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Der Pharma Chemica, 2010, 2(6):416-428
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Antihyperglycaemic effect of hydroalcoholic extract from *Punica granatum* L. peels in normal and streptozotocin-induced diabetic rats and its potent α-amylase inhibitory

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ABSTRACT

This study was designed to evaluate the antidiabetic effect of hydroalcoholic extract from *Punica granatum* peels in normal and streptozotocin-induced diabetic rats and its potent α-amylase inhibitory. Antidiabetic effect of this extract was investigated after oral administration at dose of 400 mg/kg b.w. In normal fasted rats, the test drug extract showed no hypoglycaemic effect but were found to be depressing the peak value of blood glucose after oral glucose tolerance (glucose 2.5 g/kg b.w.). After 2-weeks of daily treatment in streptozotocin-induced diabetic rats, plasma glucose levels were decreased after oral administration of hydroalcoholic extract from *Punica granatum* peels by 56% in the first week and 32.92% in the second week. However, this extract showed hypolipidemic effect (Total cholesterol and triglycerides) in streptozotocin-induced diabetic rats. *In vitro*, *Punica granatum* peels extract demonstrate a potent inhibitory effect on α-amylase activity (IC_{50}=3.65 g/L) these finding strongly suggest that PGP extract improves postprandial hyperglycaemia, at least in part, by inhibiting alpha amylase activity.

Key words: *Punica granatum*, diabetes mellitus, streptozotocin, medicinal plants, α-amylase, post-prandial hyperglycaemia.

***Abbreviations**:*
EA: enzymatic activity; IP:intraperitoneal; PG: Punica granatum; PGP: Punica granatum peels; AOA: Aspergillus oryzae alpha amylase; DW: Distilled water; TC: Total cholesterol; TG; Triglycerides; b.w.: body wight.; STZ: Streptozotocin

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia resulting from defects insulin secretion, insulin action or both [1].

It is well documented that chronic hyperglycaemia of diabetes is associated with long-term damage dysfunction and eventually the failure of organs especially the eyes, kidneys, nerves, heart and blood vessels [2-3].

Various pharmacological approaches are used to improve diabetes via different mode of action such as stimulation of insulin release, increase the number of glucose transporters and inhibition of gluconeogenesis [4]. Other therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolysing enzymes (α-amylase and α-glucosidase) in the digestive tract. Inhibition of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma rise [5].

Though all these contribute in the alleviation of diabetes but several complicities still persist and diabetes is increasing fast. Recently, the importance of biologically active substances in natural form and complementary medicine has received much attention for various reasons [6-7].
Diabetes mellitus has been treated with medicinals plant. The scientific investigation has confirmed the efficacy of many preparations of these plants, some of which are remarkably effective [8]. In this study we have selected *Punica granatum* L. from Algeria to evaluate its anti-diabetic activity.

Punica granatum L. (PG) is a small tree, belonging to the Punicaceae family, native to the Mediterranean region. All part of this species has been wildly used by traditional medicine in Africa, Europ, Asia and America for the treatment of different types of diseases [9]. The rind is valued as an astringent in diarrhoea and dysentery. The root bark is astringent and anthelmintic. The seed are considered to be stomachic and cardiotonic. The green leaves are made into a paste and applied in conjunctivitis. The powdered flower buds are useful in bronchitis. The biological activities, viz. antibacterial, antifungal, anthelmintic and antifertility of the various extracts of different parts of this plant have also been reported [10-14].

The extracts of root, rind and flowers of *P. granatum* have been reported to exert some sugar lowering action in animals [15-20].

This study was designed to examine the effect of the hydroalcoholic extract of PGP on blood glucose levels in normal and STZ-induced diabetic rats *in vivo* and to evaluate the potent α-amylase inhibitory action *in vitro*.

MATERIALS AND METHODS

2.1. Plant material

Fresh *P. granatum* fruits were collected in the Tafna region of Tlemcen (Algeria). The air-dried fruit peels were ground into fine powder and extracted (10 g) by refluxing with 100 mL aqueous-methanol solution (30:70 v/v) bath for 2 h. The extract was filtered and evaporated to dryness under reduced pressure below 60°C and suspended in distilled water (DW).

2.2. Animals

Male adults albinos rats "Wistar" weighing 200-260 g were used for this study. They were housed in air conditioned at 23°C with a 12 h light/bark cycle and were provided with standard food and water *ad libitum*.

Animals, described as fasted, were deprived of food for at least 16 h but allowed free access to water before starting the experiments.

3. Biological assay

3.1. Behavioural effect and toxicity

Extract of *P. granatum* peels (PGP) were administered orally at dose of 400, 800, 1000, 2000, 3000 and 4000 mg/kg b.w. (dosing volume 10 mL/kg b.w.).

