

How to Cite:

Al-Hamdani, M. M., & Ali, S. A.-J. (2022). Antidiabetic property of ethanol extract of pelargonium graveolens with constituents chemical screening. *International Journal of Health Sciences*, 6(S5), 10290–10298. <https://doi.org/10.53730/ijhs.v6nS5.11671>

Antidiabetic property of ethanol extract of pelargonium graveolens with constituents chemical screening

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Abstract---Objects: The phytochemical screening and the antihyperglycemic impact of Pelargonium graveolens were performed to judge the impact of ethanol extract using alloxan-induced diabetic rats. Methods: Intragastric ethanolic extract amount level of 220 mg/kg body weight were given to alloxan-encouraged diabetic male albino rats for four weeks. Diabetic control rats assumed the equivalent quantity of the vehicle (Dimethylsulfoxide, DMSO) were processed together with the former group. The serum insulin, liver glycogen assay and oral glucose tolerance tests were done. The chemical screening of P. graveolens specified the existence of carbohydrates, glycosides, triterpenes, cardenolides, saponins, sterols, tannins, flavonoids and catechins. Results: The diabetic-treated rats with the ethanolic extract of P. graveolens performed clear improvement of the declined glucose tolerance and hepatic glycogen content without associated excess in serum insulin concentration. Conclusions: ethanol extract of P. Graveolens improved the glucose tolerance of alloxan-treated diabetic rats.

Keywords---Pelargonium graveolens ethanol extract, diabetic rats, phytochemical screening, Insulin, Glycogen.

Introduction

The predominance of Type 2 Diabetes (T2DM) in individuals over the age of 65 years, increasingly evolved ¹. Clear treatment strategies and alternatives treatments are required to meet the increase in the number of patients with T2DM in the world , high cost of modern treatments and the untreatable metabolic problems of the disease.²

Antihyperglycemic effect of some plants, might form a beneficial evolution of pharmaceutical entities forming new source of modern oral hypoglycemic compounds, or as supplement in addition to the existing drugs ³. Literature review revealed that

some plants had hypoglycemic action such as: alkaloids, polysaccharides, glycosides, glycans, oils, peptides, glycoproteins, sulfide molecules, vitamins, saponin and amino acids of various plant families ⁴⁻⁸. Multiple groups medicine systems used *Pelargonium graveolens* (known also as *Geranium graveolens*), as traditional treatment of hyperglycaemia..

Affi et al., ⁽²⁰¹⁴⁾ reported that about 28 components were yielded when hydrodistilled oil of *Pelargonium graveolens* fresh leaves, particularly citronellol, citronellylformate and menthone /isomenthone (oxygenated monoterpenes). Essential gastrointestinal enzymes incorporated in carbohydrate and lipid digestion have been shown to be inhibited by *Pelargonium graveolens* phytochemicals which may lead to support controlling diabetes ⁹. Ethanolic extract of *P. graveolens* was found by Pradeepa and coworkers to contain predominantly phytochemicals such as: tannins, phenol, flavonoid, glycosides, anthraquinone, terpenoids, low sugar, saponins, and phlorotannins while starch and steroids were absent ¹⁰.

The aim of this study was to estimate the hypoglycemic activity of the ethanolic extract of *P. graveolens* in male albino alloxan rats. Moreover, to learn about the preliminary phytochemical examination of *P. Graveolens* as well as the chemical analysis of the ethanol extract.

Materials and Methods

Preparation of plant extract

Leaves of *P. graveolens* were taken from Baghdad during the flowering period late summer 2019. One of the authors conducted the expression of the plant. The leaves of the plant were sun dried until constant weight. A quantity of 100 g of the dried plant materials was soaked in 1.0 L of ethanol (95%) at room temperature for 72 hrs to prepare the ethanol extract. The extract then filtered by two steps; first step through filter paper No. 42 (125 mm, Whatmann), second step by using cotton wool. Using a rotary evaporator (Heidolph, Germany), The extract was concentrated using a modified water bath heater at 40° C. The resultant extract was used directly for the phytochemical screening, and the rest was kept in a glass container at -20°C to perform the rest of the bioassay.

Phytochemical screening

Phytochemical screening was proceeded using standard procedures ¹¹⁻¹³ to detect: carbohydrates, glycosides, saponins, cardenolides, sterols and/or tannins, flavonoids, amino acids and triterpenes.

Carbohydrates test: (Purple Ring test)

5 mL in water was put to 0.5 mL of the ethanolic extract, then add two drips of α -naphthol solution/alcoholic in assay pipe. Occurrence of the carbohydrates is indicated when a violet ring at the junction is formed.

