

The Effect Of Clove (*Syzygium Aromaticum*) Extract On Rats With Induced Diabetic Nephropathy

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Abstract

The present study aims to evaluate the effects of clove extract on rats with induced diabetic nephropathy. Thirty-five adult rat were divided into 5 groups, 1st group was fed on basal diet kept as negative control group. While the other rats were injected with streptozotocin (STZ) and glycerol to induce diabetic nephropathy then these rats were classified into 4 groups as positive control group, while the other three groups were given orally clove extract at 0.5, 1 and 2 ml/rat respectively. **Results:** The results revealed that the three tested materials exhibit significant ($P<0.05$) decrease in serum glucose level, and an improvement in serum kidney function and lipid profile, while serum insulin concentrations and HDL-C were significantly ($P<0.05$) increased. In addition, an enhancement ($P<0.05$) of the liver enzymes and antioxidant enzymes (catalase, superoxide dismutase and glutathione reductase) activities were observed while, serum MDA was significantly decreased as compared to the positive control group. This study demonstrated that clove extract is suitable for diabetic patient with nephropathy.

Key words: Diabetes, rats, clove extract, diabetic nephropathy, liver enzyme, kidney function, lipid profile.

Introduction

Diabetes mellitus (DM) is categorized by hyperglycemia due to absolute, (type 1) or relative deficiency of insulin (type 2). It has become a common illness affecting about 180 million people worldwide (**Finkel et al., 2009**). Hyperglycemia, hyperlipidemia, hyper aminoacidemia, and hypo insulinaemia are the most symptoms of DM that leads to decrease insulin secretion and action (**Maiti et al., 2004 and Wadkar et al., 2008**). The acute complication of DM include hyperglycemia, diabetic ketoacidosis, lactic acidosis, hyperosmolar non-ketotic coma (**Andrew et al., 2010**).

Diabetic nephropathy (DN) is one of the most serious microvascular complication of diabetes affecting a large population. About 15–25% of type I diabetes patients and 30–40% of type II diabetes patients. develops diabetic nephropathy (**Hovind et al., 2000**).

Diabetic nephropathy can be clinically defined by specific pathophysiological changes in renal morphology and alterations in functional efficiency, which eventually progresses into end stage renal disease and chronic kidney disease (**Abbas et al., 2013**). Insulin injections, glucose lowering drugs as well as lifestyle changes, weight control and diet therapy, are recommended for handling diabetes (**Kempf et al., (2008)**).

Clove (*Syzygium aromaticum*) is a herbal plant which belongs to the species *aromaticum* genus *Syzygium* and family *myrtaceae* (Dennehy et al., 2007). The therapeutic effects of this plant is used for alleviating gastrointestinal symptoms and acts as ant-inflammatory, insecticidal, antiplatelet, antioxidant, and antihypertensive (Alqareer et al., 2006). Eugenol, isoeugenol and caryophyllene are the most compounds in clove, that contributing to the pharmacological effect (Bensky et al. 2004 and Pourgholami et al., 2007). Moreover, plant foods rich in polyphenolic fractions have been reported to cause insulin-like effects in glucose utilization (Gruenwald et al., 2010).

Materials and Methods:-

Materials: Chemicals: Casein, vitamins, minerals, cellulose and Streptozotocin, glycerol were obtained from El-Gomhoria Company, Cairo, Egypt. Kits required for estimating parameters used in the study was purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt. Dried Cloves (*Syzygium aromaticum*) was obtained from the local herbal market. **Animals:-** Adult male Sprague-Dawley rats (n = 35 which weighing (170g) were purchased from Farm of Experimental Animals in Helwan, Egypt.

Methods:

A. Identification of clove: The scientific classification of this plant was carried out at the National Scientific Research. **Kingdom:** Plantae, **Order:** Myrtales, **Family:** Myrtaceae, **Genus:** *Syzygium*, **Species:** *S. aromaticum*.

Binomial name : *Syzygium aromaticum*

B. Preparation of clove extract: Water extract of dried clove was carried out by dissolving (5, 10 and 15) gm of ground clove with 100ml boiling water respectively.

C. Chemical composition: the gross chemical composition of clove extract was determined according to the official methods (Barre et al., 1997).

D. Induction of Diabetic rats: Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg BW). After Three days, random blood samples were taken then the level of the blood glucose was assessed and the level ≥ 250 mg/dl was considered as diabetic (Sarkar, et al., 1996).

E. Induction of nephropathy of rats: Rats was given intramuscular injections of 50% glycerol (10 ml/kg B.Wt.) in their hind limbs. Random blood samples were obtained to measure the kidney functions to insure the induction of acute renal failure (Midhun et al., 2012).

