

EFFECT OF AQUEOUS PSIDIUM GUAJAVA LEAVES EXTRACT AS ANTI - HYPERGLYCEMIC AGENT

Omima M. R. Abu Elez⁽¹⁾, M. A. Hammam⁽²⁾ and F.M. Elshony⁽²⁾

⁽¹⁾ Pathological of Biochemistry Dept. - Medical Analysis- El-Roda Hospital- Damietta - Egypt

⁽²⁾ Biochemistry Department- Faculty of Agricultural- Menuofia University-Egypt

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ABSTRACT: Background: Hyperglycemia is one of the serious problems that affect humans' lifestyle and require special treatments. Incorrect use of synthetic drugs results in side effects and other problems. Diabetic patients need safer and more effective anti-diabetic drugs that decrease diabetic complications. Natural products are harmless due to conventional medicine can be inefficient (e.g. side effects and ineffective therapy).

Objective: Evaluate anti- hyperglycemic activity of aqueous leaves extract of Psidium guajava in hyperglycemic rats.

Methods: Adult males albino rats, weighting (140-150 grams) were assigned to one of four groups of six animals each (n= 6). Hyperglycemia induced by single dose intraperitoneal injection of STZ - NA (60 mg/kg - 110 mg/kg, b.wt i.p.), the control groups was given distilled water, the experimental sub-groups were started one week later of STZ induction, then treated once daily by aqueous extract of Psidium guajava leaves until the end of experiment. Initially, blood samples were taken for measured glucose levels and HbA1c. The body weight, urine sugar and blood glucose levels were checked regularly.

Results: The polyphenolic of guava leaves extract was found to be (227.63 mg GAE /g. Ex), while flavonoids content was (79.96 mg QE/g. Ex). However, it caused a significant decrease in blood glucose levels and HbA1c and other glycemc indicators.

Conclusion: aqueous extract of Psidium guajava leaves possess significant anti-diabetic, and effective in lowering blood glucose level but lack hypoglycemic effect in hyperglycemic rats. Further studies warranted to elucidate possible mechanism(s) of action of the plant material.

Key words: Hyperglycemia, Hypoglycemia, Anti-hyperglycemic, Diabetic, Natural products, Psidium guajava, Streptozotocin and Rats.

INTRODUCTION

Hyperglycemia or abnormally high blood glucose one of the serious problems that require special treatments and can be affect humans' lifestyle. Hyperglycemia developing with impaired fasting glucose occurs when the bodies lack insulin or cannot use insulin properly causes an osmotic diuresis with significantly dehydration and electrolytes loss and directly causes glycation of proteins, lipids and nucleic acid, then

injures cells and induces lipid peroxidation (Ahmadvand *et al.*, 2014). Long- term hyperglycemia, leads to increased formation of reactive oxygen species (ROS) free radicals and increasing diabetic nephropathy complications associated with oxidative stress leads to serious macro-vascular and micro-vascular diabetic complications such as neuropathy, nephropathy, cardiac failure stroke,

kidney failure and premature death (CDC, 2014).

The prevalence of diabetes higher in men than in women (CDC, 2015). Four out of five people with diabetes live in low- and middle-income countries and half of these people who died from diabetes under the age of 60. Africa has the highest mortality rate due to diabetes compared to other parts of the world (IDF, 2012).

Incorrect use of synthetic drugs results in side effects and other problems (WHO, 2004). It is necessary to evaluate medicinal plants to support existing drugs in treating diabetes, which is very cheap, effective, harmless, safe and have biological activities to treat life-threatening ailments with no side effects, low toxicity and economic (Grasas *et al.*, 2018). Currently, many herbal medicines therapeutic available recommended for the treatment and improve diabetes (Altalhi *et al.*, 2018). Among these plants is common guava leaves tree is a member of *Myrtle* family (*Myrtaceae*) (Naseer *et al.*, 2018), genus: *Psidium*, species: *guajava* and common names of the plant are guava, goiaba, guayaba, *etc.* (Godebo *et al.*, 2018). The leaves are wide, clear green in color, and have prominent veins (Rouseff *et al.*, 2011).

