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Anti Hyperglycemic Effects Of *Citrullus Vulgaris* (Colocynth) And *Rosmarinus Officinalis (* Rosmary) In Streptozotocin-Induced Diabetic Rats

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Abstract

Citrullus vulgaris (Colocynth) and *Rosmarinus officinalis* (Rosmary) has great potential as an antioxidant and an anti hyperglycemic agent in vitro.The plants are rich in many pharmaceutical active ingredients like tannins,flavonal and lignans.Thus the present study was designed to investigate anti hyperglycemic effects of *Citrullus vulgaris* (Colocynth) and *Rosmarinus officinalis* (Rosmary) powder in streptozotocin-induced diabetic rats. Twenty five Sprague-Dawley rats were randomized into 5 equal groups. Group1 negative control group and the other groups were injected with twice intraperitoneal (i.p) injection with Streptpzotocin (STZ) (30 mg/kg body weight).The difference between first and second injection was three days to induce diabetes.Group 2 positive control, group and groups 3,4 and 5 were given (Colocynth) , (Rosmary) and mixture of them at concentiration of 5% after the second injection with STZ for 4 weeks. Blood samples were collected for serum biochemical analyses, also the Liver and kidney were taken for histopathological examination. The (Colocynth) , (Rosmary) powder and mixture of them significantly reduced body weight gain, feed intake and feed efficiency ratio, normalized serum levels of liver enzymes and kidney function parameters, improved lipid profile, decreased blood glucose in diabetic rats.Histopathological examination showed amelioration of histopathological lesions seen in the liver and kidneys of experimental diabetic rats.Plants exhibited hepatoprotective, hypolipidemic and antidiabetic effects in diabetic rats.The study recommends that the (Colocynth) , (Rosmary) powder should be further investigated as a new supplement for the management of diabetes.

KeyWords: hyperglycemic, antidiabetic, colocynth, rosemary,, hepatoprotective, biochemical analyses and Rats.

Introduction :

Diabetes mellitus (DM) is often associated with dyslipidemia. Elevated levels of plasma free fatty acids play a pivotal role by contributing significantly to insulin resistance **(Ramach *et al.*, 2012).** A patient with elevated low-density lipoprotein (LDL) will have a high risk of experiencing cardiovascular disorders **(Kaur and Singh, 2002)** that is why diabetic patients usually experience various disease, such as atherosclerosis, diabetic nephropathy and neuropathy **(Sheetz, 2002).** Treating will be important to reduce macrovascular disorders in diabetic patients. Because DM control side effects is a challenge, drugs derived from plants may play an important role in the treatment of DM. Medical herbs has been popular from ancient times among people, and in recent years a multilateral broach has emerged on using medicines with natural and especially herbal origin **(Mohajeri *et al.*, 2008).**

Diabetes is a common disease and has its side effects **(Sarah *et al.*, 2011).** Diabetes increases blood glucose, and impaired metabolism of proteins and lipids. Altered cellular metabolism caused by hyperglycemia has been suggested to play any important role in increasing the risk of cardiovascular, renal, ophthalmic and neurological complications of diabetes mellitus **(Anfenan, 2014).**Today, diabetes is regarded as one of the important problems of people with diabetes in the world will reach 334000000 people by 2025 **(Irvine and Taylor, 2009),** which will increase to 552 million by 2030 **(IDF , 2011).**

Citrullus vulgaris commonly known as (Colocynth), has great potential as an antioxidant and an antihyperglycemic agent in vitro **(Hashim *et al.*, 2013).***C. vulgaris* is also rich in tannins, flavonal, norlignan compounds **(Huang *et al.*, 1998),** lignans **(Huang *et al.*, 1996).**

Rosmarinus officinalis (Rosmary) is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, native to the Mediterranean region **(Room and Adrian,1988)**

Rosemary contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol. In traditional medicine, extracts and essential oil from flowers and leaves are used in the belief they may be useful to treat a variety of disorders ***(Vallverdú-Queralt et al.,*2014)**

Rosemary essential oil contains 10-20% camphor, though the chemical composition can vary greatly between different samples, according to in vitro studies **(Rašković and Aleksandar, *2014*)**

The present study was carried out to investigate anti hyperglycemic effects of *Citrullus vulgaris* and *Rosmarinus officinalis* uses as apowder in streptozotocin- induced diabetic rats.