Animals were observed during first 6 h and continued for 15 days for behavioural changes and any signs of mortality.

3.2. Effect of PGP extract on oral glucose tolerance in normal rats

Fasted rats were divided into three groups of five animals each. Group I: serving as control, received NaCl (0.9% in distilled water) orally in a volume of 10 mL/kg b.w. Group II: received PGP extract at dose of 400 mg/kg b.w. orally. Group III: received glibenclamide (5 mg/kg b.w.) suspended in the DW.

All the animals were given glucose (2.5 g/kg b.w.) orally 30 min after test drug administration and plasma glucose levels were determined at 0, 30, 60, 120, 180 min.

3.3. Effect of PGP extract on blood glucose in normal fasted rats

Fasted rats were divided into three groups of five animals each: Group I: received NaCl (0.9% in DW) orally (dosing volume of 10 mL/kg b.w.). Group II: received PGP extract at dose of 400 mg/kg b.w. suspended DW. Group III: received glibenclamide (5 mg/kg b.w.) suspended in the DW.

Blood glucose was estimated at 0, 30, 60, 120, 180 min for the three groups and continued for 7 and 15 days after daily administration of PGP extract for group I and II.

3.4. Effect of PGP extract on blood glucose in STZ-induced diabetic rats

Rats were made diabetic by a single intraperitoneal injection of STZ (*Sigma Aldrich*) at dose of 50 mg/kg b.w dissolved in citrate buffer (0.1 M, pH 4.5) (Gupta et al., 2005) [21]. Development of diabetes was confirmed by polydipsia, polyuria and measuring blood glucose concentrations 48 h after injection of STZ. Rats with blood glucose level of 2 g/kg or higher were considered to be diabetic.

Fasted rats were divided into three groups comprising of five animals in each:

Group I: received 10 mL/kg b.w. of NaCl (0.9% in distilled water) orally. Group II: received PGP extract at dose of 400 mg/kg b.w. suspended in the DW. Group III: received glibenclamide (5 mg/kg b.w.).

Blood glucose was estimated at 0, 30, 60, 120, 180 min and continued for 7 and 15 days after daily administration of PGP extract.

3.5. Effect of PGP extract on hyperlipidemia in STZ-induced diabetic rats

Plasma total cholesterol and triglycerides were estimated at 7 and 15 days after daily administration of PGP extract in diabetic rats compared with normal control group.

3.6. Analytic procedures

Blood glucose was estimated by One Touch Ultra Glucometer.

Plasma total cholesterol [22] and triglycerides [23] were estimated by using enzymatic cholorimetric kits (Sprinreact) (Blood samples from the retro-orbital plexus were collected and centrifuged at 3000 rpm for 10 min).

3.7. Statistical analysis

For anti-diabetic activity, the results have been expressed as mean±SEM, and the significance of the results were analysed by student's *t*-test compared with control. *P*-value of 0.05 or less was considered to be significant.

3.8. Effect of PGP extract on α-amylase activity *in vitro*

The α-amylase inhibition assay was performed using chromogenic method adapted from Bernfeld (1955) [24]. *Aspergillus aryzae* α-amylase (AOA) [EC.3.2.1.1 Fluka; specific activity 26UI/mg)] was dissolved in phosphate buffer (0.02 M, pH 6) to give a final activity of 1.3 UI/mL solutions. Potato starch (type Merk) dissolved in the same buffer, was used as a substrate solution at different concentrations.

1 mL of plant extract (at different concentrations) or acarbose (used as positif control) dissolved in phosphate buffer (0.02M, pH 6) and 1 mL of starch solution (at different concentrations) were mixed. The reaction was started by the addition of 1 mL of the enzyme solution (1.3UI/mL in reaction mixture) at 25°C.

1 mL mixture was added into a separate tube containing 1 mL DNSA colour reagent solution (interval of 1 min between each tube) and placed into 100°C water bath. After 5 min, these tubes were cooled in an ice water bath and diluted with 10 mL of DW. α-Amylase activity was determined by measuring the absorbance of the mixture at 540 nm. Control incubations, representing 100% enzyme activity were conducted an identical fashion in the absence of inhibitor.

From the net absorbance obtained, the maltose generate was calculated from equation obtained from the maltose standard calibration curve.

- **Statistical analysis**

– Kinetic parameters were calculated using *Lineweaver-Burk* and *Dixon plots*.

– The type of inhibition was determined by *Lineweaver-Burk* plot.