Phytochemical analysis of guava leaves, revealed the presence of more than 20 isolated compounds include carotenoids, essential oils, fatty acids, anthocyanin's, coumarins, luteolin, and quercetin-like compound, terpenoids and triterpenes (Verma *et al.*, 2018). Guava leaf contains high levels of copper, vitamins [C, B3, B6 and A], iron, tannin, saponins, alkaloid, glycosides, Morin, carbohydrates and dietary fiber. It contains also low calorie, minerals, antioxidants, polyphenol and flavonoids especially (quercetin) for prevention of cancers, aging, cell differentiation and

apoptosis and immune enhancing, and prevent weight loss, maintain dental protection, reduce cholesterol levels, prevent stomach, prostate cancer and very effective in diarrhea treatment (Olaniyan, 2017).

The aim of work: Evaluate the hypoglycemic and anti- hyperglycemic activity and to prove in vivo antioxidant potential of aqueous leaves extracts of *Psidium guajava* in hyperglycemic rats.

MATERIALS AND METHODS

Preparation of plant leaves extract

Freshly, green leaves of *psidium guajava* obtained from the farm of (Agricultural of Research Centre- Giza-Egypt). The leaves washed with water then dried in the shade away. The air-dried plant material was ground using a mechanical grinder. 300 gm of fine powdered material macerated in 3000 ml of methanol (80%) overnight for 72 hours, with frequent agitation. Then filtered, the residues were re-extracted in the solvent for another 2 times. The supernatant filtered twice; through cheese cloth then through filter paper (whatman No.1). Then evaporated by rotary evaporator under pressures at 40°C and dried using incubator at 40 °C for 24 hours to solid yield extract of guava leaves. The extract was preserved in sterile dark bottle at 4°C until further experiments.

Determination of total polyphenols

Total polyphenol contents of leaves were determined in ME using Folin-Ciocalteu reagent assay based on a colorimetric oxidation / reduction reaction, described by Chandra *et al.*, (2014). The purplish compound absorbance was measured at 750 nm, against distilled water as blank. Total phenolic contents expressed as mg Gallic acid equivalents (mg GAE) /g dry weight extract.

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Determination of total flavonoids content: The estimation of total flavonoids content was performed by aluminum chloride colorimetric assay with quercetin as a reference, described by Chang et al., (2002). The absorbance of the reaction mixture was measured at 415 nm, using UV/ VIS- 1800 Series, spectrophotometer (Perkin Elmer, Lambda 2S, USA) against blank.

Reducing Power assay

Reducing power was performed as described in the method by (Ferreira et al., 2007). Ascorbic acid was used as reference standard. Absorbance was measured at 700 nm against blank solution.

The experimental animals

Healthy; Twenty four adult males albino rats have average weighted (140 - 150) gm. All animals housed in the clean plastic ventilated cages and steel wire tops. Cages floor were covered with sawdust, which replaced every 2 days. All rats allowed acclimatizing to a new laboratory environmental for 7 days under laboratory conditions (12h-light / 12h-darkness cycle) in air-conditioned room ($22 \pm 2^{\circ}\text{C}$, 45-75 % relative

humidity). During the experimental period animals had *ad libitum* with free access to tap water and basal diet.

Seventy-two hours later of injection of STZ, the glucose levels measured from the tail vein after overnight fast. Hyperglycemia confirmed by using glucometer (Accu- Check, Germany). Only the rats considered as diabetic if fasting glycemia levels higher than 250 mg/dl. Hyperglycemia symptoms appeared after 72 hours as weight loss, polydipsia and polyuria. More assurance was obtained after detecting glucosuria. All studies were carried out after one week of STZ injection. Untreated Diabetic Group ($n = 6$) received basal diet and Treated Diabetic Group ($n = 12$) fed with the same diet supplemented with aqueous extract of guava leaves for 8th weeks.