Materials & Methods

Materials

- **Colocynth and Rosmary** were obtained from agricultural Seed, Spices and Medicinal Plants Co. (Harras), Cairo, Egypt.

- **Experimental animals**

Twenty five adult male albino rats, Sprague Dawley, weighting (150±10g) were purchased from Medical Insects Research Institute, Doki, Cairo, Egypt. Rats were housed in environmentally controlled atmosphere and were fed on basal diet according to AIN-93 guidelines **(Reeves *et al.*, 1993)** in animal laboratory in Faculty of Home Economics.

-**All chemicals**, solvents and buffers in analytical grade, Streptozotocin , vitamin and salt mixtures components used for rats feeding were purchased from El-Gomhoria Company for Chemicals and Drug Trading, Cairo, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt.

Methods

Colocynth and Rosmary preparation: Colocynth and Rosmary were powdered by electric grinder (Moulinex, France), packed in dusky Stoppard glass bottles at 4ºC until use.

Basal diet: The basal diet prepared according to the formula mentioned by **(AIN, 1993)** as follow: protein (10%), corn oil (10%), vitamins mixture (1%), mineral mixture (4%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamin mixture component was that recommended by (**Campbell, 1963**) while the salts mixture used was formulated according to (**Hegested *et al.*, 1941).**

Induction of diabetic rats

Normal healthy male albino rats were injected by intra-peritoneal injection of STZ at a low dose (30 mg/kg of body weight , dissolved in 0.05 M citrate buffer, pH 4.5, immediately before use **) (Ji *et al.*, 2011).** One week after injection, fasting blood glucose (FBG)

levels were determined from tail blood using an specific kit (AlGomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt). The rats with FBG levels above 126 mg/dL were considered to be diabetics and included in the experiment.

Experimental design

All rats were fed on basal diet for one week for adaptation, then rats were randomly divided into two main groups, the first group, negative control group (n=5) fed on basal diet, and the second main group; diabetic groups (n=20) were divided into four groups (5 rats each), Group1: Positive control group fed on basal diet and groups 2,3 and 4 fed on basal diet containing 5% rosemary,colocynth and mixture of them respectively. At the end of the experiment (28 days), rats were fasted overnight (12 hours) and anesthetized with diethyl ether. Blood samples were collected into a dry clean centrifuge glass tubes. Serum was separated by centrifugation at 4000 rpm at room temperature according to **Drury and Wallington (1980)**. Serum was carefully aspirated and transferred into clean quiet fit plastic tubes and kept frozen at (-20o C) until analysis.

Biochemical analysis

Different tested parameters in serum were determined using specific methods as follow: Serum Glucose according to ***Trinder (1969),*** Lipid profile; Cholesterol, Tri Glycerides **(T.G)**, High Density Lipoprotein **(H.D.L-c)** , Low Density Lipoprotein **(L.D.L-c)** and Very Low Density Lipoprotein **(V.L.D.L-c)** were determined according to ***Allain (1974)*** , ***Fossati and Prencipe (1982) , Lopez (1977),*** and ***Lee and Nieman, (1996)*** respectively. Glutamic Oxalic Transaminase (**GOT**), Glutamic Pyrofic Transaminase (**GPT**) and Alkaline phsphatase (**ALP**) were determined according to **Yound, (1975), Tietz, (1976)** and **Belfield and Goldberg, (1971)** respectively**.**

Statistical analyses

The data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean ± SD. Differences between treatments at $\mathbf{p \leq 0.05}$ were considered significant.

