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Evaluation of antidiabetic and antihyperlipidimic activity of aqueous extract of Iraqi propolis in alloxan induced diabetes mellitus in Wister Albino rat

تقييم الفعالية المضادة لداء السكري وارتفاع مستوى الدهون في الدم للمستخلص المائي للعكبر العراقي في ذكور الجرذان المستحدثة بداء السكري

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Abstract

The present study was undertaken to investigate the antihyperglycemic and antihyperlipidemic effects of aqueous extract of Iraqi propolis (AEIP) in alloxan-induced diabetic rats. Diabetes was induced in Wister rats by single intraperitoneal administration of alloxan monohydrate (150 mg/kg). Animals were divided into five groups (n=6) in each receiving different treatments. Graded doses (150mg/kg and 300 mg/kg) of AEIP were studied in alloxan-induced diabetic rats for a period of 28th days. Glibenclamide (600 µg/kg) was used as a reference drug. For histomorphological study of pancreas , the animals were killed after 28 days , routine haematoxylin and eosin stain. In alloxan-induced diabetic rats, the daily oral treatment with AEIP significantly recover the weight ($p>0.01$) with respect to the control group, also a significant reduction in blood glucose. Besides, administration of AEIP for 28th days significantly($p>0.01$) decreased serum contents of total cholesterol, triglycerides, low density lipoprotein LDL and very low density lipoprotein VLDL whereas HDL-cholesterol was effectively increased. The histopathological studies also indicated that AEIP is effective in regeneration of insulin secreting β-cells. Studies clearly demonstrated that AEIP possesses hypoglycemic and antihyperlipidemic effects mediated through the restoration of the functions of pancreatic tissues and insulinotropic effect.

المستخلص:

أجريت الدراسة الحالية لتقييم الفعالية المضادة لداء السكري وارتفاع مستوى الدهون في الدم للمستخلص المائي لمادة العكبر العراقية المنشأ في ذكور الجرذان المستحدثة بداء السكري عن طريق إعطاء جرعة مفردة خلال الغشاء البريتوني بمادة الالوكسان (150ملغم/كغم).قسمت الحيوانات إلى خمسة مجاميع أعطيت مختلف العلاجات، جرعات مدرجة 150 و 300ملغم / كغم من المستخلص المائي لمادة العكبر العراقية درست لكلا الجرذان الطبيعية والمعاملة بمادة الالوكسان لفترة 28 يوما.كليبينكلامايد (600مايكروغرام)استعمل كمصدر دواء علاجي.لدراسة المقطع النسيجي للبنكرياس قتلت الحيوانات بعد 28 يوما استعمات صبغة الايوسن والهيماتوكسلين. أظهرت النتائج بوجود استجابة مهمة إحصائيا في الحيوانات المستحدثة بداء السكري والمعالجة بالمستخلص المائي لمادة العكبر العراقية من خلال استعادة وزن الجسم مقارنة بمجموعة السيطرة و انخفاض مستوى السكر بالدم.كذلك أشارت النتائج إلى وجود نقصان مهم إحصائيا في الكوليسترول الكلي والترايكليسترول والبروتينات الدهنية القليلة الكثافة وزيادة معنوية في البروتينات عالية الكثافة في جميع المجاميع المعالجة مقارنة بمجموعة السيطرة ،أيضا أشارت الدراسة النسيجية بان المستخلص المائي لمادة العكبرذو تاثي في إعادة تكوين وإفراز الأنسولين من خلايا بيتا .استنتجت الدراسة الحالية امتلاك المستخلص المائي لمادة العكبر العراقية المنشأ تأثيرات مضادة لارتفاع مستوى السكر والدهون بالدم من خلال أعادة وظيفة أنسجة البنكرياس وامتلاك فعالية الأنسولين.

