Effect of phenolic compounds extract of *S. melongena* peels on Sugar levels and biochemical parameters in alloxan-induced Diabetic rats

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ABSTRACT

Diabetes mellitus is a serious complex chronic condition that is a major problem of health worldwide. Recent studies focused on the ability of phenol compounds in the treatment of diabetes mellitus and its complications. In the present study, phenol compounds of *Solanum melongena* peels were extracted and diagnosed using HPLC, and the extract administrated to alloxan induced diabetic rat. The aim of this study is to investigate the effect of phenol extracts of *S. melongena* peels on sugar, lipid and liver enzymes in alloxan induced diabetes in rats. Serum glucose level, lipid profile, GOT and GPT enzymes were estimated and results were statistically calculated by ANOVA and the differences were considered significant at *p* value ≤ 0.05. Results showed that the administration of phenols extract decreased the glucose level in the phenols administrated diabetic rat. Also phenols extract decreased cholesterol, triglyceride and LDL level, and increased serum HDL level diabetic rat. The phenol compounds also showed the ability to detoxify liver via lowering GOT and GPT enzymes in phenols administrated diabetic rat.

تأثير مستخلص مركبات فينولية قشور باذنجان *S. melongena* على مستوى سكر ومؤشرات كيموحيوية في جرذان مصابة بالسكري مستحث بالالوكسان

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كلمات مفتاحية: الوكسان, متعدد الفينول,السكري, مستوى الدهون

خلاصة

يعتبر مرض السكري واحداً من أهم الأمراض المزمنة المنشرة في أنحاء العالم.وتُركز الدراسات الحديثة على إستخدام الفينولات في علاج مرض السكري. في هذه الدراسة تم استخلاص الفينولات من قشور الباذنجان *S. melongena* , وتم تشخيص الفينولات بتقينة الكروماتوغرافيا عالية الاداء HPLC وأُعطيت هذه المستخلصات لذكور الجرذان المصابة بالسكري المستحث بمادة الالوكسان.إن الهدف من هذه التجربة هو دراسة تاثير المستخلص على مستوى السكروالدهون وأنزيمات الكبد في الجرذان المصابة بالسكري. تم قياس مستويات السكر, الدهون واانزيمات الكبد في مصل الجرذان,وتم تحليل النتائج التي حصلنا عليها احصائياً وأوجدت الفروقات باستخام تحليل التباين (ANOVA) وأُعتبرت الفروقات ذات قيمة معنوية عند مستوى دلالة ≤ 0.05.أظهرت النتائج أن إعطاء جرع من مستخلص الفينولات للفئران المصابة بالسكري يعمل على خفض نسبة السكر في الدم وتعمل على خفض مستويات الدهون الضارة وزيادة الدهون النافعة (عالية الكثافة), كذلك تعمل الفينولات على خفض فعالية انزيمات الكبد GOT و GPT في الفئران المصابة.

1. INTRODUCTION

Last years there has been an increased awareness of the importance of traditional medicine in the health care worldwide because of their natural origin and less side effects(1), (2). Many traditional medicines in use are derived from medicinal plant minerals and organic matter (2). Despite the large use of synthetic organic drugs in the twentieth century, over 25% of prescribed medicines in many industrial countries derived from plants, and up to 80% population of developed countries nowadays use medicinal plants as remedies (3).Among the great structural diversity of plants compounds, phenols have attracted interest and the most attention for their wide variety of bioactivities (4). Phenolic compounds exhibit a wide range of biomedical effects (anticarcinogenic, or antimutagenic, antibacterial, antiviral, anti-inflammatory and anti-allergic) (4) (5).

Diabetes mellitus (DM) is the more distributed human health disorder which, according to the World Health Organization (WHO, 2004), affects more than 176 million people worldwide (6). May causes of DM were reported including oxidative stress, DM occurs due to reduction of body endogenous antioxidant and increase of oxidative stress (7). Phenolic compounds from plants belong to a class of bioactive components with antioxidant activities (5). Crude extracts of fruits, vegetables, cereals, herbs, and other plant materials rich in phenolic compounds (8).