F. Experimental design: The basal was formulated according to Reeves et al., (1993). The Biological experiments and the biochemical analysis were carried out at the post graduated lab, Home Economic Faculty, Helwan University. Thirty five adult male rats was housed in well aerated cages under hygienic conditions, and was fed on basal diet for one week for adaptation. After acclimatization period, rats were divided into 5 groups (n=7) as follows: Group-1 (negative control group), were fed on standard diet. Group-2 (diabetic control) was fed on standard diet. Groups (3- 5) are diabetic rats, were fed on basal diet and given orally clove extract by 0.5, 1 and 2 ml/rats, respectively. At the end of experiment, blood samples were taken from the animals then centrifuged to obtain serum.

The feed intake (FI) was recorded every day throughout the experimental period. Body weight gain % (BWG) and feed deficiency ratio (FER) were determined according to **Chapman et al., (1959)**, using the following equations

$$\text{BWG \%} = \frac{\text{initial body weight} - \text{final body weight}}{\text{Initial body weight}} \times 100$$

$$\text{FER} = \frac{\text{weight gain (g)}}{\text{feed intake (g)}}$$

Biochemical Analysis: Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) were determined according to **Allen, 1974; Fassati and prencipe, 1982** and **Lopez, 1977**, respectively. Low density lipoprotein –cholesterol (LDL-C) and very low density lipoprotein (VLDL-C) were determined according to the equation of **Friedwable et al., (1972)**. $\text{VLDL-C} = \text{TG}/5$, $\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C})$

Determination of aspartate amino transferase (AST) and alanin amino tranferase (ALT) were determined according to **Reitman and Frankel, (1957)**. Serum Urea and uric acid were determined according to **Pattn and Crouch (1977)**, the determination of Creatinine was according to **Henry (1974)**, whereas glucose and insulin were determined according to **(Trinder, 1959 and Matthews, 1985)**, respectively .

Statistical Analysis : All obtained data was analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20. Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to All differences was considered significant at $P < 0.05$ (**Armitage and Berry, 1987**).

Result and Discussion:

Results of Table (1) shows the effect of clove extract on body weight of rats with induced diabetic nephropathy. There is no significant different in IB Wamong all rats. The injection with glycerol and STZ caused a significant decrease ($P < 0.05$) in the FBW, BWG% and FER as compared to the normal rats. There is significant difference in FBW, BWG% and FER among the groups given clove extract. The highest increased in FBW were recorded at the group given orally clove extract (2 ml/rats). FI was numerically increased due to clove extract among the tested groups as compared to the control group.

The obtained results agreed with some researchers who reported a significant decrease in body weight of diabetic rats due to the body's inability to store or use glucose that causes hunger and weight loss (**Narasimhulu et al., 2014** and **Kota et al., 2012**) and may be the result of protein wasting due to absence of carbohydrate as an energy source (**Al-Attar, 2010**). The damage of β - cells and disorder of insulin secretion in the diabetic state causes reduction in BWG and increase in food and water intake and urine volume (**Kang et al., 2006**).

Moreover, **Al- Attar and Zari, (2007)** reported that supplementation clove oil caused higher body weight change than diabetic rats giving control diets, may be due to the insulin like action of clove on muscle, adipose tissue and hepatocytes. **Chaudhry et al., 2013** and **Srinivasan et al., (2014)** found that administration of eugenol for 30 days significantly improved glycemic control which prevented the loss of body weight and excess of food and fluid intake on diabetic control rats.

Results of table (2) shows the effect of clove extract on blood glucose and insulin concentrations in diabetic nephropathy rats. The treatment with clove extracts significantly ($P < 0.05$) decreased the level of glucose compared to the positive control group. There is significant change ($p < 0.05$) in serum glucose among the groups that given oral clove extract. The highest reduction in glucose level was recorded at the groups that fed basal diet and given clove extract (2 ml/rats). Clove extract at the three tested levels caused glucose reduction by (39.38, 47.25 and 54.30 %) respectively.

On the other hand, the treatment with clove extract increased ($p < 0.05$) insulin significantly by (43.38, 89.94 and 123.46%) respectively as compared to the control group. The highest increase in insulin concentration was recorded at the groups that fed basal diet and given clove extract (2 ml/rats).

Diabetes mellitus is a disease with impaired carbohydrate, fat, and protein metabolism caused by limited insulin secretion or lower tissue sensitivity to insulin (**Abel-Salam, 2011**). Clove is one of spices that reportedly modulated physiological responses in diabetic rats and reduced glucose level (**Nangle et al., 2006 and Zari and Al-Attar, 2007**) due to the antioxidant and hypoglycaemic effects of clove essential oil contents especially eugenol and eugenyl acetate (**Lee and Shibamoto, 2001**) that facilitating glucose usage via extra pancreatic ways (**Hassanen, 2010**).

