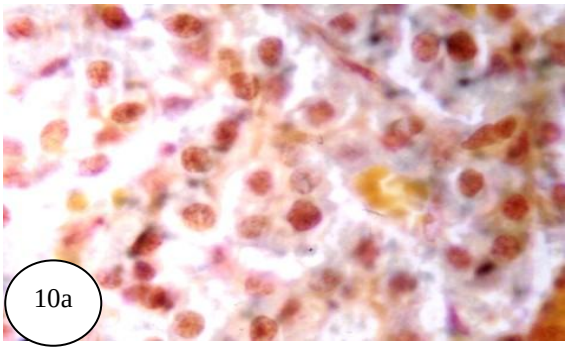

M.A.F X 1000

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التأثير المعالج لمرض السكر بالمستخلص المائى لقشر الرمان فى الجرذان المصابه
بمرض السكر المستحدث بالألوكزان

أيناس على مهدى خليل
الهيئة القوميه للرقابه والبحوث الدوائيه

تظهر الأدوية المعالجه لمرض السكر أعراضا جانبيه غير مرغوب فيها تضمن تغير صورةالدم والغيبوبه وأضطرابات فى وظائف الكبد والكلى بالأضافه الى إرتفاع تكلفتها ويواجهة الطب مشكلة كيفية القضاء على مرض السكر بدون أعراض جانبيه, مما تطلب بذل الجهد للبحث عن نباتات تعالج مرض السكر أكثر فاعليه وآمانا وأقل تكلفه

يهدف هذا البحث تقييم فاعلية الستخلص المائى لقشرة الرمان بجرعته العلاجيه على عدد خلايا بيتا وسكر الدم والانسولين فى الجرذان المصابه بمرض السكر وأخرى غير المصابة لمدة أربعة أسابيع.

وأظهرت هذة المعامله إنخفضاً فى مستوى الجلوكوز وأرتفاعاَفى مستوى الأنسولين وأظهرالبنكرياس ذيادة عدد خلايا بيتا فى الجرذان المصابه وغير المصابه بمرض السكر.

ويستنتج هذا البحث أن المستخلص المائى لقشر الرمان يؤدى إلى ذيادة عدد خلايا بيتا وبالتالى الى خفض مستوى السكر فى الدم.