– The α-amylase inhibition was expressed as percentage of inhibition and calculated by the following equation:

$$\%I = \frac{Vi_{control} - Vi_{test}}{Vi_{control}} \times 100$$

– The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assays conditions specified. These values were calculated from the dose-inhibition curve.

RESULTS

4.1. Behavioural effect and toxicity

The 15 days observation period during the oral toxicity study did not reveal any toxic effects.

After administration of the extract, the rats appeared weakened (from 1000 and 3000 mg/kg b.w.) but are fed regularly. From the sixth hour, all the rats have found behaviour consistent with

that in controls. A very high dose (4000 mg/kg b.w.) caused a mortality of three rats from six after 24 and 48 h in test drug administration.

This extract was found to be safe for further biological studies as no mortality was observed at 3000 mg/kg b.w. in rat. These observations from oral toxicity study suggest that the extract for PGP is practically non-toxic.

4.2. Effect of PGP extract on oral glucose tolerance in normal rats

The effects of PGP extract on oral glucose tolerance are shown in table 01. After 30 min starting the glucose tolerance test, blood glucose concentrations were increased from its initial value of control (group I) and then glycaemia started to decrease gradually till the end of the studies (180 min) with a value of 0.97±0.07 g/L.

PGP extract (400 mg/kg b.w.), inhibited the increase in glucose levels at 60 and 120 min after glucose loading, he was found to be effective in depressing the peak value of blood sugar. At 60 min starting test, plasma glucose levels were increased by 77.46% of control group and only by 40.12% of group II.

Administration of glibenclamide (5 mg/kg b.w.) induced time dependent hypoglycaemic effect, plasma glucose levels were decrease by 48.84% at the end of the test.

Table 01: Effect of oral administration of PGP extract on oral glucose tolerance.

	Blood glucose levels (g/L)				
Time (min)	**0**	**30**	**60**	**120**	**180**
Groups					
Group I (Control)	0.91±0.067	1.30±0.193	1.62±.0.170****	1.36±0.110*	0.97±0.072
Group II **(** 400 mg/kg p.c. PGP extract)	1.01±0.047	1.40±0.127*	1.42±0.060****	1.37±0.098*	1.28±0.106$^{\bullet}$
Group III **(** 5 mg/kg p.c. glibenclamide »	0.86±0.064	0.75±0.043$^{\bullet}$	1.13±0.080$^{*\bullet}$	0.59±0.098$^{*\bullet\bullet}$	0.44±0.062$^{**\bullet\bullet}$

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value
($^{\bullet}$) : statistically significant difference to the control group

4.3. Effect of PGP extract on blood glucose levels in normal fasted rats

Table 02 shows the effect of PGP extract on blood glucose levels in normal fasted rat for 3 h. From these results, PGP extract did no effect on blood glucose levels. Compared with control group, the blood glucose in rats treated with PGP extract remained within normal limits with a slight increase during the 1st and 2nd hour (1.18 g/L and 1.24 g/L, respectively).

Rats treated with glibenclamide (5 mg/kg b.w.), show a gradually decrease in blood glucose after 30 min starting test. Blood glucose was decrease by 37.61% with a hypoglycaemic effect at the end of the experiment (0.55 ± 0.025 g/L).

Table 02: Effect of oral administration of PGP extract on blood glucose levels in normal fasted rats

	Blood glucose levels (g/L)				
Time (min)	**0**	**30**	**60**	**120**	**180**
Groups					
Group I (Control)	1.03±0.035	1.14±0.055	1.16±0.015*	1.13±0.068	1.06±0.073
Group II (400 mg/kg p.c. PGP extract)	0.99±0.048	1.18±0.016*	1.24±0.009$^{***\bullet\bullet}$	1.15±0.053	1.07±0.033
Group III (5mg/kg p.c. glibenclamide »	0.89±0.072	0.81±0.036$^{\bullet}$	0.77±0.041$^{\bullet\bullet\bullet}$	0.59±0.0013$^{*\bullet\bullet}$	0.55±0.025$^{**\bullet\bullet}$

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value
($^{\bullet}$) : statistically significant difference to the control group

After two weeks of daily treatment, a highly significant decrease in blood glucose (26.33%) is observed in normal rats treated with PGP extract but no hypoglycaemic effect during the first week, during the second week this decrease was only 13.07% (table 03).

