

البحث رقم (٤)

Anti-diabetic Effect of Cinnamon and Cloves oils in Alloxan-Induced Diabetic Rats

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Abstract

Diabetes is the world's largest endocrine disease associated with increased morbidity and mortality rate. Diabetes mellitus (DM) comprises a group of common metabolic disorders characterized by hyperglycemia. The effect of different concentrations (0.25 and 0.5%) of cinnamon oil, cloves oil and their mixture on diabetic rats was evaluated. Forty eight male Albino rats were used in this study and divided into 8 groups, each group contain 6 rats. Rats were treated by alloxan (150mg/kg B.W) to induced diabetic. Serum liver functions (ALT, GOT, and GPT), total cholesterol, triglycerides, lipoprotein fraction (HDL-c, LDL-c, VLDL-c), glucose level, kidney functions (urea, uric acid and creatinine) were determined, Data from diabetic rats revealed that the 0.5 % mixture oils (cinnamon and cloves) showed significant changes in the tested biochemical parameters. As conclusion, diabetic rats treated with 0.5% mixture oils powders had significantly improvement glucose level, lipid profile, liver and kidney functions compared with other concentrations.

Key words: Spices oil, Rats, Anti-diabetic and Biochemical analysis.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves (**Nagappa *et al.*, 2003**).

Since, ancient times, plants have played an important role in the treatment of many diseases. Different parts of medicinal plants such as leaf, root, flower and seed are used as extracts and chemical compounds to produce drugs (**Ozgen *et al.*, 2009**).

According to world Health Organization (WHO), 80% of the World's population is dependent on the traditional medicine (**Maiyo *et al.*, 2010**).

Diabetes is the world's largest endocrine disease associated with increased morbidity and mortality rate. Diabetes mellitus is also associated with long term complications including retinopathy, nephropathy, neuropathy and angiopathy and several others (**Sharma *et al.*, 2010**).

A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have potential to impart therapeutic effect in complicated disorders like diabetes and its complications (**Tiwari and Rao, 2002**).

Medicinal plants are gradually gaining global acceptability given their potential as bioactive agents to be used as pharmaceuticals. New hypoglycemic agents derived from plants have shown both hypoglycemic action and the ability to improve some of the secondary complications of diabetes such as kidney damage, fatty liver, and oxidative stress. In addition, some tropical herbs offer both benefits as it has been recently informed in experimental models (**Fonseca et al., 2012**).

Cinnamon is the bark of the *Cinnamom cassiae*, it contain cinnamon anhydride, tannin, cinnamic acid and methyl-hydroxychalcone polymer (MHCP) etc. Cinnamon has a long history as an anti-diabetic spice, but trials involving cinnamon supplementation have produced contrasting results (**Kirkham et al., 2009**).

Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products. The most important constituents of cinnamon are cinnamaldehyde and *trans*-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (**Goni et al., 2009**).

Raol and Gan, (2014) mentioned that cinnamon has been used as a spice in daily life without any side effects. Several reports have dealt with the numerous properties of cinnamon in the forms of bark, essential oils, bark powder, phenolic compounds, flavonoids, and isolated components. Each of these properties plays a key role in the advancement of human health. The

antioxidant, antimicrobial activities and anti-diabetic activities occur indirectly via receptor-mediated mechanisms. The significant health benefits of numerous types of cinnamon have been explored.

Mang *et al.*, (2006) show that cinnamon extract seems to have a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycaemic control.

Clove belongs to a tree *Eugenia caryophyllata* (*Syzygium aromaticum*), is used as a spice in almost all the world's fare. Bud Oil of Clove has natural behavior and the main properties include antioxidant, insecticidal, antifungal and antibacterial properties. By tradition, it has been used in food preservation as flavoring and antimicrobial sub-stance (**Velluti *et al.*, 2003**).

Material and Methods

Materials:

Cinnamon (*Cinnamon zeylanicum*) and Cloves (*Syzygium aromaticum*) oil were obtained from local market, Shibin El-Kom City, Menoufia Governorate, Egypt.

Cholesterol powder:

Alloxan, it was pure chemical fine product (DBH) were purchased from SIGMA Chemical Co., (USA), and was used for induction of diabetes among rats.

Casein, cellulose, choline chloride, and DL Methionine:

Casein, cellulose, choline chloride powder, and DL methionine powder, were obtained from Morgan Co. Cairo, Egypt.

Experimental animals:

A total of 48 adult normal male albino rats Sprague Dawley strain weighing 140 ± 10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

The chemical kits:

Chemical kits used for determination the (TC, TG, HDL-c, ALT, AST, ALP, urea, uric acid and creatinine) were obtained from Al-Gomhoria Company for Chemical, Drug and Instruments, Cairo, Egypt.