Introduction

Diabetes mellitus is one of the endocrine glands diseases in human and animal which involves the gland circulatory system. About 6.3% of world populations live with diabetes. Diabetes creates the following common symptoms during its chronic length: thirst, polyuria, increased appetite and weight decrease, heart and coronary problems, kidneys problems, ketosis, and blood glucose increase among others (1). In addition, lipid disorders and lipid per-oxidation together with diabetes

play a crucial role in the development of cardiovascular disease (2). Although, insulin and hypoglycemic drugs constitute the main treatment in diabetes, the use of nutritional methods and medicinal plants are increasing in some countries (3).This metabolic disorder can be induced chemically using alloxan monohydrate or streptozotozine. Alloxan-induced diabetes mellitus is caused by the selective pancreatic beta cell toxicity (4).Propolis or "bee-glue" contains a number of natural active constituents that have been shown to exert a variety of medical properties, such as anti-microbial activity protective effect against radiation-induced damage anti-mutagenic effect, anti-hyperalgesic action and anti-inflammatory activity. Most of these effects have been related to the anti-oxidant and free radical scavenging properties of propolis (5)The purpose of this study was to assess the effects of aqueous propolis extract treatment on alloxan - induced diabetic rats by following the variations of the blood glucose levels, and serum chemistry profiles and the body weight. Glibenclamide is an hypoglycemic agent which is also used as a standard medication in the treatment of diabetes. At the end, treatment with glibenclamide and aqueous propolis extract will be compared.

MATERIAL AND METHODS

Origin of propolis sample: Propolis samples were collected from hives of honey bees of Karbala, Iraqi during spring and summer seasons of 2013. Propolis samples were cleaned, free of wax, paint, wood, cut into small pieces, and placed in clean container.

Preparation of aqueous extract of propolis : Aqueous extract of propolis was obtained as described by Nagai et al (11) ,with slight modification. In brief, 100.0 g of propolis was suspended and extracted with 10 volumes of distilled water with shaking at 80 °C for 1 day. The extracts were vacuum filtered using Buchner assembly and filtrates were pooled. The residue was re-extracted under the same conditions. Finally all the extracts were pooled together and solvent was evaporated using rotary evaporator. The dry mass obtained was collected and 100 ml of 10 mg/ml stock solution was prepared. This solution was utilized to prepare different concentration of extract for further assessments.

Experimental animals: Thirty male Wister albino rats (150-200 g) were used in this study. They were obtained from the Iraqi National Center for Drugs Safety and Evaluation. The animals were housed in plastic cages (groups of six mice/cage) in a room with controlled temperature (22±2 °C) under a 12 h light/dark cycle with access to standard certified rodent diet and water ad libitum.

The rats were divided into five consisting of six animals each as following:

Group 1 Normal control

Group 2 Diabetic control

Group 3 Diabetic rats treated with 150 mg/kg (body weight) AEIP.

Group 4 Diabetic rats treated with 300 mg/kg (body weight) AEIP.

Group 5. Diabetic rats treated with 600 µg/kg of Glibenclamide orally.

Induction of diabetes: Diabetes was induced by a single intraperitonial injection of alloxan monohydrate in citrate buffer (pH 4.5) at a dose of 150 mg/kg , body weight of the rat. The diabetic state was confirmed 48 h after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 150 mg/dl were included in the study (17).

Parameters: Body weight were monitored at regular weekly intervals for four weeks.

Biochemical Estimation of blood glucose level and lipid profil: Blood was collected and serum was separated by centrifugation at 5000 rpm for 10 min. Collected serum was used for biochemical analysis. Fasting blood glucose level was measured on day 0, 7, 14, 21 and 28. Blood was collected from the tail vein and fasting blood glucose level was measured by O-Toludine method. The results were expressed in terms of milligram per deciliter of blood .A lipid profile is a group of blood tests that tells how our body uses, changes, or stores lipids. The lipid profile was measured after 28th day and include:Total cholesterol (TC).Triacylglycerol (TAG).High density lipoprotein cholesterol (HDL-C).Low density lipoprotein cholesterol (LDL-C).Very low density lipoprotein cholesterol (VLDL-C).