Table 03: Effect of oral daily administration of PGP extract on blood glucose levels in normal fasted rats

	Blood glucose levels (g/L)		
Time (weeks)	**Week 0**	**Week 1**	**Week 2**
Groups			
Group I (Control)	0.89±0.04	0.92±0.070	1.08±0.034 **
Group II (400mg/kg p.c. PGP extract)	1.08±0.03	0.80±0.025***	0.94±0.057

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value

4.4. Effect of PGP extracts on blood glucose levels in STZ-induced diabetic rats

Table 04 shows the effect of PGP extract on blood glucose levels in STZ-diabetic rats for 3 h. The diabetic control rats (group I) have a significant hyperglycaemia increases gradually during the experiment. Blood glucose in rats treated with PGP extract also increases but with a smaller rate compared to control.

The blood glucose of diabetic rats treated with glibenclamide decreases non significantly during the experiment. To 3 h., this decrease is relatively large (18.02%).

Table 04: Effect of oral administration of PGP extract and glibenclamide on blood glucose levels in STZ-induced diabetic rats.

	Blood glucose levels (g/L)				
Time (min)	**0**	**30**	**60**	**120**	**180**
Groups					
Group I (Control)	2.45±0.34	3.05±0.31	3.28±0.32*	3.49±0.28*	3.37±0.35**
Group II (400mg/kg p.c. PGP extract)	2.48±0.37	2.96±0.16	2.83±0.20	3.06±0.23*	2.85±0.32
Group III (5mg/kg p.c. glibenclamide »	2.40±0.71	2.54±0.62	2.36±0.78	2.23±0.69	1.97±0.60

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value

Table 05 shows the effect of oral daily administration of PGP extract (400 mg/kg b.w.) on blood glucose levels in diabetic rats for two weeks. These results displayed significantly lowered blood sugar level. After the 1st week, blood glucose decreased significantly (56%) compared to initial value, it decreased from 2.48 g/L to 1.09 g/L. After the 2nd week, blood glucose was only reduced by 32.92%. Also, blood glucose in rats treated with glibenclamide is reduced by 50.55% in the first week to be increased during the second week by 37.36% (3.3 g/L). Compared with control rats, blood glucose was increased by 50.92% during the second week.

Table 05: Effect of PGP extract on blood glucose levels in STZ-induced diabetic rats after oral daily administration.

	Blood glucose levels (g/L)		
Time (weeks) / **Groups**	**Week 0**	**Week 1**	**Week 2**
Group I (Control)	2.45±0.34	2.48±0.67	3.70±0.41**
Group II **(** 400 mg/kg p.c. PGP extract)	2.48±0.32	1.09±0.41*	1.66±0.41•
Group III **(** 5 mg/kg p.c. glibenclamide »	2.40±0.71	1.19±0.75•	3.30±1.28

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value
(•) : statistically significant difference to the control group

4.5. Effect of PGP extract on hyperlipidemia in STZ-induced diabetic rats

Table 06 and 07 shows the plasma levels of total cholesterol and triglycerides index in normal and STZ-induced diabetic rats. STZ treatment resulted in significant elevation of TC and TG. There was a significant reduction index of diabetic rats treated with PGP extract.

Table 06: Effect of PGP extract on TG levels in STZ-induced diabetic rats after oral daily administration

	Triglycérides (g/L)		
Time (weeks) / **Groups**	**Week 0**	**Week 1**	**Week 2**
Group I: Diabetic control	2.08±0.5	2.01±0.43	4.00±0.44 **▲▲
Group II: Diabetic + 400 mg/kg p.c. PGP extract	2.00±0.63	1.55±0.31	1.92±0.72 •
Group III: Diabetic + 5mg/kg p.c. glibenclamide	3.89±1.2▲	1.70±0.83	2.78±1.29•
Group IV: Normal control	1.82±0.43	1.74±0.2	2.0±0.23

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value
(•) : statistically significant difference to the diabetic control group
(▲) : statistically significant difference to the normal control group

Table 07: Effect of PGP extract on TC levels in STZ-induced diabetic rats after oral daily administration

	Cholestérol (g/L)		
Time (weeks) / **Groups**	**Week 0**	**Week 1**	**Week 2**
Group I: Diabetic control	0.65±0.052▲▲	0.40±0.094*	0.64±0.096▲
Group II: Diabetic + 400 mg/kg p.c. PGP extract	0.65±0.067▲	0.40±0.068*	0.38±0.054 *●
Group III: Diabetic + 5mg/kg p.c. glibenclamide	0.62±0.130	0.41±0.021*	0.40±0.1
Group IV: Normal control	0.45±0.06	0.42±0.083	0.39±0.045

Values are expressed as mean±SEM (n=5)
(*) : statistically significant difference to the zero time value
(●) : statistically significant difference to the diabetic control group
(▲) : statistically significant difference to the normal control group

4.6. Effet of PGP extract on α-amylase activity

4.6.1. Enzyme kinetics in the absence of inhibitor

- **Detremination of initial velocities**

The effect of different concentrations of substrate on the activity of α-amylase is shown in figure 01. The initial velocities calculated from this curve are the slopes of the lines traced through the experimental points. The initial velocities obtained are shown in the table 08.