More than 400 traditional plant treatments for diabetes mellitus have been recorded (9), such as *Murraya koenigii*, *Aegle marmelos*, *Ocimum sanctum*, *Mentha piperitae*, *Cajanus cajan*, *Coccinia indica*, *Gymnema sylvestre*, *Momordica charantia*, *Brassica juncea*, and *Eugeniajambolana* (10). A hypoglycemic action of plant extracts has been confirmed in animal models and non-insulin-dependent diabetic patients, and various hypoglycemic compounds have been identified (9).

The aim of this study is to investigate the curative effect of phenol extracts of *Solanum melongena* peels in alloxan induced diabetes in rats.

2. MATERIALS AND METHODS

Materials

Solanum melongena obtained from local markets in samara in October. Plants were washed with tap water then with D.W. plants peels were obtained in a deep (4.5-5 mm), the ratio of peels was (18 %) form total plant weight. Peels then dried in the shadow for 3 days. Peels were powdered using a blinder ad sieved in a sieve (1 mm), powder saved in a plastic container in order to avoid humidity.

Methods

Extraction of Phenols

Phenolic compounds were extracted from peels using a method by (G.anon, 1979) (11). 200 gm of peels powder and 800 ml of 2% acetic acid were mixed and left for 24 hours in a mixing incubator (up to 50° C). After that the mixer cooled and filtered using vacuum filtration, and then equal volume of *n*–propanol was added with an amount of NaCl until saturation. The mixture then separated into two layers, the upper layer that contains phenolic compounds was isolated and concentrated using rotary evaporate. An adequate was taken for chemical analysis and the other were used in the treatment.

HPLC analysis

Polyphenols of *S.melongena* peels has been identified by High performance liquid chromatography (HPLC) technique (Shimadzu, Japan). The optimized conditions for HPLC analysis were; The chromotographic column (phenomenex C-18, 50 long × 2mm i.d. and 3

µm particle diameter) , the mobile phase was (A): formic acid (0.1%) and (B) acetonitrile: methanol: formic acid (6:3:1v/v) , gradient program 20% to 100 % B for 10 min. flow rate 1.1 ml/min .Ultra violet (UV) detection has been recorded at the wave length (280 nm) . concentration were calculated depending on the peak area (12).

Estimation of Antioxidant Activity

Antioxidant scavenging activity for extracts has been estimated using a method depending on the inhibition of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. 0.1 ml of sample was to 2.9 ml (0.002 %) of DPPH solution (Sigma-Aldrech, Germany), and incubated for 30 min at room temperature in dark place (The radical stock solution was prepared fresh daily). The color developed read at 517 nm and scavenging activity were calculated from the following equation (13):

$$\text{Inhibition \%} = \frac{[(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

Animal Models

Albino rat weighing 310±10 grams were obtained from the laboratory animal center of the state company for drugs industry & medical appliances in samara. The animals were reared in a polypropylene cages (30X 25 X15 cm diameter) covered with a steel grid covers, maintained at controlled temperature (22±3 °C) and a period of 12 h light and 12 h darkness. Rats were fed a required supplementation of rodent diet and drinking water every day, with changing the Sawdust every 2 weeks during one month of the experiment.

Animals were divided into three groups (five animals in each), all were feed standard food and water. Group I (control), Group II (alloxan induced DM) animals were injected with alloxan subcutaneously (50 mg/Kg), Group III (treatment group) animals injected with alloxan (50 mg/Kg) and phenol extract were given daily (100 mg/Kg) (14). After 1 month animals were fasted for one day then killed and blood were drown then serum separated by centrifugation and kept at (-20 °C) for biochemical analysis.

Biochemical analysis

The following biochemical parameters were estimated in animal serum including;

Estimation of Glucose

Glucose estimated using colorimetric method provided by (Biomaghreb, Tunsia). 10 µl of samples were added to 1 ml of enzyme reagent and incubated at 37°C for 5 min, H_2O_2 elaborated by the action of the enzyme reacts with 4-aminoantipyrin to develop a red color which read at 500 nm and results were expressed as mg/dl.