Blood Samples:

Initially, blood samples taken for measured glucose levels and HbA1c. The urine sugar, body weight and blood glucose levels were checked regularly. Blood examination at 8th - weeks revealed difference between the diabetic control group and treated groups.

Experimental protocol:

N	Group	Treatment
I	Normal Control (NC)	Non-diabetic rats, received daily basal diet and tap water followed by a single orally 1ml of distilled water only, twice weekly during the experimented period of eight-weeks.
II	Diabetic Control (DC)	STZ-diabetic rats (60 mg/kg. b.wt. I.P.), received daily basal diet and tap water followed by a single orally 1mL of distilled water only during the experimented period.
III	D+ PGLE- 0.2 gm.	STZ-diabetic rats received basal diet and tap water and treated daily with single orally with aqueous guava leaves extract only at a dose (0.2 g/kg b.w/ day).
IV	D+ PGLE- 0.4 gm.	STZ-diabetic rats received basal diet and tap water and treated daily with single orally supplemented with aqueous guava leaves extract only at a dose (0.4 g/kg b.w/ day).

Estimation of biochemical parameters

Determination of fasting blood glucose levels

At the Initial of the experiment, following the induction of STZ- induced rats, blood samples were taken from the base of rats' tail. The blood glucose level estimated using a standard glucometer (Accu – Check, Germany), then monitored periodically at end of (2nd weeks, 4th weeks and 8th weeks) up to eight- weeks according to the enzymatic colorimetric method described by (Trinder, 1969).

Estimation of glycosylated hemoglobin (HbA1c)

Initial screening and final experiment, blood samples were obtained from the retro-orbital region of each rat into tubes containing EDTA as anticoagulant for estimation of HbA1c in whole blood according to method described by (Tietz, 1999).

Urine glucose levels

Urine glucose levels were weekly checked by urine glucose strips according to method described by NCCLS, (1992). Unpreserved urine samples may lose up to 40% of their glucose after 24 hours storage at room temperature.

Determination of body weight gain

The body weighed pre-treatment and once a weekly for all animals to adjust the dose of treatments. The body weight gain estimated by subtracting the initial body weight (before STZ induced) from the final body weight (post-treatment) of each rat.

Statistical analysis

Quantitative data results expressed as mean \pm SD, one-way (ANOVA) test was

employed for comparison each parameter among groups before and after the treatment. $P \leq 0.05$ considered significant and $P > 0.001$ considered highly significant for comparison the results.

RESULTS AND DISCUSSION

Total polyphenols and flavonoids content

Natural Polyphenols are secondary metabolites act as defense against different types of the stress; Protection of the plants against ultra-violet radiation, low soil fertility and changes of environmental temperature (Rodríguez *et al.*, 2014). While flavonoids high redox potential, act as reducing agents, hydrogen donors, and scavengers of singlet oxygen (Tsao and Yang 2003).

Our results of total polyphenols and flavonoids content were illustrated in (Fig. 1). Guava leaves possess the highest antioxidant activity with higher total phenolic contents (227.63 mg GAE/g. Ex), and flavonoids content (79.96 mg QE/g. Ex) in the present analysis.

Our results were accordance with those of El-Sayed and Said, (2017), who noted the extracts of guava leaves possess highest antioxidant capacities with higher total phenolic contents as well as the largest concentration of flavonols.

Reducing power of various concentrations of guava leaves extract

Reducing power assay measures the total reducing capability of antioxidants based on methanolic extract or Vit.C ability to reduce yellow ferric complex (Fe^{+3}) to blue ferrous complex (Fe^{+2}) ions by electron-donating antioxidants in acidic medium. The reducing power capacity of methanolic

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leaves extract of *p. guajava* as absorbance value of (0.606) was significantly lower than vitamin C (0.692) at 20 $\mu\text{g} / \text{ml}$ (Fig. 2). At the highest concentration of phenolic up to the level 80 $\mu\text{g}/\text{ml}$ guava leaves extract showed linearly highest activity (1.675). The reducing power of methanolic leaves extract of guava relatively increased with graded concentration. Increasing absorbance at 700 nm indicates an increase in reductive ability (Benzie and Strain, 1999).