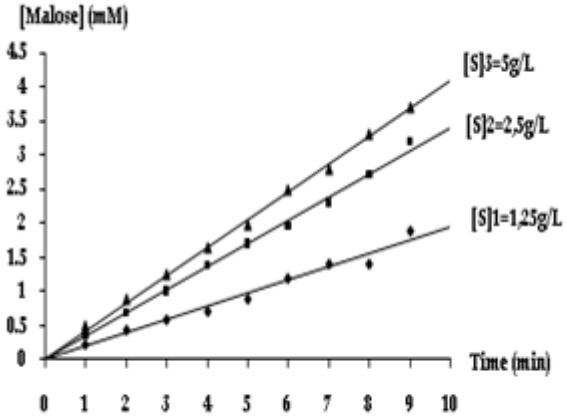


Figure 01: Effect of different concentrations of starch on the activity of AOA (EA=1.3 UI/mL).

Table 08: The initial velocities of the enzymatic reaction at different concentrations of starch.

Concentrations of substrat	**1.25 g/L**	**2.5 g/L**	**5 g/L**
Initial velocities (mM/min)	0.200	0.330	0.410

- **Determination of kinetic parameters of α-amylase**

The double-reciprocal plots « *Lineweaver-Burk* » of the reaction of AOA with different concentrations of starch in the absence of inhibitor is shown in figure 02.
The extrapolated value of kinetic parameters for AOA gives a *Vmax* of 0.714 mM/min and *Km* of 3.4 g/L.

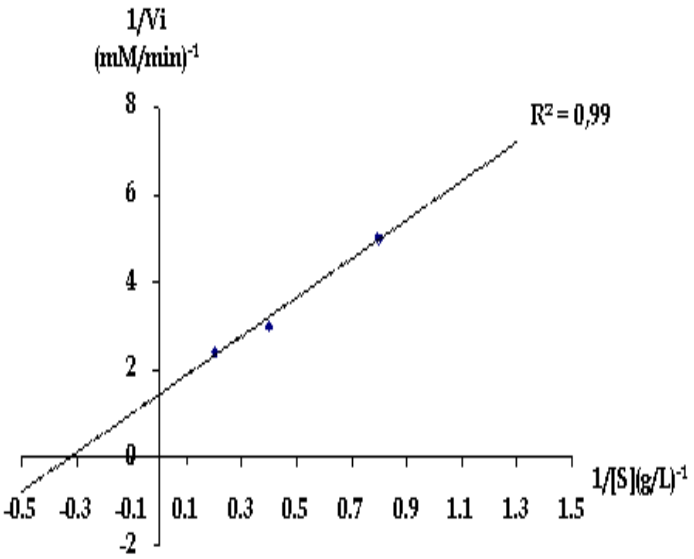


Figure 02: Double-reciprocal plots of the starch concentrations on the activity of AOA.

4.6.2. Enzyme kinetics in the presence of inhibitor

- **Detremination of initial velocities**

Following the same procedure, after adding different concentrations of inhibitor (PGP extract or acarbose), the initial velocities are determined graphically. The values obtained were shown in Table 09 compared with control kinetic.

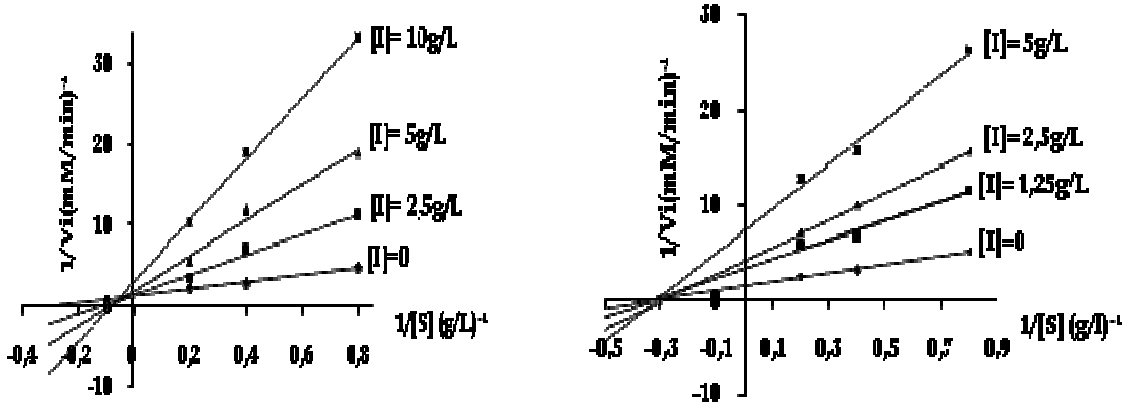
Table 09: The initial velocities obtained after enzymatic hydrolysis of different concentrations of starch in the absence and presence of inhibitor (EA=1.3 UI/mL).