Cardenolides test: (Keller-Killiani test)

A quantity of 5 mL water was put then 3 mL of glacial acetic acid was added having just 1 drip of the FeCl_3 sol was put to 1 mL of the ethanolic extract, then, 1 mL of centered sulfuric acid was added very gradually, though the assay pipe is kept awry. Forming of the brown circle at the border designated the occurrence of a deoxy sugar typical of cardenolides. Below the brown circle, a violet ring might form, though a greenish circle may appear just beyond the brown circle and slowly feast through in the acetic acid layer.

Saponins Test

A quantity of 5 mL of distilled water was added, on one mL of extract, then the solution, strongly, was shaken and detected to be a stable tenacious froth. Frothing then mixed with five olive oil drops, then mixed energetically to monitor an emulsion that will form.

Phytosterols the test of: (Liebermann Burchard's test)

A filtrate was prepared by mixing of one mL of the extracts with 10 mL chloroform and filtered. The filtrate was added to one mL of acetic anhydride, boiled then left to cool. A quantity of 5 mL centered sulfuric acid was added. The brown circle creation at the intersection means that there are phytosterols.

Tannins Test

In a test tube, one gram of ethanol extract put in 10 mL of water was boiled and then filtered. Then, few drips of 0.1% FeCl_3 were added to form a blue-black or green-brown color.

Flavonoids Test

Two techniques were performed to detect flavonoids. Firstly, using a steam bath, a volume of two mL of the extract was warmed up together with volume of 10 mL of ethyl acetate for five minutes. Then, the blend was filtered, 5 mL of the filtrate was taken and blended together with 1 mL of dilute ammonium hydroxide solution.

Formation of the yellow color specified the existence of flavonoids. Second, a volume of 4 mL of dilute ammonia was added to a part of an ethanolic filtrate of the extract then a volume of 1 mL of centered sulfuric acid was added. A yellow color that disappeared on standing indicated the existence of flavonoids.

Amino acid Test

A concentration of 0.25% w / v of ninhydrin reagent was added to a volume of P. graveolens ethanolic extract and then boiled for ten minutes, the appearance of blue color indicate the occurrence of amino acids in the extract.

Experimental animals

In the current study, the black rat (*Rattus rattus*) weighing from 175 to 220 g was used as an experimental animal. Rats were monitored for approximately 14 days prior to the start of the experiment to rule out any new infections that might affect them. A stainless steel cages were used to kept the selected animals. The animals were preserved at room temperature ($25 \pm 2^\circ \text{C}$), using 12 hours a day of natural light from the sun, and give them arrival to food and water according to a standard diet of the specified composition and weight (13.2% fiber, 17% protein., 3.2% fat, 66.6% carbohydrates and few vitamins and minerals).

Diabetes Induction in the rats

Diabetes induced in rats using single intraperitoneal injection of alloxan monohydrate (2, 4, 5, 6-Tetraoxohexa-hydropyrimidine; Sigma-Aldrich) at a level of 150 mg / kg body weight dissolved in solution Jackets for pH 4 (Misra and Aiman, 2012). Rats were forbade of food for 24 hours pre the injection of alloxan.

The rat were prevented from eating overnight (12-14 hours) and then given glucose (3 g / kg of body weight) through gastric intubation for a period of six days post the aloxan injection. Blood sample were drawn from the retro-orbital venous plexus of each rat two hours post glucose administration, then centrifuged. Blood glucose level for each rat was measured then. The rat with blood glucose concentrations ranging from 180 to 300 mg / dL two hours after glucose administration were regarded as diabetic; and participated in this experiment.

Ethical approval

The project proposal and sampling method were approved by animal ethics committee of Al-Ayen university.

Animal Grouping

The selected rats were alienated into four groups; diabetic rats divided in to treated and untreated groups, control non-diabetic rats also divided in to treated and untreated groups. The diabetic treated group administered daily with *P. graveolens* ethanol extract dissolved in dimethyl sulfoxide (DMSO) as a vehicle , at a level of 220 mg / kg of body weight via tube into the stomach for a period of 6 weeks. The other corresponding group given the same equivalent amount of the vehicle solvent (DMSO) for the same duration and also the same pathway as the diabetic treated animals. The two groups of the control animals treated in the same way.