Estimation of Cholesterol

Cholesterol estimated using colorimetric method (15), (Biolabo, France) commercial kit. 10 µl of Samples were added to 1 ml of enzyme (cholesterol oxidase) reagent and incubated at 37° C for 5 min. resulted H_2O_2 by the action of the enzyme reacts with 4-aminoantipyrin to develop a red color which read at 500 nm and results were expressed as mg/dl.

Estimation of Triglyceride

Triglyceride (T.G) estimated using colorimetric method (16) ,(Biolabo, France) commercial kit. 10 µl of Samples were added to 1 ml of enzyme (Lipase and glycerol phosphate oxidase) reagent and incubated at 37° C for 5 min. H_2O_2 by the action of the enzyme reacts with chromagen to develop a red color which read at 500 nm and results were expressed as mg/dl.

Estimation of HDL

HDL estimated using commercial kit(17) from (Biolabo, France).500 µl of samples were added to 50 µl of precipitant (phosphotungstic and magnesium chloride) reagent and incubated 10 min at room temperature. After that the mixture has been centrifuged for 10 min

and 25 µl of the supernatant were mixed with 1 ml of reagent and incubated for 5 min at 37° C, and read at 500 nm. Results were expressed as mg/dl.

Estimation of LDL and VLDL

LDL and VLDL were estimated mathematically; when VLDL = TG/5, and LDL were calculated form the equation; Total Cholesterol= HDL+ LDL + VLDL (18).

Estimation of GOT and GPT enzymes

GOT and GPT enzymes has been estimated using commercially available kits (Biomerieux, France). 100 µl of serum samples were added to 0.5 ml of enzyme buffer and incubated at 37° C for 30 min. after that 0.5 ml of DNPH has been added and incubated at room temperature for 25° C. finally 5 ml of NaOH were added and brown color formed has been read at 546 nm and results were expressed as IU/ml (19).

Statistical analysis

All data were analyzed using SPSS software program. Tables were presented as mean±SE. Statistical analysis was carried out using one-way ANOVA. The criterion for statistical significance was ≤0.05.

3. RESULTS

HPLC analysis of *S.melongena* peels extract showed high levels of different phenols (cinnamic acid, caffic acid, Caffeoylputrescne, 5- Caffeoylquinic acid and querecetin). As shown in (table1). The highest level was 5-Caffeoylquinic acid approximately (3.5-5 times) higher than the other phenols (figure 1).

Table 1: phenol compounds with their retention time and Area

No.	Phenol	t_R	Area	Conc. (ppm)
1.	Cinnamic Acid	1.307	17.2263	284.67
2.	Caffic Acid	2.547	16.1029	199.21
3.	Caffeoylputrescne	3.392	18.506	201.24
4.	5-Caffeoylquinic Acid	4.445	18.9124	995.05
5.	Querecetin	5.295	16.692	251.60