The changes in fasting blood glucose levels induced by leaves extracts

As shown in Table (1), before first-week of induction of diabetes in rats, there is not any significant difference among the groups in blood sugar levels. At initial of experiment (Zero- Time), blood sugar level after given STZ, increased to 282.3 mg/ dL in diabetic control. According to the experiment results, normal fasting blood glucose in rats of this experiment was determined as 84.92 mg/dl. Serum glucose level significantly increased ($p \leq 0.05$) in diabetic control group as compared to normal control rats after second weeks, 4th weeks and final weeks of treatment.

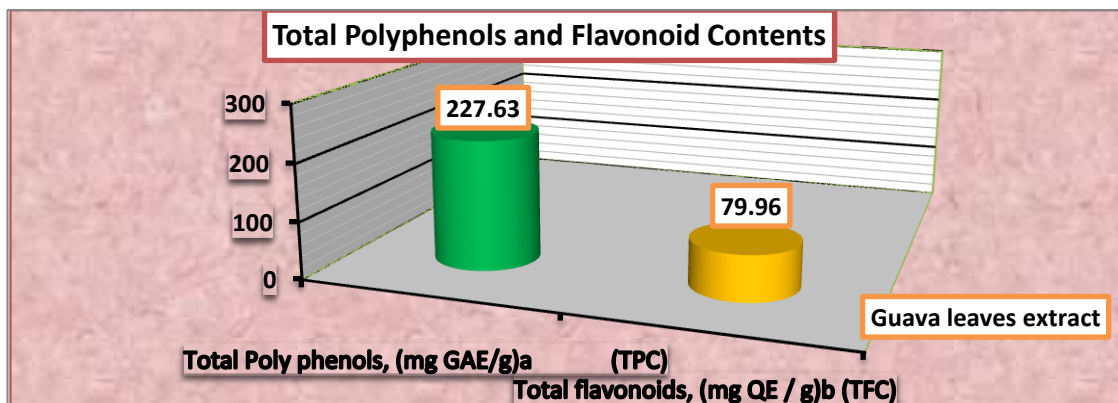


Fig. (1): Total polyphenols and flavonoids content in methanolic guava leaves extract

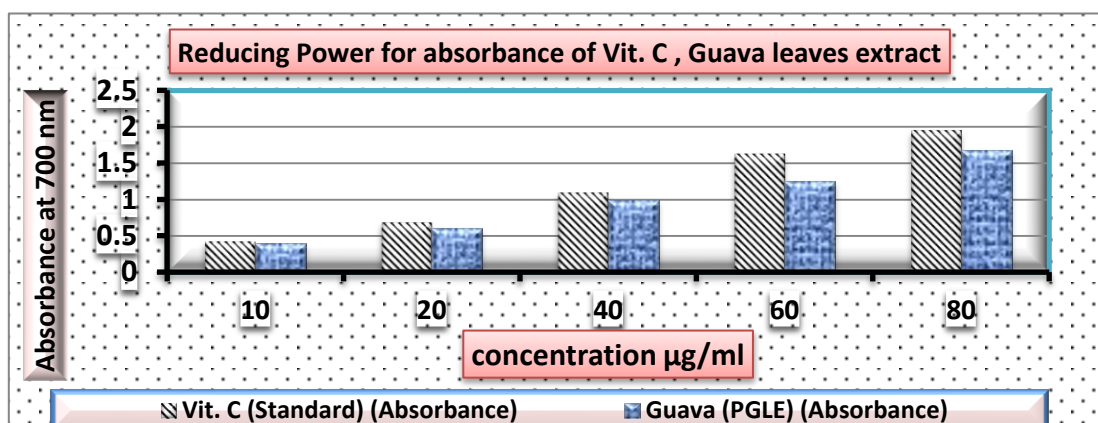


Fig. (2): Reducing power of various concentrations for guava leaves and olive leaves extract ($\mu\text{g}/\text{ml}$)