Inhibitor Concentrations / Substrat		1.25 g/L	[2.5 g/L	5 g/L
[I]=0		**0.200**	**0.330**	**0.410**
PGP extract	**2.5 g/L**	0.089	0.143	0.280
	5 g/L	0.052	0.085	0.178
	10 g/L	0.030	0.052	0.097
Acarbose	**1.25 g/L**	0.087	0.151	0.175
	2.5 g/L	0.064	0.105	0.143
	5 g/L	0.038	0.063	0.077

According to the results shown in the table 09, the initial velocities of the enzymatic reaction decreases in the presence of different concentrations of the inhibitor (PGP extract or acarbose) compared to that obtained in the absence of inhibitor, this result shows that there is an inhibition of the α-amylase induced by the PGP extract compared with positive control.

- **Determination of kinetic parameters and mechanism of inhibition**

The double-reciprocal plots « *Lineweaver-Burk* » of AOA in the absence and presence of inhibitor is shown in figure 03. The limiting velocitie (*Vmax*) and Michaelis Menten constant (*Km*) obtained from these representations are shown in Table 10.



(A) (B)

Figure 03 : Double-reciprocal plots « *Lineweaver –Burk* » in the absence and presence of PGP extract (A) and acarbose (B) (EA=1.3 UI/mL)

Table 10: Limiting velocities and Michaelis Menten constant obtained in the absence and presence of inhibitor

PGP extract (g/L)	0	2.5	5	10
Vmax (mM/min)	**0.714**	0.685	0.591	0.310
Km (g/L)	**3.4**	8	10	11.11
Acarbose (g/L)	**0**	**1.25**	**2.5**	**5**
Vmax (mM/min)	**0.714**	0.306	0.240	0.136
Km (g/L)	**3.4**	**3.4**	**3.4**	**3.4**

From these results, the limiting velocitie (*Vmax*) was decrease gradually in the presence of PGP extract and acarbose, but Michaelis Menten (*Km*) constant was increase in the presence of PGP extract and remains constant in the presence of acabose.

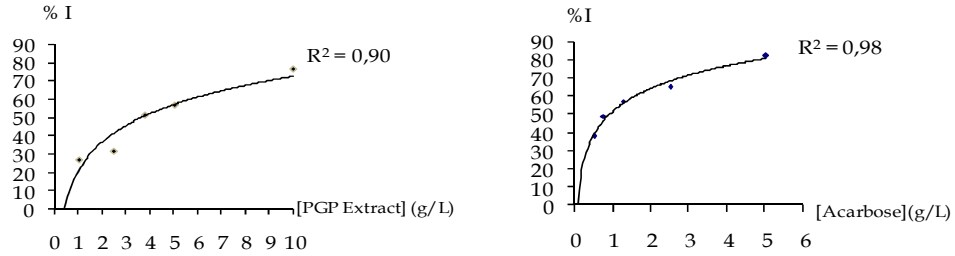
The *Lineweaver-Burk* plots found that the mechanism of inhibition exerted by PGP extract and acarbose is a mixed noncompetitive and non-competitive mechanism, respectively.

- **Determination of IC_{50} and The inhibition constant (*Ki*).**

To calculate the percentage of inhibition and IC_{50}, the concentration of substrate is sat at 5 g/L in the presence of different concentrations of inhibitors.

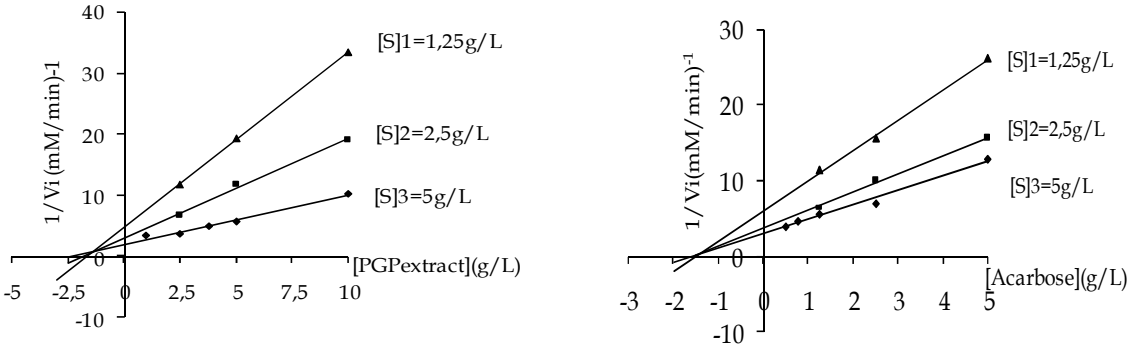
The IC_{50} exerted by the PGP extract or acarbose is determined graphically using logarithmic regression of percentage inhibition versus inhibitor concentration (Fig 04). The inhibition constant is determined from the Dixon plot (Fig.05).