Blood Sampling

At the finale of the test duration, healthy mice with diabetes were eliminated with diethyl ether / chloroform. Then the blood samples were withdrawn and centrifuged at 4000 rpm for 20 minutes. After this, the clear floating sera was rapidly detached and kept at -20°C for later use. Also, the day before the anesthesia of mice, an oral glucose tolerance test was performed, using an oral administration of 3.0 g glucose / kg of body weight, and blood samples were withdrawn from the orbital venous plexus after 0, 30, 60, 90 and 120 minutes from ingestion of glucose.

Biochemical analyses

Depending on the enzymatic technique, blood glucose was tested with the Bio-Merieux groups. The radioactive serum insulin was tested by Radioimmuno-assay kits provided by Diagnostic Products Company, Los Angeles, USA. The glycogen content of the liver was assessed according to the method adopted by Kemp and Adrienne (Kemp and Van Heijningen, 1954) using reagents prepared in the laboratory.

Statistical analysis

All data were symmetrically generated and statistically analysed with One-Way or two-way ANOVA from a SPSS v. 24. The significance interval was cited at $P < 0.05$, with a 95% confidence level.

Results and Discussion

Chemical screening of *P. graveolens* ethanol extract

The hypoglycemic effect of the plant extract was studied according to the primary phytochemical analysis of *P. graveolens* which showed the presence of tannin, glycosides and /or carbohydrates, flavonoids, sterols and /or triterpene, saponins, amino acids, and cardenolides. Previous study indicated that flavonoids, alkaloids, peptides, terpenoids, amino acids, sulfur-containing compounds, inorganic ions, vitamins, and carbohydrates may positioning in this group ¹⁴.

The antidiabetic effect noticed in these animals may be due to the interpretation proposed by Prabhakar, P. K et al who reported that the mechanism for sulfur-containing compounds involves the interaction of SH groups with inactivated insulin compounds by competing with insulin for these compounds, the treatment protects insulin from damage¹⁵. However other mechanisms did not excluded which may attribute the effect of extract to the stimulation of insulin action at the receptor or post-receptor level, or may have a direct effect on carbohydrate metabolism pathways. Although sulfur-containing compounds had insufficient evidence to support the effect of its as antidiabetic agents; The proposed mechanism for the activity of this sulfur-containing compounds, one of the predominate compounds in the extract, involves the interaction of SH groups with inactivated insulin compounds by competing with insulin for these compounds, thus, protects insulin from damage.

Biochemical effects

Figure 1: The curves showed glucose tolerance in normal rat, and control in diabetic rats. The blood glucose concentration in all periods (0,30,60,90 and 120 min) of the glucose tolerance test was very important ($P < 0.005$), but in diabetic rats it was high when compared with the values in control rats. Taking the extract for four weeks promoted a significant improvement in glucose tolerance in the diabetic rats. The result was observable at all times of the glucose tolerance assay due to the ethanol extract of *P. Graveolens*.

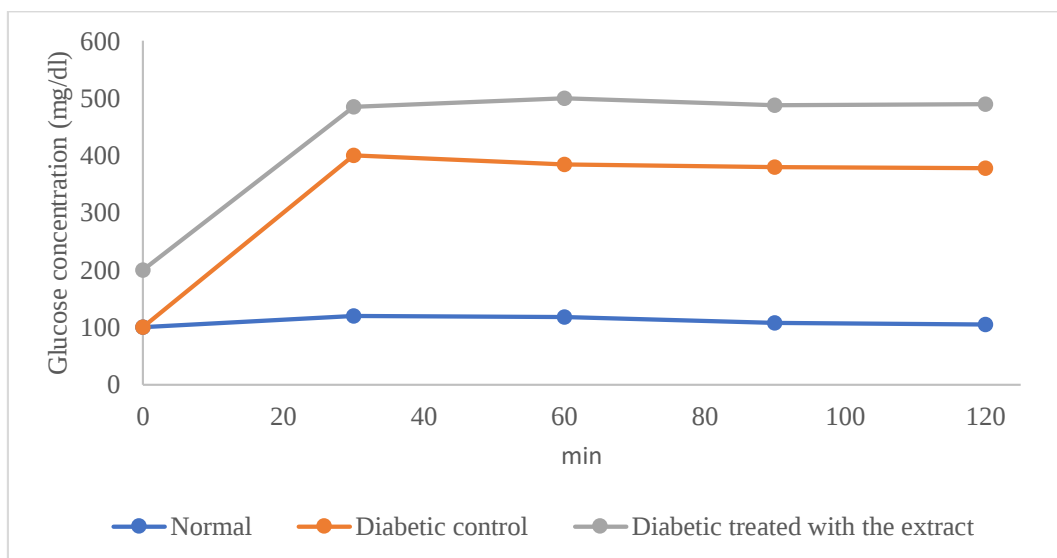


Figure 1: Effect of oral administration of *P. graveolens* extract on the glucose tolerance test of the alloxan diabetic male albino rats