Table (1): Effect of graded doses of aqueous leaves extract of *p. guajava* on glycemic indices (fasting bl. glucose) in severe diabetic rats

Experimental rats	No. Rats	Fasting plasma glucose levels (mg/dl)			
		Pre- Treatment Blood glucose levels (mg/100 ml blood) (Zero -Time) Initial week	Post – Treatment Blood glucose levels (mg/100 ml blood)		
			2 nd weeks	4 th weeks	8 th weeks Final week
Normal Control (NC)	6	84.92 ± 6.8	83.63 ± 4.2	84.34 ± 2.3	79.61 ± 7.2
Diabetic Control (DC)	6	282.3 ± 37.6	396 ± 47.1	425.3 ± 12.2	465.0 ± 27.3
D + PGLE- 200 mg	6	264.0 ± 13.9	169.4 ± 5.3*	115.4 ± 3.9**	89.22 ± 2.5**
D + PGLE- 400 mg	6	306.2 ± 23.4	138.6 ± 3.2*	102.6 ± 4.8**	78.13 ± 2.6**

Mean in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at* (P ≤ 0.05) between DC group & at ** (P > 0.001) between NC group and other groups, compared to pre- treatment levels.

Co-treated daily with administration of Aq. leaves extract of *p.guajava* significantly reduced the blood glucose levels in all the treated groups compared to the diabetic control. Glucose level in rats' model of diabetes treated daily with guava leaves extract (400 mg/kg b.w) decreased to 138.6 mg /dl after 2 weeks and restored to control value 78.13 mg/dl after 8th – weeks.

Two extracts groups had a relatively similar decrease (200 mg and 400 mg) of Aq. *p. guajava* leaves extract (89.22 ± 2.5 mg/ dL and 78.13 ± 2.6 mg/ dL) respectively at final week. Thus, the dose of (200 mg/kg. b.wt) sufficient for reduction in blood glucose levels.

Glycosylated Hemoglobin (HbA1c %) Values

Maintaining glycemic control by treating well- HbA1c targets. The HbA1c levels of normal rats ranged from (5.07 ± 0.04 % and 5.11 ± 0.02 %). HbA1c level of the control untreated group was increased an approximately 2 times (6.24 ± 0.33%) to (11.4 ± 0.28 %). In current

investigate at the end of 8th-weeks in clinical trial, reported that HbA1c significantly decreased from (5.97 ± 0.14 % to 5.81 ± 0.03%) in PGLE-200 mg treated subjects (Table 2). At final- week after feeding, HbA1c levels of diabetic rats treated with herbal extract (0.4 g/kg b.wt.) was (6.34 ± 0.02%).

Urine glucose levels in variable experimental rats

Urine glucose measured once commonly no longer used for diagnosis or monitoring, because it's neither sensitive nor specific (Ardawi et al., 2000; and Alto, 2005). Table (3) shows glucose not detected in the urine of control rats. But diabetic untreated rats were highly glucosuria following induction of diabetic was (+) increased to (+++) at day 7, then increased further to (++++) at day 14 of the experiment. Diabetic treated with Aq. PGLE- 400 mg had initial urine glucose level (Nil) 24-hours after STZ treatment. It increased to (+) at day 7. The diabetic with Aq. PGLE- 200 mg treated rats had urine glucose levels of Trace and (++) at days 1 and 7 respectively after treatment

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with STZ. In this study, Aq. Guava leaves extract (200 & 400) mg/kg b.w/ day were equally effective in abolishing glycosuria, since there were no traces of glucose in the urine of plant leaves extract treated diabetic rats at the end of the study.

Our results were compatible with (Antai *et al.*, 2010), when comparative effects of Rothmannia Hispida leaves extract on alloxan-induced diabetic rats. They indicated that glucose not detected in the urine of normal control, and diabetic untreated rats were glycosuria; their mean urine glucose concentration at the end of the study was significantly higher than that of the treated groups.