PGP extract was showed inhibitory activity against α-amylase with IC_{50} of 3.65 g/L and Ki of 1.25 g/L compared with the positif control (IC_{50}=0.89 g/L, Ki=1.5 g/L).



(A) (B)

Figure 04: Logarithmic regression of percentage inhibition for different concentrations of PGP extract (A) and acarbose (B).



(A) (B)

Figure 05: Representation of Dixon plot: (A) PGP extract; (B) acarbose.

DISCUSSION

Many herbal medicines have been used in the prevention and treatment of diabetes. They have in general not been associated with marked toxic or other side effect. As for most natural medicines, however, their action mechanisms need to be clarified [25-26].

This study investigated the potential antidiabetic activity of *P. granatum* medicinal plant used in traditional treatment of diabetes.

For the first time, this study reports the antihyperglycaemic effect of the extract of PGP. In normoglycaemic fasted rat, this extract marked no hypoglycaemic activity but in oral glucose tolerance test (OGTT), PGP extract lowered the peak of blood glucose levels after administration of glucose. These effects were compared with glibenclamide, the reference hypoglycaemic drug of sulphonylurea type [27]. Administration of glibenclamide induced time-dependent hypoglycaemic effect in normal rats by stimulating β-cells to release insulin. From these results, lowering effect of blood glucose levels of PGP extract not be due to potentialise of insulin release from pancreatic cells.

It is reported by Nogueira and Pereira (1986b) [17], that an infusion of the epicarps of *P. granatum* inhibited the intestinal absorption of glucose in rats. Thus a possibility exists that

retardation of intestinal glucose absorption may also be partly responsible for inhibition of hyperglycaemia in glucose-fed rats.
In addition, PGP extract significantly reduced the blood glucose levels in STZ-induced diabetic rats in the first and second week after daily administration, compared to the control group where blood glucose remains high. In STZ diabetic rats treated with glibenclamide, the lowering of blood glucose was observed only in first week, because STZ causes diabetes by destruction of β-cells pancreatic. The effects suggest that PGP extract lowers glucose levels by increasing the peripheral glucose utilization or by improving the sensivity of insulin receptors, inhibition of the proximal tubular reabsorption mechanism for glucose.

It is roported by Khalil (2004) [18] that an aqueous extract of PGP at dose of 430 mg/kg b.w lowered blood glucose levels in alloxan-induced diabetic rats after 4 weeks of daily administration.

Dyslipidemia is one of the major cardiovascular risk factors, it has been demonstrated that insulin deficiency in DM leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulations of lipids such as TC and TG in diabetic patients [28]. Diabetic patients have problems in packaging cholesterol and tend to have higher serum TG levels. Our data were in line with this notion as the STZ treated diabetic rats exhibited clear-cut abnormalities in lipid metabolism as evidence from the significant elevation of serum TC, TG. Treatment with PGP extract for 15 day was sufficient to produce a significant reduction in the TC and TG. These results indicate that PGP extract has a lipid-lowering effect on the diabetic rats.

In second time, this study was designed to establish the inhibitory activity of PGP extract against α-amylase, digestive enzyme related to diabetes. PGP extract was exerted the inhibitory activity with IC_{50} value of 3.65 g/L compared with acarbose inhibitory activity (IC_{50}= 1.86 g/L) a second type of inhibitory of α-amylase (Wong et Robertson, 2003) [29]. The result suggest that PGP extract inhibits carbohydrate digestion rather than the transit and absorption of glucose and other sugar in the digestive tract and improves post-prandial hyperglycaemia, at least in part by inhibiting α-amylase activity.

Prashanth *et al.* [30] reported that an ethanolic extract of PGP inhibited the α-amylase activity by 68.2% at 1 mg/mL of the reaction mixture.

CONCLUSION

In conclusion, the PGP extract, in totality, was effective in lowering the blood glucose levels, TC, TG and inhibiting α-amylase activity.