Result in table (3) illustrates the effect of clove extract on kidney functions. The supplementation with clove extract significantly decreased ($P < 0.05$) serum urea, creatinine and uric acid as compared to the positive group. There are significant differences in serum kidney functions among the groups given orally clove extract. The highest improvement of kidney function is observed at the group that given orally clove extract (2 ml/rats).

In the present study, the diabetic rats had increased levels of creatinine and urea which are considered as significant markers of renal function and this agrees with the results of **Sacan et al., (2006)**. The kidneys of diabetic rats become enlarged and maybe associated with membrane damage caused by hyperglycaemia mediated oxidative stress (**Ramesh et al., 2007**), these changes are reversed with clove supplementation in addition to enhance the kidney function related to its essential oil contents that decreased membrane damage and reversed fatty acid changes as evidenced by improved insulin level, and also supported by regulated glycoprotein components (**Hassanen, 2010**).

Result in table (4) the results show that liver functions are significantly increased at the positive control group as compared to negative control group. The supplementation with the tested materials significantly decreased ($P < 0.05$) the level of liver function compared to negative control group. There are significant differences in serum liver functions among the groups given orally clove extract. The highest improvements in liver function are observed at the group that given orally clove extract (2 ml/rats).

In diabetic rats, the activity of serum ALP, ALT and AST were significantly ($P < 0.05$) increased. Supporting these findings, it has been found that the liver was necrotized in diabetic rats mainly due to the leakage of these enzymes from the liver into the blood stream (**Mansour et al., 2002**). On the other hand, the administration of clove to diabetic rats reduced ALP, ALT and AST activity towards their normal values (**Hassanen, 2010**).

The present study showed that there were higher levels of TC, TG, LDL-C and VLDL-C accompanied by low level of HDL-C in diabetic rats (table 5). The level of lipids is usually raised in

diabetes due to an increase in adipose tissue lipolysis in absence of insulin and mobilization of free fatty acids from the peripheral depots(Pacheco et al., 2001), since insulin inhibits the hormone sensitive lipase (Hassanen, 2010).

The mean value of lipid profile is significantly lowered at the group fed on clove extract as compared with the positive control group. The highest improvement in lipid profile is recorded at the group fed on basal diet and clove extract (2 ml/rats).The continuous administration of clove to diabetic rats reduced the elevation of serum lipids and enhanced the level of HDL-C; these results are in consequence with those found by Badee et al., (2005). The hypolipidaemic effect could be probably attributed to decreasing 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase activity), a key enzyme of cholesterol biosynthesis and/or by reducing the NADPH required for fatty acids and cholesterol biosynthesis (Sharma et al., 2003 and Vessal et al.,2003). Also, the clove might stimulate the production of insulin which in turn inhibits lipoprotein lipase activity (Ravi et al., 2005)

Table (1): Effect of clove extract on body weight of rats with induced diabetic nephropathy

Parameters Groups	IBW(g)	FBW (G)	BWG%	FI(g/d/rat)	FER
Control (-ve)	169.00±1.08 ^a	204.16±1.55 ^a	20.80±0.46 ^a	16.7	0.035±0.08 ^a
Control (+ve)	168.56±0.61 ^a	149.93±0.44 ^e	-11.04±0.59 ^e	11.4	-0.027±0.01 ^d
clove extract (1 ml/rats)	169.16±0.92 ^a	186.53±1.22 ^d	10.27±0.99 ^d	14	0.021±0.01 ^c
clove extract (1 ml/rats)	166.33±1.64 ^a	191.40±1.23 ^c	15.10±1.32 ^c	14.5	0.029±0.02 ^b
clove extract (2 ml/rats)	169.20±0.94 ^a	199.16±1.96 ^b	17.70±0.54 ^b	14.8	0.034±0.01 ^{ab}

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at (P < 0.05).

Table (2): Effect of clove extract on glucose and insulin concentrations of rats with induced diabetic nephropathy

Parameters Groups	Glucose (mg/dl)	Glucose reduction (%)	Insulin (uIU/ml)	Insulin increment(%)
Control (-ve)	88.30±0.96 ^e	-	1.29±0.02 ^a	
Control (+ve)	277.36±2.60 ^a	-	0.537±0.05 ^e	
clove extract (0.5 ml/rats)	168.11±1.69 ^b	39.38	0.77±0.01 ^d	43.38
clove extract (1 ml/rats)	146.30±2.56 ^c	47.25	1.02±0.02 ^c	89.94
clove extract (2 ml/rats)	126.73±2.12 ^d	54.30	1.20±0.006 ^b	123.46

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at (P < 0.05).