Effect of leaves extract on the changes of body weight gain in variable rats

The normal control rats had initial body weight of (144 ± 5.0 g), but at end of the experiment, their body weight increased to (173 ± 2.3 g). The diabetic untreated group had initial body weight of (143 ± 5.0 g). Their final body weight was (94 ± 2.1 g) which significantly (P≤0.05) lower compared to the initial body weight (Table 4). The initial mean

body weight of diabetic treated with PGLE-200 mg was (143 ± 4.3 g). They had a significant (P≤ 0.05) weight lost at 7 day (129 ± 2.1) prior to treatment with the extract. At the end of the experiment, their mean body weight increased significantly (P>0.001) to (153 ± 2.5 g). PGLE- 400 mg treated groups, increased significantly (P>0.001) to (159 ± 2.3) compared with their weights of control values at zero – time.

We noted that *P.Guajava* leaves have more potent in diabetic rats for improving their body weight gain, might be a result of its high ability to control high blood glucose levels. The present results showed that the STZ-induced rat model of diabetes associated with a significant decrease in weight gain. These results agree with Husni *et al.*, (2016), who stated that STZ-induced diabetes characterized by a severe loss of body weight that explained by increase in fat and protein catabolism due to loss and ineffective utilization of glucose, and absolute or relative deficiency of insulin and decreased production of ATP and protein synthesis in all tissues.

Table (2): Effect of graded doses of aqueous leaves extract of p. guajava on HbA1c

Parameters Groups	N	Glycated Hemoglobin HbA1c (%)	
		Initial – week	Final – week
Normal control (NC)	6	5.11 ± 0.02	5.07 ± 0.04
Diabetic control (DC)	6	6.24 ± 0.33	11.4 ± 0.28
STZ+ PGET -200 mg	6	5.97 ± 0.14	5.81 ± 0.03**
STZ+ PGET-400 mg	6	6.67 ± 0.03	6.34 ± 0.02**

Table (3): Urine glucose level of variable experimental rats

Variable (Experimental rats)	N	Urine glucose		
		1 day	7 days	14 days
Normal control/NC	6	Nil	Nil	Nil
Diabetic untreated/ (DC)	6	+	+++	++++
D + PGET-200 mg /kg b.w/day	6	Trace	++	Nil
D + PGET-400 mg /kg b.w/day	6	Nil	+	Nil

Table (4): The effect of daily orally administration of aqueous extract of *P. guajava* leaves, within eighth- weeks on the change in body weight gain (gm) in hyperglycemic rats.

Experimental (Groups)	Body weight (gm.) (140 : 150)						
	N	Pre-treatment (before STZ - induced)	Pre-treatment (after STZ - induced)	Post- treatment levels			
				7- days M±SE	2 nd - Weeks M±SE	4 th -weeks M±SE	6 th - weeks M± SE
Normal Control (NC)	6	144 ± 5.0	148 ±1.2	151 ±1.1	154 ± 1.3	160±2.0	173±2.3
Diabetic Control(DC)	6	143 ± 5.0	137 ±1.0	130 ± 0.8	126± 2.0	107±1.6	94 ±2.1
STZ +PGL-200 mg	6	143 ± 4.3	129 ±2.1*	131 ± 1.4*	133 ± 2.3 *	140 ±2.4 **	153 ±2.5 **
STZ+ PGL-400 mg	6	145 ± 5.0	132 ±1.0*	135 ± 2.1*	140 ± 2.1 **	147 ±1.6 **	159 ±2.3**

Mean in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at* (P ≤ 0.05) between DC group and other groups or at** (P > 0.001) between NC group and other groups.

Conclusion

In conclusion, this study revealed that aqueous extracts of *psidium guajava* leaves possess significant anti-diabetic and effective in lowering blood glucose level, but lack hypoglycemic effect on hyperglycemic rats.