Studies are underway to further elucidate the mechanism of antihyperglycaemic effect of the extract of the peels of *P. granatum* an ancient medicine for diabetes and isolation of the active components may pave the way to development of new agents for the treatment of diabetes and its complications.

Acknowledgments
I thank M^r Azzi rachid, M^r Rahmoun Mohamed Nadjib and Benariba Nabila for their suggestions and technical assistance.

REFERENCES

[1] WHO: World Health Organisation Consultation, *Report of a WHO Consultation*, Geneva, **1999**.
[2] T. Matsui, T. Tanaka, S. Tamura, A. Toshima, Y. Miyata, K. Tanaka *et al.*, *Journal of Agricultural and Food chemistry.*, **2007** (55), 99, 105.
[3] L.H. Smith, S.O. Their, Fisiopatologıa: Principios biologicos de la enfermedad. Editorial Medica Panamericana, *Buenos Aires*, **1999**, 1549, 1600.
[4] D. Syiem, G. Syngai, P.Z. Khup, B.S. Kharbuli, H. Kayang, *Journal of Ethnopharmacology.*, **2002** (83), 55,61.
[5] R. Rhabasa-Lhoret, J.L. Chiasson, α-Glucosidase inhibitors (third ed), John Wiley & Sons Ltd., UK, **2004**, 901–914.
[6] J. Maroo, V.T. Vasu, R. Aalinkeel, S. Gupta, *Journal of Ethnopharmacology.*, **2002** (83), 317, 320.
[7] H. Fujita, T. Yamagami, *Life science*., **2001** (70), 219, 227.
[8] J.K. Grover, S. Yadav, V. Vats, *Journal of Ethnopharmacology.*, **2002**, 81, 100.
[9] N.K. Murthy, V.K. Reddy, J.M. Veigas, U.D. Murthy, *Journal of Medicinal Food.*, **2004** (7), 256, 259.
[10] C.L. Chopra, M.C. Bhatia, I.C. Chopra, *J. Am. Pharm. Assoc*., **1960**, 49, 780.
[11] V.B. Trivedi, S.M. Kazmi, *Ind. Drugs.*, **1979**, 16, 295.
[12] M.A. Charya, M.M. Reddy, B.P. Kumar, S.R. Reddy, *New Botany.*, **1979**, 6, 171.
[13] K.C. Singhal, *Ind. J. Pharmacol*., **1983**, 15, 119.
[14] M.L. Gujral, D.R. Varma, N.K. Sareen, *J. Med. Res.*, **1960**, 48, 46.
[15] D.G. Nogueira, N.A. Pereira, *Rev. Bras. Farm*., **1984**, 65,46.
[16] D.G. Nogueira, N.A. Pereira, *Rev. Bras. Farm*., **1986**a, 67,59.
[17] D.G. Nogueira, N.A. Pereira, *Rev. Bras. Farm.*, **1986**b, 70,129.
[18] E.A. Khalil, *The Egyptian Journal of Hospital Medecine.*, **2004** (16), 92, 99.
[19] M.A. Jafri, M. Aslam, K. Javed, S. Singh, *Journal of Ethnopharmacology.*, **2000** (70), 309, 314.
[20] Y. Li, S. Wens, P.B. Kota, G. Peng, G.Q. Li, J. Yamahara, B.D. Roufogalis, *Journal of Ethnopharmacology.*, **2005** (99), 239,244.
[21] R.K. Gupta, A.N. Kesari, P.S. Murthy, R. Chandra, V. Tandon, G. Watal, *Journal of Ethnopharmacology.*, **2005** (99), 75, 81.
[22] C.P. Fasce, *Clim Chem*., **1982**, 18, 901.
[23] P. Fossati, L. Prencipe, *Clim Chem*., **1982** (28), 2077, 2080.
[24] P. Bernfeld, Amylase α and β, in Methods in Enzymology. (Colowick S. and Kaplan N.O. eds.), *Academic Press*, *New York*., **1955**, 149,158.
[25] F. Ye, Z. Shen, M. Xie, *Phytomedicine.*, **2002** (9), 161,166.
[26] Y.M. Kim, M.H. Wang, H. Rhee, *Carbohydrate Research.*, **2004** (339), 715, 717.
[27] E. Larger, Mécanisme d'action des antidiabétiques oraux. Médecine thérapeutique, Paris, **1997**, 97-102.
[28] R.B. Goldberg, *Diabetes Care.*, **1981** (4), 561, 572.
[29] D.W. Wong, G.H. Robertson, α-Amylases, *U.S. Department of Agriculture*, California, **2003**.
[30] D. Prashanth, R. Padmaja, D.S. Samiulla, *Fitoterapia.*, **2001** (72), 179, 181.