Results and Discussion

Results

Data recorded in Table (1) showed that hyperglycemic rats had significant increases ($P<0.05$) in blood glucose (BG) level when compared to the negative control group.Consumption of Colocynth ,

Rosmary and their mixture at(5%) percent had significant decreases in blood glucose (BG) level when compared to the positive control group. Best group recorded for 5% mixture.

Table (1). Effect of Colocynth and Rosmary on blood glucose (BG) level in diabetic rats

Parameters	Negative Ve^-	Positive Ve^+	Colocynth	Rosmary	Mixture
			5%	5%	5%
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
BG (mg/dl)	119.25^{d} ± 2.72	254.47^{a} ± 2.35	176.04^{b} ± 1.42	154.60^{c} ± 2.81	122.60^{d} ± 3.21

Data are presented as means ± standard deviation, (n= 5 for each group) Values with different superscripts within the column are significantly different at P <0.05. values with similar or partially similar superscripts are non-significantly different.

Table (2). Effect of Colocynth and Rosmary on serum levels of total cholesterol (TC) and triglycerides (TG) in diabetic rats

Groups	T.C (mg\dl)	T.G (mg\dl)
	Mean±SD	Mean±SD
Control V-	121.63^{b}± 1.9	120^{cd} ± 2
Control V+	171^{a} ± 2	179.8^{a} ±1.8
5%Colocynth	106.7^{c} ± 2	115.2^{d} ± 2.5
5% Rosmary	93.7^{d} ± 2	118.9^{cd} ± 1. 9
5% Mixture	79.5 e± 2	135b ± 17.3

Data are presented as means ± standard deviation, (n= 5 for each group) Values with different superscripts within the column are significantly different at P <0.05. values with similar or partially similar superscripts are non-significantly different.

Data recorded in Table (2) showed that Hyperglycemic rats had significant increases ($p<0.05$) in the serum levels of total cholesterol (TC) and triglycerides (T.G). Consumption of Colocynth and Rosmary and their mixture at(5%) percent caused significant decreases in serum levels of TC and TG when compared to the positive control group. Best group in T.C and T.G recorded for 5% mixture and 5% colocynth respectively.

Table (3). Effect of Colocynth and Rosmary on serum levels of HDL-c , LDL-c and VLDL-c in diabetic rats

Groups	HDL(mg\dL)	LDL (mg\dL)	VLDL(mg\dL)
	Mean±SD	Mean±SD	Mean±SD
Control (-)	85[a]± 2.06	12.63 [e] ±7.08	24.0 [c] ± 0.20
Control (+)	20 [e]± 2	115 [a] ±7.38	36.0 [a] ± 0.30
5% Colocynth	55 [b] ±2.1	28.7 [b] ± 1.9	23.0 [c] ± 0.52
5% Rosmary	46.[c] ± 2.1	23.9 [c] ± 2	23.8 [c] ± 0.41
5% Mixture	33.9 [d] ± 1.8	18.6 [d]± 2.08	27.0 [b] ± 0.30

Values are expressed as mean ± SD. Means in the same row with different litters are significantly different ($p \leq 0.05$).**HDL**: High Density Lipoprotein, **LDL**: Low Density Lipoprotein, **VLDL**: Very Low Density Lipoprotein.

Data recorded in Table (3) showed that Hyperglycemic rats had significant increases ($p<0.05$) in the serum levels of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) , and had significant decrease in the serum of (HDL-c) when compared with positive group, while consumption of Colocynth and Rosmary and their mixture at(5%) percent had significant decreases in serum levels of LDL, VLDL,and had significant increase of HDL when compared to the positive control group.For LDL best group recorded for 5% mixture.