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تأثير المستخلص المائي لأوراق الجوافة كمضاد للداء السكري

أميمة محمد رشاد أبو العز^(١)، مصطفى عبدالله همام^(٢)، فؤاد مطاوع الشونى^(٢)

(١) قسم الكيمياء الحيوية الباثولوجية - للتحاليل الطبية - مستشفى الروضة المركزي - دمياط

(٢) قسم الكيمياء الحيوية - كلية الزراعة - جامعة المنوفية

المخلص العربي

Hyperglycemia: "الإرتفاع الغير طبيعى في سكر الدم"

هو أحد المشكلات الصحية الخطيرة التى تؤثر على أسلوب حياة، هذا المرض نتيجة نقص إنتاج الأنسولين المسئول عن تنظيم سكر الدم في الخلايا. . Hyperglycemia يسبب زيادة الأسموزية مع زيادة الجفاف، إنتاج الشقوق الحرة بالتالى زيادة الأكسدة مع فشل وظائف معظم أعضاء الجسم خاصة العين، الكلى، الشرايين وزيادة تكوين AGES المسئول عن المضاعفات الصغرى والكبرى وأمراض القلب المصاحبة للمرض السكرى..! يمكن السيطرة على هذا المرض بتشخيص أعراضه بطريقة سليمة ، إكتشافه مبكرا...! لكن في نفس الوقت فان الأدوية المصنعة لعلاج السكرى أصبح غير مناسب لعدة أسباب منها " الآثار الجانبية الغير مرغوبة وعدم كفاءة الادوية المصنعة " خاصة عند إستخدامها على المدى الطويل .. ! مما أدى إلى البحث عن مصادر طبيعية للعلاج والتي لها تأثير فعال لعلاج السكرى، قليلة السمية وإقتصادية كما تقلل الآثار الجانبية ولا تسبب أى مشكلات صحية من بينها أوراق الجوافة كبدايل طبيعية.

أهداف الدراسة: التحقق من التأثير الحيوى لأوراق الجوافة كمضاد للداء السكرى وذلك بإنخفاض مستوى سكر الدم وغيرها من مؤشرات السكرى إلى المعدل الطبيعى باجراء التحليل البيوكيميائى لفئران التجربة المختبرة.....!

نتائج الدراسة الحالية فيما يلى :

- أوضحت النتائج أن المستخلص الميثانولى لأوراق الجوافة غنى بالمركبات الفينولية، غنية بالفلافونويد.
- تأثرت كل المؤشرات الحيوية نتيجة الإصابة بالداء السكرى لذلك أجريت التجربة البيولوجية لدراسة تأثير المستخلص المائي لأوراق الجوافة على خفض مستوى سكر الدم. حيث أشارت النتائج أن المجموعات المعالجة بأوراق الجوافة بتركيزاتها المختلفة قادرة على خفض مستوى سكر الدم، لذلك الجرعة (PGLE - ٢٠٠ مجم) كافية للتحكم فى مستوى سكر الدم كما أنها قادرة على خفض مستوى HbA1c. إنخفاض الوزن من مساوى الداء السكرى، انعكس ذلك على إنخفاض الوزن للمجموعات المصابة وعلى العكس تماما سجلت مجموعات الغذاء التجريبي إرتفاعا معنويا فى الوزن ، تشير المجموعة الثالثة [PGLE - ٤٠٠مجم] إرتفاعا معنويا فى الوزن (159 ± 2.3) ، بالتالى أفضل من الناحية الغذائية.

الخاتمة: أوضحت النتائج ان مستخلص أوراق الجوافة مؤثر على خفض مستوى سكر الدم فى فئران التجربة حيث يضم العلاج التحكم فى السكرى من أعراض ومنع حدوث مضاعفات مع تقليل انخفاض السكر عن المعدل الطبيعى.

أسماء السادة المحكمين

أ.د/ صلاح محمد القوصى كلية العلوم - جامعة المنوفية

أ.د/ مدحت مصطفى أبوزيد كلية الزراعة - جامعة المنوفية