Histopathological study:On day 28, when the animals were sacrificed, the pancreas tissues were removed and stored in 10% formalin after washing with normal saline. The tissues embedded in paraffin and sectioned with 5 µm thickness and then stained with hematoxylin eosin for microscopic assessment. For the quantitative analysis of pancreatic islets, the number of pancreatic islets was counted under microscope (40 ×).(18)

STATISTICAL ANALYSIS: Results were expressed as Mean ± S.D. Statistical significance was calculated by using One Way Analysis of Variance (ANOVA) by SPSS software version 12.0. $P < 0.01$ was considered as significant. Values bearing different letters as superscripts showed significant differences ($p < 0.01$).

Results and Discussion :

Body weight There was a significant reduction in body weight of the animals in diabetic group in comparison to control. After administration of AEIP for 28days, the body weight was recovered significantly (p>0.01) with respect to the control (table 1).

Table-1:Effect of AEIP on body weight (g) in control and all treated groups.

Groups	0^{th} day	7^{th} day	14^{th} day	21^{th} day	28^{th} day	mean TRT
Control non Diabetic	180.0±1.12 Aa	181.0±1.20 Aa	183.3±4.05 Aa	205.6±5.16 Bb	210.0±12.4 Bb	191.80
Control Diabetic	185.0±1.30 Aa	181.7±4.07 Aa	175.0±6.07 Aa	168.2±3.7 Cc	159.7±3.72 Bb	173.65
AEIP 150mg /kg	193.3±4.45 Aa	186.6±1.7 Aa	213.6±10.80 Bb	216.7±12.14 Bb	220.0±11.14 Bb	206.20
AEIP 300mg /kg	200.0±2.12 Aa	185.7±1.8 Aa	206.7±8.15 Bb	220.0±12.12 Bb	223.0±14.13 Bb	207.08
Gilbenclomide	195.0±1.15 A	188.3±8.14 A	195.0±2.11 A	196.3±8.16 B	201.7±7.12 B	194.42
Mean days	190.86	184.96	144.42	201.04	202.87	

Different small letters refer to significant differences between groups horizontally P<0.01.
-Different capital letters refer to significant differences within group vertically P<0.01.

Fasting blood glucose level : Fasting blood glucose levels of all animals before treatment were within the normal levels. Fasting blood glucose level was significantly elevated after 3 days of Alloxan treatment with respect to control level. Treatment of AEIP for 28 days resulted restoration of fasting blood glucose level near to normal P<0.01, when compared with diabetic control and normal control (table 2).

Table-2.Effect of AEIP on fasting blood glucose in control and all treated groups :

Groups	0^{th} day	7^{th} day	14^{th} day	21^{th} day	28^{th} day	Mean TRT
Control non Diabetic	96.25±2.80 Aa	99.70±2.50 Aa	99.80±15.0 Aa	98.5±28.0	96.70±2.3 Aa	98.19
Control Diabetic	99.00±3.60 Aa	154.1±3.90 Bb	210.0±6.61 Bc	296.2±6.90 Bd	370.0±10.3 Be	225.86
AEIP 150mg /kg	100.70±2.4 Aa	134.2±2.60 Cb	170.2±3.60 Cc	224.6±5.9 Cd	266.0±11.5 Ce	179.14
AEIP 300mg /kg	99.2±2.30 Aa	127.4±2.60 Cb	161±4.10 Cc	202.8±4.7 Ed	236.5±5.60 Ee	165.30

Gilbenclomide	98.6±2.20 Aa	117±1.60 Db	140.5±2.70 Dc	167.3±3.00 Dd	183.2±4.15 De	141.32
Mean days	98.75	126.48	156.30	197.80	230.48	

Different small letters refer to significant differences between groups vertically P<0.05.
Different small letters refer to significant differences between groups horizontally P<0.01.
-Different capital letters refer to significant differences within group vertically P<0.01.