Table (4). Effect of Colocynth and Rosmary on serum levels of GOT(AST) , GPT(ALT) and ALP in diabetic rats

Groups	GOT U\L	GPT U\L	ALP U\L
	Mean±SD	Mean±SD	Mean±SD
Control (-)	59.28 [d]± 1.45	54.64 e ± 0.26	68.71 [c] ± 2.90
Control (+)	149.82 [a] ± 1.21	130.21 [a] ±0.32	116.73 [a] ± 1.55
5 % Colocynth	80.45 [b] ± 2.14	88.01 [b] ± 0.13	89.51 [b] ± 1.34
5 % Rosmary	77.13 [b] ± 2.76	79.15 [C] ± 0.48	70.01 [c] ± 21.78
5 % Mixture	64.54 [c] ±1.43	61.37 [d]± 0.33	70.46 [c] ± 14.91

Values are expressed as mean ± SD. Means in the same row with different litters are significantly different ($p \leq 0.05$). **GOT**: Glutamic Oxalic transaminase, **GPT**: Glutamic Pyrofic transaminase, **ALP**: Alkaline phosphatase.

Data recorded in Table (4) showed that Hyperglycemic rats had significant decrease ($p<0.05$) in the serum levels of Glutamic Oxalic transaminase **(GOT),** Glutamic Pyrofic transaminase(**GPT**),and Alkaline phosphatase **(ALP)**. when compared with positive group**,** while consumption of Colocynth and Rosmary and their mixture at (5%) percent had significant decreases in serum levels of **GOT, GPT ,**

and **ALP.** when compared to the positive control group**,** For GPT best group recorded for 5% mixture.

Discussion :

The present study was conducted to demonstrate antihyperglycemic effects of ***Citrullus vulgaris*** and ***Rosmarinus officinalis*** in streptozotocin-induced diabetic rats.

In the current study, it was observed that there was a significant decrease in serum glucose concentrations in diabetic groups treated with ***Citrullus vulgaris*** and ***Rosmarinus officinalis*** as compared with control diabetic group. These results confirmed the result of **(*Huseini et al.*,2009)** who reported that this may be due to the hypoglycemic effect of the ***Citrullus vulgaris*** to proactive and pharmacological compounds which may help in suppressing the free radical in serum, this ultimately lead to decrease the level of blood glucose, and Clinical studies have shown medicinal benefits of colocynth in patients with diabetes, diabetic neuropathy, and hyperlipidemia. In a randomized clinical trial (RCT), HbA1c and fasting blood glucose levels were decreased in patients using 300 mg of *C. colocynthis* dry fruit powder daily for 2 months.

In the present study, it was found that the diabetic group showed significant decrease in HDL and also showed significant increase in LDL, cholesterol and triglycerides compared to control (+) group. These results agreed with the previous studies which suggested that the lipoprotein abnormalities are higher in diabetics than in non-diabetics **(Mahfouz *et al.*,2009).Veiraiah (2005)** suggested that hyperglycemia lead to an increase in LDL cholesterol by reducing the ability of the body to remove cholesterol.In addition, hyperglycemia also leads to inhibition of lipoprotein lipase and further aggravating hyperlipidemia.

After treatment with plants at similar doses and their mixture , a significant reduction was observed in TG,TC and LDL while there was a significant increase in HDLc level of diabetic rats.The finding of this study is in agreement with previously published data that illustrated the antioxidant and anti hyperglycemic effect of ***Citrullus vulgaris*** (***Rahbar, and Nabipour,2010).***

The present study showed that the AST,ALT and and ALP levels were significantly increased in the liver of hyperglycemic STZ-treated animals compared to control negative group.Serum ALT,AST and ALP

levels were determined to evaluate the hepatic functions **(Degirmenchi *et al.*, 2002).**

Merzouk *et al.*, (2000) reported that the increase in aminotransferases levels may be due to cellular damage in the liver caused by STZ-induced diabetes .Although ALT is also present in mitochondria, the mitochondrial from is low in activity and is very unstable.The detailed mechanism by which enzymes are passed from the cytosol and mitochondria of hepatocytes is not completely known.However,ALT elevation could also have been due to overweight **(Harris,2005).**