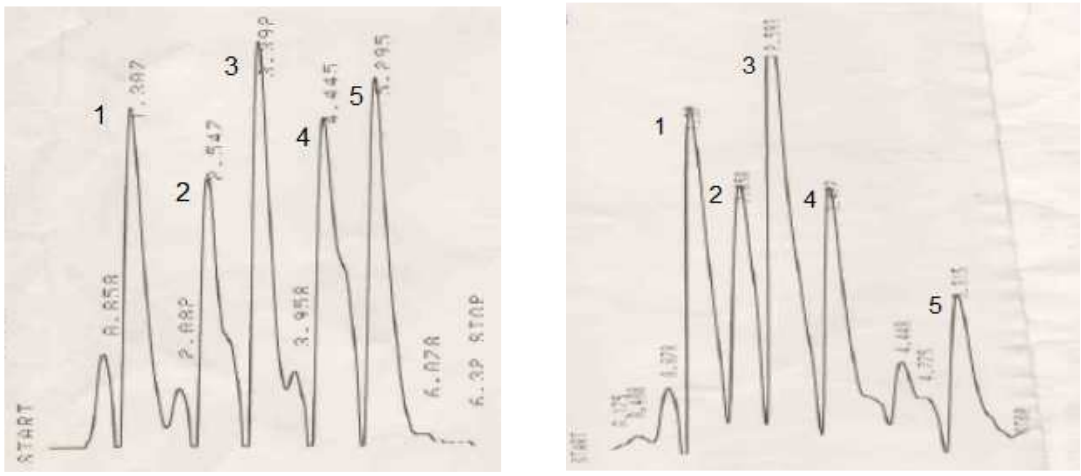

Figure 1: HPLC peaks of phenols standards (left) and *S. melongena* peels extract (right)

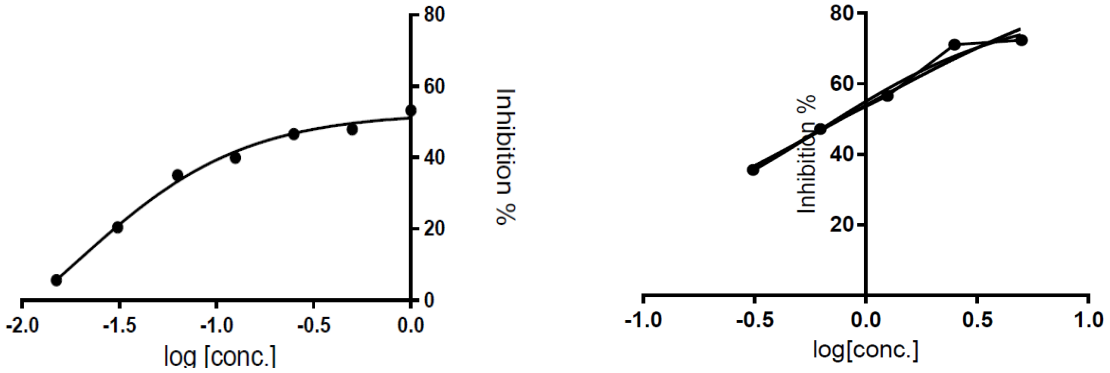

Figure 2: Antioxidant activity of ascorbic acid as standard (left) and *S. melongena* peels extract (right)

Results of biochemical parameters showed in (table 2). The glucose levels of alloxan induced diabetic rats (173.25 ± 37.00 mg/dl) were significantly higher compared with those of the control group (75.19±4.87 mg/dl) (P≤0.05) (Table 2). After *S.melongena* peels extract administration, a significant decrease in blood glucose was observed (61.66 ± 2.08 mg/dl) compared with that of the diabetic group.

Table 2: Biochemical parameters of control, diabetic, phenol administrated rat

parameter	Glucose (mg/dl)	Cholesterol (mg/dl)	T.G (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	GOT IU/ml	GPT IU/ml
Control	75.19 ± 4.87 B	120.80 ± 5.84 C	45.4 ± 2.70 C	97.20± 9.90 A	26.32 ± 3.67 C	9.62 ± 0.83 C	17.6 ± 1.67 C	17.20± 4.147 C
Alloxan 50mg\kg	173.25 ±37.0 A	180.5± 10.40 A	72.35 ± 1.92 A	45.775 ± 6.82 C	118.33±22.42 A	13.33 ± 0.50 A	72.25 ± 3.77 A	32.25 ± 3.59 A
Alloxan + polyphenol extract	61.66 ± 2.08 C	140.66± 5.85 B	58.0 ± 2.00 B	62.33±10.969 B	69.166±18.17 B	11.20 ± 0.916 B	28.00 ± 2.64 B	24.66 ± 0.577 B

Serum cholesterol in diabetes was (180.5± 10.40 mg/dl) as compared to non-diabetic (120.80 ± 5.84mg/dl), and serum T.G were (72.35 ± 1.92 mg/dl) as compared to non-diabetic (45.4 ± 2.70 mg/dl). HDL was (97.20± 9.90 & 26.32 ± 3.67mg/dl) and LDL (45.775 ± 6.82 & 118.33±22.42 mg/dl) for control and alloxan induced diabetic rat respectively.