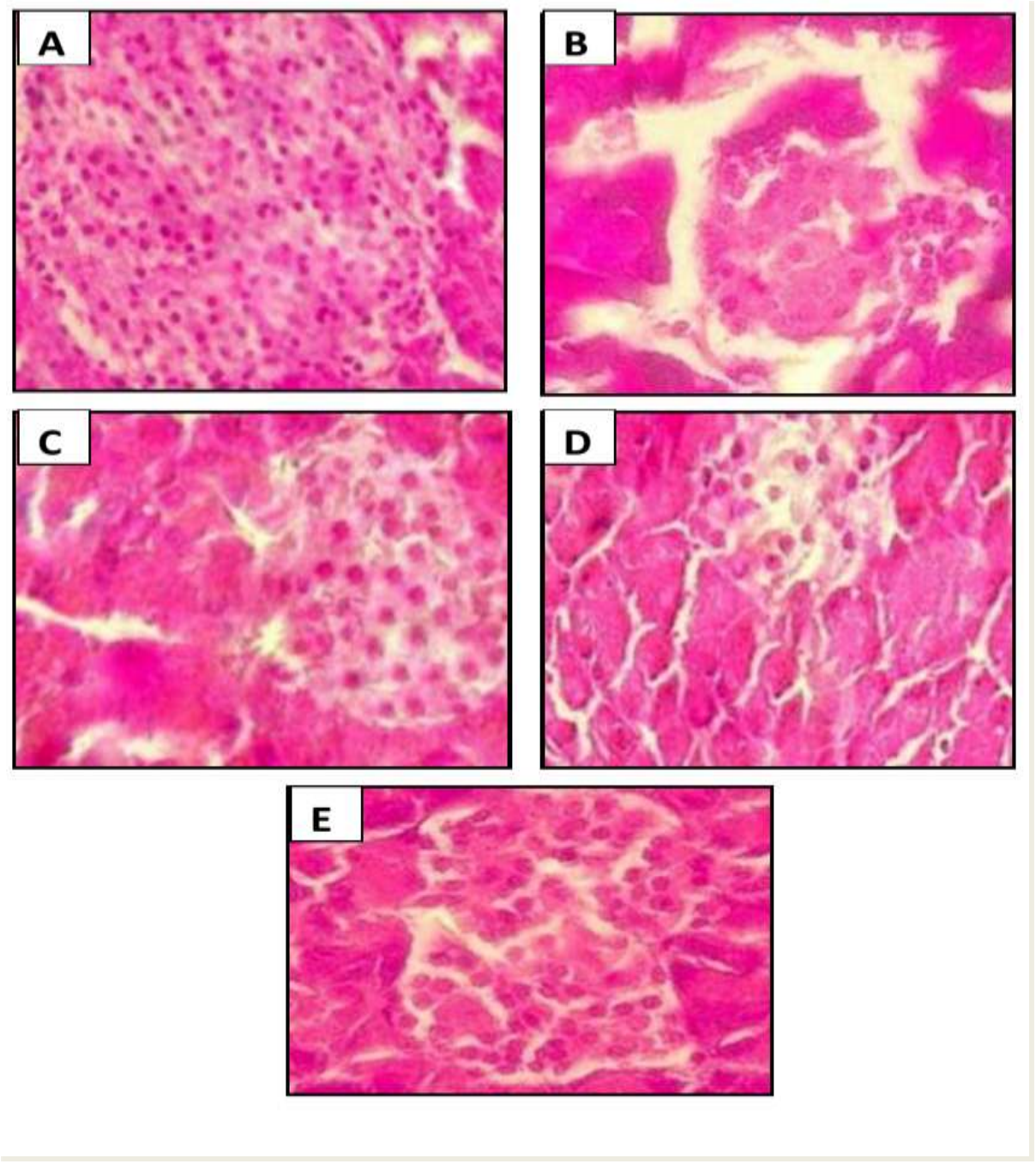
Effect of extracts on lipid profile

After 28 days of diabetes induction, vehicle treated diabetic rats showed very significantly (p<0.01) increase in serum TG, TC, VLDL and LDL level except significantly (p<0.01) decrease level of HDL as compare to control animals. AEIP 150mg /kg, 300mg /kg and Glibenclamide 6 mg/kg treated animals showed significant(p<0.01)reduction in serum TG, TC, VLDL and LDL level, and significant increase level of HDL as compare to vehicle treated diabetic control (table 3).