Table 1. Show that the variances serum insulin concentration and liver glycogen content because of diabetes and its treatment in the tested extract. Both variables were strongly ($P < 0.0$) dwindling after the 4th week of the experimental period in the diabetic group. However, the insulin concentration in the blood did not show any significant alteration in all the diabetic treated groups, unlike the diabetes control group, the liver glycogen content showed a significant increase.

The consequence of ethanol extract was significant ($P < 0.05$) on the glycogen content in the liver. Both the ratios of increased blood insulin to decreased glucose activity and increased blood insulin to increased liver glycogen activity showed positive values for diabetes control rats, however, the values of those treated with diabetes were negative (Table 1).

Table 1. Fasting serum glucose and insulin concentration and liver glycogen content of normal, diabetic non-treated and diabetic treated rats

Parameter	Normal-not treated	Normal-treated	Diabetic-not treated	Diabetic-treated
glycogen from Liver (mg/g tissue)	8.67±0.57	10.5±1.70	1.26±0.06	11.0±1.78*
Fasting glucose test (mg/dl)	92.6±2.98	101.6±1.02+	174.5±8.44	94.3±6.33
Insulin (μIU/mL)	25.5±1.24	33.3±4.60	12.5±1.56	11.5±1.24

* Data are expressed as mean ± standard error (SE). Each group has five animals. the values are significant compared to those of normal rats: + $P < 0.05$. Values are significant compared to those of diabetic non-treated rats: * $P < 0.05$

In the current study, an alloxan test was performed to encourage hyperglycemia condition in albino rats. The effect of Streptozotocin and Alloxan on the glycemic state of various animal species has been reported in several studies ¹⁶. These studies reveal that both substances have a cytotoxic effect on beta cells in the islets of the pancreas and may encourage chronic or persistent diabetes in these animals. Despite the structural difference between aloxane and streptozotocin, their direct influence on co-mechanisms such as generation of free radicals from oxygen, rather than endogenous scavengers of these reactive species, improvement of DNA strand breaks, suppression of nicotinamide adenine dinucleotide (NAD) level and ultimately inhibition, Cell functions or cell destruction (Braidy et al., 2019). Several trials were tried with different doses of alloxan, however, a single intraperitoneal amount of 150 mg / kg of body weight of male albino rats was used in the current study, this resulted in chronic hyperglycemia and a marked decrease in blood insulin levels (45%).

The results of this study are consistent with the practical and theoretical considerations that stated that the diabetic dose of alloxan could have an effect on beta cells and insulin secretion, leading to impaired glucose tolerance and elevated blood glucose levels.

Treatment of diabetic mice in this experiment with alloxan and ethanol extract *P. Graveolens* led to a significant improvement in tolerance to impaired glucose, and the results of the study showed that insulin concentration in blood was not significantly affected due to alloxan treatment in diabetic rat with the measured extract. Moreover, the diabetes-promoted reduction in liver glycogen content was significantly mitigated after administration of *P. Graveolens* ethanol extract for 4 weeks. Another possible example of increased liver glycogen is that treating diabetic mice with alloxan with sulfur-containing ingredients from *P. Graveolens* extract may lead to glucose-6-phosphatase inhibition followed by inhibition of glycogen phosphorylase activity, thereby reducing glycogenolysis (Canada et al., 2011). The process of improving glucose tolerance and glycogen content in the liver as a result of treatment with the extract although there is no significant change in the level of insulin in the blood leads to the suggestion that the extract improves the action of insulin and / or mimics the action of insulin rather than stimulating the secretion of insulin. This results in the lowering of blood glucose and increased liver glycogen activity of the ethanol extract *P. Graveolens*, independent of the insulin hyperactivity process. It is noteworthy that the extract may have powerful effects outside of the pancreas.

Conclusion

Finally, this work showed that an ethanol extract of *P. Graveolens* improved the glucose tolerance of alloxan-treated rats, which encouraged diabetes in these rats. The critical indicator presented by the current study supports additional pancreatic actions of the measured extract.

Acknowledgements

I would like to thank all the people who aided and encouraged us in this work.

Competing Interests

Authors have declared that no competing interests exist.

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