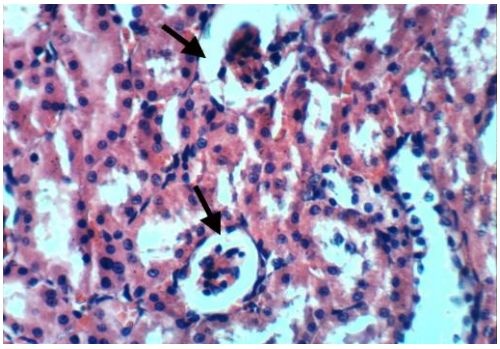

Photo. (1): Kidney of rat from group 2 showing atrophy of glomerular tuft (H & E X 400).

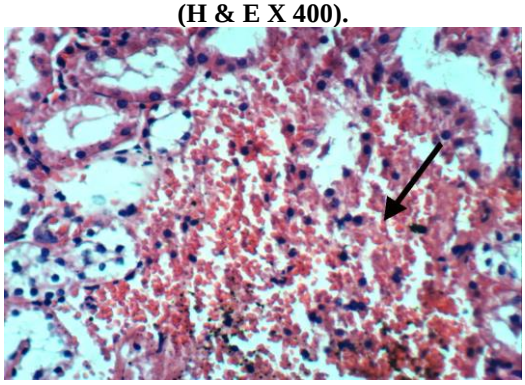

Photo. (2): Kidney of rat from group 2 showing renal haemorrhage with deposition of haemosidrin pigment (H & E X 400).

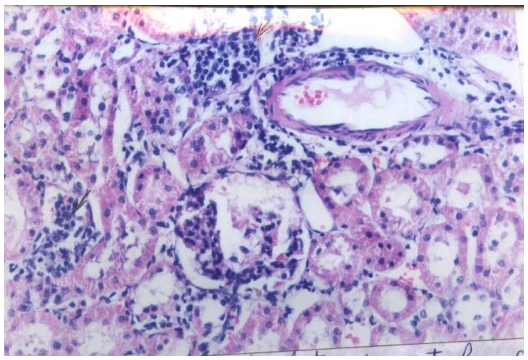

Photo (3): Kidney of rat from group 3 showing local leucocytic cells infiltrations arrows (H and E X 200).

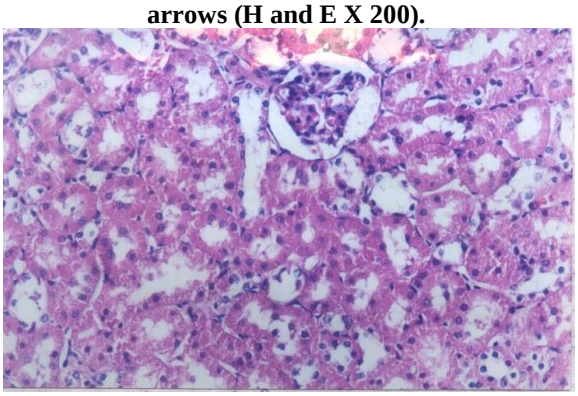

Photo (4): kidney of rat from group 4 showing no histopatholgical changes (H and E X 200).

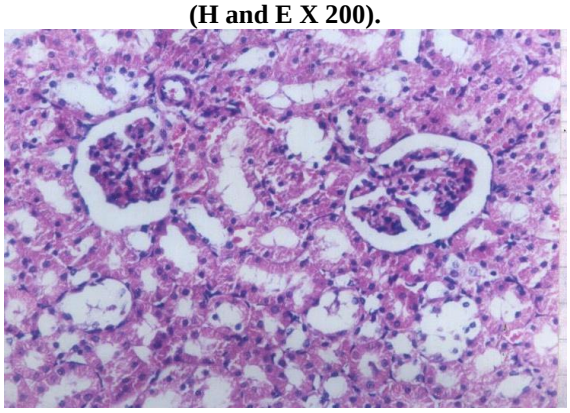

Photo (5): kidney of rat from group 5 showing no histopatholgical changes (H and E X 200).