Effect of AEIP on lipid profile in control and all treated groups on 28th days Table-3

Groups	Total cholesterol mg/dl	Triglyceride mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl
Control non Diabetic	78.82±4.1 B	58.40±1.45 C	40.57±1.6 B	26.57±1.06 B	11.68±0.24 B
Control Diabetic	107.60±1.50 A	104.60±2.90 A	34.8±3.52 C	51.88±1.12 A	20.92±0.58 A
AEIP 150mg /kg	86.20±3.70 B	76.80±2.6 B	42.6±3.33 B	28.24±2.24 B	15.36±0.52 B
AEIP 300mg /kg	76.43±4.30 B	54.75±3.2 C	51.66±4.7 A	13.82±1.47 C	10.59±0.64 C
Gilbenclomide	82.18±2.55 B	57.62±4.3 C	43.6±4.2 B	27.06±2.24 B	11.52±0.86 C

Different small letters refer to significant differences between groups horizontally P<0.01.
-Different capital letters refer to significant differences within group vertically P<0.01.



Figer(1)Micrographs of rat pancreas stained by haematoxylin and eosin of (A) Untreated . Normal control; (B) Alloxan induced diabetic rats . Diabetic control; (C)AEIP 150mg/kg body weight; (D) AEIP 300 mg/kg of body weight); and (E) Glibenclamide 600 µg//kg of body weight.

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increased demand for natural products with antihyperglycaemic activity, having fewer side effects. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas (12-13). In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (14-15). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (16). In our present study, administration of AEIP to diabetic rats for 28 days caused significant reduction in blood glucose, triglycerides and cholesterol level and improvement in HDL.In diabetic rats, decreased body weights were observed. This indicates the polyphagic condition and loss of weight

due to excessive breakdown of tissue proteins, the decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins . Increased catabolic reactions leading to muscle wasting might also be the reason for the reduced body weight in diabetic rats . Oral administration of AEIP for consecutive 28 days to diabetic rats improves the body weight. This could be due to a better control of hyperglycemic state in the diabetic rats and decreased fasting blood glucose level could improve body weight in alloxan-induced diabetic rats .Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid. Changes in the concentrations of the lipid with diabetes mellitus contribute to the development of vascular disease . Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body . In type II diabetic condition the glucose itself acts as a toxic substance and produce number of Reactive oxygen species, which may cause damage to the islet cells. The islet is the least endowed tissue in terms of intrinsic anti-oxidant enzyme expression, including SOD-1, SOD-2, Catalase and glutathione peroxides . According to earlier studies, AEIP cause antihyperglycemic effect by promoting regeneration of ß-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on ß-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption (17). In Diabetic rats, administration of bee propolis extracts led to decreased levels of blood glucose (FBG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDL-C) in serum of fasting rats; and to increased serum levels of high density lipoprotein cholesterol (HDL-C) . This suggests that propolis can control blood glucose and modulate the metabolism of glucose and Blood lipid, leading to decreased outputs of lipid peroxidation and scavenge the free radicals in rats with Diabetes mellitus(17). The histopathological studies also indicated that AEIP is effective in regeneration of insulin secreting β-cells and thus possesses antihyperglycaemic activity (figer1) slide A and B represents islets of langerhans from normal and alloxan-induced diabetic rats, respectively. Comparison of these two slides clearly indicates the reduction in the number of β-cells in the islet of langerhans of pancreas of diabetic rats. As it is evident from slide B the islet is irregularly shaped, relatively small and atrophic. Most cells of the islets are small, degranulated and dark with scanty cytoplasm. However, compared to the untreated diabetic rats, histophatological examination of the AEIP-treated diabetic rats revealed an increase in the number of β-cells within the pancreatic islets, along with a reduction in the vacuolation (slides C, D and E). In other words, the AEIP treated diabetic samples histopathologically tend to approach the histopathology of the healthy pancreatic samples. The group C however shows less β-cell regenerative efficacy in comparison to group D, showing that this extract might be producing hypoglycaemic effect in some other way. The standard treated group also shows recovery and tends to approach the histopathology of the normal rat pancreas.The antidiabetic activity of it may be due to the presence of flavonoids. It is reported that flavanoids constitute the active biological principles of most medicinal plants with hypoglycemic and antidiabetic properties.[17]

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