Table (3): Effect of clove extract on kidney functions of rats with induced diabetic nephropathy

Parameters Groups	Urea	Creatinine	Uric acid
	(mg/dl)		
Control (-ve)	24.03±0.99 ^e	0.67±0.02 ^e	2.69±0.14 ^e

Control (+ve)	56.96±1.38 ^a	1.31±0.03 ^a	5.93±0.19 ^a
clove extract (0.5 ml/rats)	40.30±1.50 ^b	1.10±0.05 ^b	4.09±0.07 ^b
clove extract (1 ml/rats)	35.50±1.24 ^c	0.94±0.02 ^c	3.56±0.04 ^c
clove extract (2 ml/rats)	28.33±1.07 ^d	0.80±0.04 ^d	3.21±0.02 ^d

Data are expressed as mean ± SE. Means with different superscript letters in the column are significantly differences at (P < 0.05).

Table (4): Effect of clove extract on liver functions of rats with induced diabetic nephropathy

Parameters Groups	ALT	AST
	(μ/L)	
Control (-ve)	42.03±0.81 ^d	30.93±1.10 ^d
Control (+ve)	117.60±1.02 ^a	96.03±1.82 ^a
clove extract (0.5 ml/rats)	103.73±2.05 ^b	59.56±0.69 ^b
clove extract (1 ml/rats)	98.17±1.73 ^c	49.10±1.25 ^c
clove extract (2 ml/rats)	95.86±1.65 ^c	47.73±0.81 ^c

Data are expressed as mean ± SE. Means with different superscript letters in the column are significantly differences at (P < 0.05).

Table (5): Effect of clove extract on lipid profile of rats with induced diabetic nephropathy

Parameters Groups	TC	TG	HDL-C	LDL-C	VLDL-C
	(Mg/dl)				
Control (-ve)	123.13±1.22 ^e	81.73±0.62 ^e	58.02±0.88 ^a	48.76±2.22 ^e	16.34±0.12 ^e
Control (+ve)	189.63±2.06 ^a	146.16±1.75 ^a	28.26±1.04 ^e	132.13±2.40 ^a	29.23±0.35 ^a
clove extract (1 ml/rats)	167.10±1.22 ^b	123.10±1.27 ^b	37.26±2.40 ^d	105.21±1.70 ^b	24.62±0.25 ^b
clove extract (1 ml/rats)	142.30±1.28 ^c	117.86±1.17 ^c	42.23±1.18 ^c	76.49±0.62 ^c	23.57±0.23 ^c
clove extract (2 ml/rats)	130.66±1.21 ^d	103.93±2.07 ^d	49.80±2.12 ^b	60.08±2.92 ^d	20.78±0.41 ^d

Data are expressed as mean ± SE. Means with different superscript letters in the column are significantly differences at (P < 0.05).

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المستخلص العربي

تأثير مستخلص القرنفل علي الفئران المصابة باعتلال الكلي السكري

The Effect of Clove (*Syzygium Aromaticum*) Extract on Rats with Induced Diabetic Nephropathy

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تهدف الدراسة الحالية إلى تقييم تأثير مستخلص القرنفل على الفئران المصابة باعتلال الكلي السكري. تم تقسيم خمسة وثلاثين فأر بالغ إلى 5 مجموعات ، تم تغذية المجموعة الأولى على النظام الغذائي الأساسي كمجموعة ضابطة سالبة.بينما تم حقن باقي الفئران بمادة الستربتوزوتوسين والجلسرين لاحداثاعتلال الكلي السكري , ثم تم تصنيف الفئران المصابة الي 4 مجموعات المجموعة الاولى صنفتم كمجموعة ضابطة موجبة بينما اعطيتباقي المجموعات الثلاثة الاخرى مستخلص القرنفل عن طريق الفم بمقدار 0.5مل و 1مل و2مل /فار علي التوالي . النتائج : اظهرت النتائج ان المجموعات الثلاثة التي تم علاجها بمستخلص القرنفل عن طريق الفم اظهرت انخفاضاً معنوياً في مستوى الجلوكوز في الدم ووظائف الكلي ومستوي الدهون بينما زادت تركيزات الانسولينوالليبوبروتينات عالية الكثافة بشكل ملحوظ، بالإضافة الي ذلك لوحظ تحسن في انزيمات الكبد والانزيمات المضادة للاكسدة مقارنة بالمجموعة الضابطة الموجبة.أظهرت هذه الدراسة أن مستخلص القرنفل مناسب لمرضى السكري الذين يعانون من اعتلال الكلي.