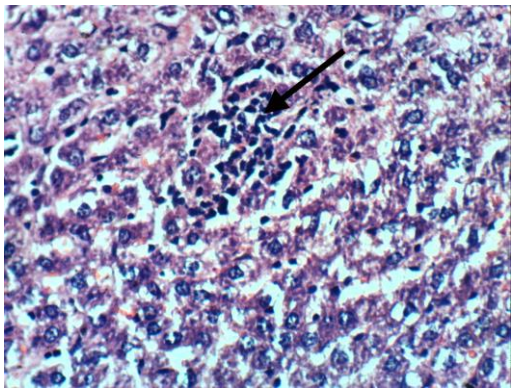

Photo. (6): Liver of rat from group 1 showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

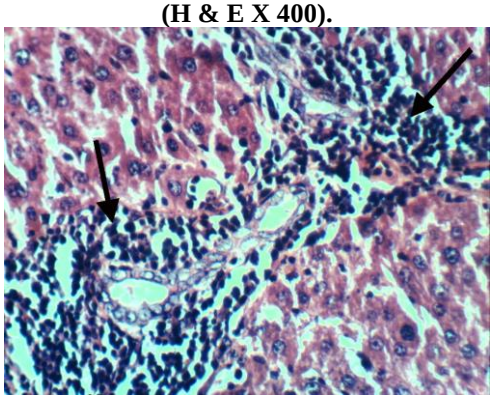

Photo. (7): Liver of rat from group 2 showing portal infiltration with massive inflammatory cells (H & E X 400).

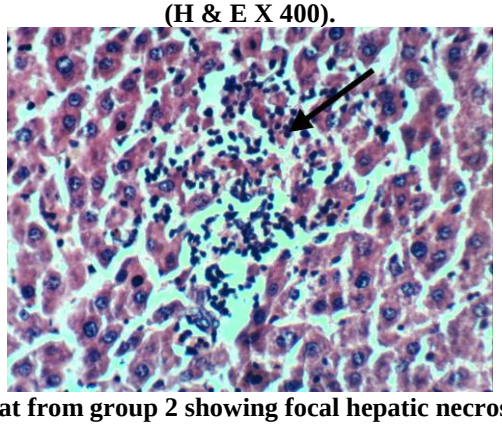

Fig. (8): Liver of rat from group 2 showing focal hepatic necrosis associated with inflammatory cells infiltration (H & E X 400).

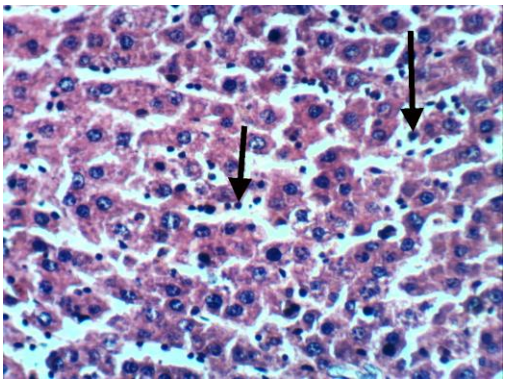

Fig. (9): Liver of rat from group 2 showing sinusoidal leucocytosis (H & E X 400).

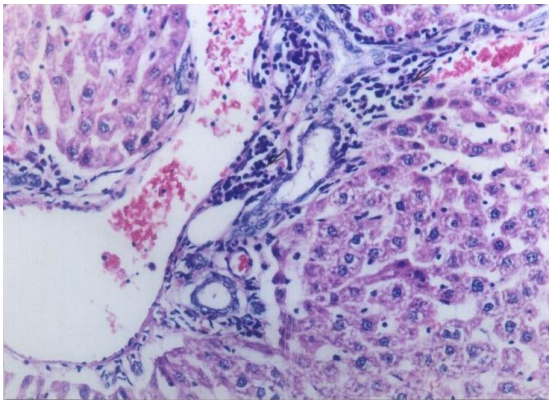

Photo (10): liver of rat from group 3 showing portal infiltration with leucocytes arrows (H and E X 200).