Also results showed that GOT and GPT enzymes of treated group (28.00 ± 2.64 and 24.66 ± 0.577 U/ml) were lower than diabetic group (72.25 ± 3.77 and 32.25 ± 3.59 U/ml).

4. DISSCUSION

The extract showed high antioxidant activity (IC_{50} = 0.7751) as compared to standard ascorbic acid (IC_{50}= 0.4874), figure 2. Thus high antioxidant activity of *S. melongena* peels extract is due to high phenolic content in the peels as phenols has antioxidant activity (20).

Table 2 showed that glucose levels of alloxan induced diabetic rats are significantly higher compared with control group .Intraperitoneal injection of alloxan which is toxic to

β-cells induces the production of reactive oxygen species (ROS) responsible for diabetes complications produced in animals (21). Various hypoglycemic agents reduce (ROS) levels indirectly by lowering blood glucose level and preventing hyperinsulinemia, and directly by acting as free radical scavengers (22). Phenol compounds have powerful antioxidant and radical scavenging activities (23). *S. melongena* peels contain high phenols concentration as shown in (Table 1). The ability of phenol compound to scavenging ROS delete the toxic effect of alloxan on β-cells and lowering the glucose levels in polyphenols administrated rats (61.66 ± 2.08 mg/dl).

Disturbances in lipid metabolism are an essential part of the deranged metabolism in diabetes mellitus, extremely increase in serum lipid levels have been observed occasionally with diabetes mellitus (24), this increase is attributable to associated risk factors, including and dyslipidemia which is characterized by elevated serum cholesterol, triglyceride and LDL levels, and low levels of HDL (25).Such an increase represents a risk factor for cardiovascular disease (21). High levels of serum lipids in diabetes and low HDL were showed in table 2.

The administration of *S. melongena* peels extract decreased the total cholesterol levels in diabetic rats due to its phenolic content, which is important in preventing or treating the complications of diabetes. Phenols reduce both cholesterol synthesis and absorption. Synthesis reduces via inhibition the activity of hydroxy methylglutaryl-CoA reductase in liver microsomes (26). Also Phenols may reduce cholesterol absorption due to the interaction of these compounds with cholesterol carriers and transporters present across the brush border membrane (27). Phenols were shown to alter lipoprotein metabolism by decreasing serum triglycerides and apoprotein-B concentrations. Studies using Hep-G2 cells showed that the phenolic compound decreased apoprotein -B secretion, thereby reducing the concentration of triglycerides (28).

phenols has an ability reduced the number of VLDL particles secreted by the liver, which resulted in lower concentrations of VLDL cholesterol in the phenol administrated group (11.20 ± 0.916 mg/dl) as compared to diabetic group(13.33 ± 0.50mg/dl) (28).

Hepatocellular lipid accumulation caused by elevated glucose can be inhibited by phenols and eventually inhibition of high-fat–induced hepatic steatosis (29), (30). This was demonstrated through the decreased plasma GOT and GPT enzymes as compared to diabetic group also reflects decreased high-fat– induced hepatocyte injury (30).Hepatocellular AMP activated protein kinase (AMPK) activity has a role in the accumulation of lipid in liver ,high glucose level inhibits AMPK and caused hepatocellular lipid accumulation. Phenols inhibit fat accumulation in liver Via effecting AMPK activity in human HepG2 hepatocytes , AMPK regulates fatty acid oxidation and lipid synthesis, two important determinants of tissue lipids and hyperlipidemia in diabetes (29),the phenols inhibit microsomal triglyceride transfer protein(MTP) , thereby reducing overall TG accumulation within the endoplasmic reticulum, Because of the inhibitory effects of phenols on MTP and consequent reduction in the accumulation of TG in the liver (28).

5. CONCLUSION

In this study we concluded that phenols may be used in the treatment or management of D.M or hyperlipidemia as its ability in the reducing of blood sugar level and decreasing lipid levels in the serum of diabetic rats, also phenols may be used in the liver detoxification resulting from drug toxicity as its ability to decrease live enzyme activity.

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