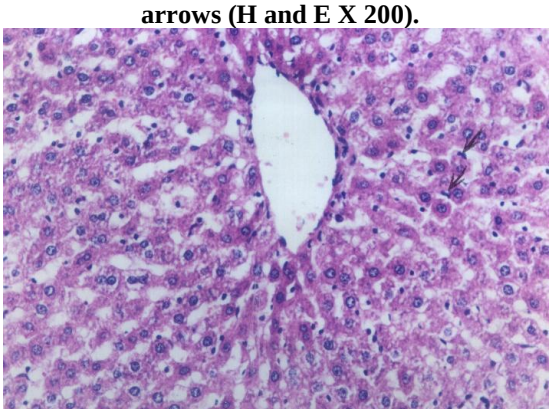

Photo (11): liver of rat from group 5 showing slight activation of kupffer arrows cells (H and E X 200).

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التأثير المضاد للحنظل وحصاالبان على ارتفاع السكر في الفئران المصابة بداء السكري المحدث بالإستربتوزوتوسين

مي محمود الحسيني خفاجي
قسم التغذيه وعلوم الأطعمه - كلية الاقتصاد المنزلى – جامعة المنوفية

يلعب كلا من نبات الحنظل وحصاالبان دورا هاما كمضاد للأكسدة وعامل خافض لسكر الدم . وهم نباتات غنية بالعديد من المركبات مثل التانين والفلافونال واللجنان ولذلك تم تصميم هذه الدراسة للتحقق من تأثير هذه النباتات على خفض مستوى سكر الدم في الفئران المصابة بالسكري المحدث بواسطة الإستربتوزوتوسين.

تم توزيع خمسة وعشرون من الفئران الألبينو من سلالة Sprague-Dawley بطريقة عشوائية إلى ٥ مجموعات متساوية.المجموعة الأولى :مجموعة ضابطة سالبة, أما المجموعات الأخرى فتم حقنها بالاستربتوزوتوسين مرتين (٣٠ملجم/ كجم من وزن الجسم). كان الفرق بين الحقنة الأولى والثانية ثلاثة أيام وذلك لإحداث مرض البول السكري. وبعد ذلك تم تقسيم الفئران المصابة إلى : المجموعة الثانية مجموعة ضابطة موجبة,أما المجموعات الثالثة والرابعة والخامسة فأعطيت مسحوق نبات الحنظل وحصاالبان ومخلوط منهم بتركيز٥% على التوالي لمدة أربعة أسابيع.تم جمع عينات الدم لإجراء التحاليل الكيميائية الحيوية في الدم.وكذلك الفحص الهستوباثولوجي لكل من الكبد والكلى.أدى إعطاء مسحوق الحنظل وحصاالبان إلى تحسين مستويات انزيمات الكبد ووظائف الكلى إلى المستويات الطبيعية في الفئران المصابة بداء السكري.كما أدى تناول المسحوق وخليطه إلى تحسن في أنسجة كبد وكلى الفئران المصابة للمجموعات المعالجة مقارنة بالمجموعة الضابطة الموجبة.

وأخيرا تؤكد الدراسة بأن هناك دورا هاما لكل من الحنظل وحصاالبان في خفض مستوى السكر في الدم. كما أوصت الدراسة باجراء العديد من الدراسات الاخرى التي تؤكد دوره كمكمل جديد في التحكم في مستويات السكربالدم.

الكلمات المفتاحية:
الحنظل- حصاالبان- انخفاض سكر الدم- التحاليل الكيميائية- الفئران.