Methods:

Experimental design:

Forty eight adult male white albino rats, Sprague Dawley Strain, 10 weeks age, weighing (140 ± 10 g) were used in this experiment. All rats were fed on basal diet (casein diet) prepared according to AIN, (1993) for 7 consecutive days. After this adaptation period, rats are divided into 5 groups, each group which consists of six rats as follows: group (I): rats fed on basal diet as negative control. Group (2): Injected by alloxan a dose of 150 mg /kg of rat's body weight and used as a positive control group. Group (3): A group infected diabetic fed on cinnamon oil by 0.25% of the weight of basal diet. Group (4): A group infected diabetic fed on cinnamon oil by 0.5% of basal

diet. Group (5): A group infected diabetic fed on cloves oil by 0.25 % of basal diet. Group (6): A group infected diabetic fed on cloves oil by 0.5 % of basal diet. Group (7): A group infected diabetic fed on mixture of cinnamon and cloves oil by 0.25 % of basal diet. Group (8): A group infected diabetic fed on mixture of cinnamon and cloves oil by 0.5% of basal diet. During the experimental period, the body weight and feed intake were estimated weekly and the general behavior of rats was observed. The experiment period was take 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered and collect blood samples. Blood samples were centrifuged at 4000 rpm for ten minute to separate blood serum, and then kept in deep freezer till using.

Blood sampling:

After fasting for 12 hours, blood samples in initial times were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of each experiments. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 30 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen in deep freezer till analysis according to method described by **Schermer (1967)**.

Biochemical analysis:

Lipids profile:

Determination of total cholesterol:

Serum total cholesterol was determined according to the colorimetric method described by **Thomas (1992)**.

Determination of serum triglycerides:

Serum triglyceride was determined by enzymatic method using kits according to the **Young, (1975) and Fossati, (1982)**.

Determination of high density lipoprotein (HDL-c):

HDL-c was determined according to the method described by **Friedewaid (1972) and Grodon and Amer (1977)**.

Calculation of very low density lipoprotein cholesterol (VLDL-c):

VLDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** using the following formula:

$$\text{VLDL-c (mg/dl)} = \text{Triglycerides} / 5$$

Calculation of low density lipoprotein cholesterol (LDL-c):

LDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** as follows:

$$\text{LDL-c (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-c}$$

Determination of total lipids:

Determination of total lipids in serum was colorimetrically determined according to **Schmitt and Drevon (1964)**.

Liver functions:

Determination of serum alanine amino transferase (ALT), serum asparatate amino transferase (AST), serum alkaline phosphatase (ALP) were carried out according to the method of **Hafkenscheid (1979)** , **Clinica Chimica Acta (1980)**, and **Moss (1982)**, respectively.

Kidney functions:

Determination of serum urea:

Serum urea and serum creatinin were determined by enzymatic method according to (**Henry (1974)** and **Patton & Crouch 1977**).

Determination of blood glucose:

Enzymatic determination of plasma glucose was carried out calorimetrically according to the method of **Tinder (1969)**.

Statistical analysis:

The data were analyzed using a completely randomized factorial design (**SAS, 1988**) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

RESULTS AND DISCUSSION

Data given in Table (1) show the changes of body weight, feed intake and feed efficiency ratio of diabetic rats fed diet supplemented with cinnamon, cloves oils and their mixture. The obtained results showed that the body weight gain (BWG) g/28 day of negative control recorded the higher

value when compared with positive control with significant difference. The mean values were 23.60 and 22.31 g/28 day, respectively. From diabetic rat groups, it is clear to notice that the highest (BWG) % recorded for 0.25 % cinnamon oil, while the lowest BWG% recorded for 0.25 % cloves oil with significant difference. The mean values were 26.37 and 16.99 g/28 day, respectively.

In case of feed intake, it could be notice that the feed intake (FI) g/ day of positive control recorded the higher value when compared with negative control with significant difference. The mean values were 18.46 and 18.39 g/ day, respectively. While, 0.25 % cinnamon oil recorded the higher FI while the lowest value recorded for 0.25 % cloves oil with significant difference. The mean values were 20.29 and 16.07 g/ day, respectively.

On the other hand, feed efficiency ratio (FER) of negative positive control recorded the higher value when compared with positive control with significant difference. The mean values were 0.046 and 0.043 %, respectively. In case of treated rat groups, it clear to mention that 0.5 % cinnamon oil recorded the higher FER while, the lower value recorded for 0.25% cloves oil and 0.5% mixture oils. The mean values were 0.057 and 0.038 %, respectively. These results are in agreement with **Kota et al., (2012)**, they found that there were an association between hyperglycemia and decrease body weight of diabetic animals, diabetes induced reduction in body weight, and the body's inability to store or use glucose causes hunger and weight loss.

On the other hand, GOT liver enzyme of positive control rats group recorded the higher value when compared with negative control group with significant difference. The mean values were 54.82 and 8.22 U/L, respectively. While, the highest GOT liver enzyme of treated group recorded for group fed on 0.25 % cinnamon oil fruits but, the lowest value recorded for group fed on 0.5% mixture oils with significant difference. The mean values were 38.4 and 11.21 U/L, respectively.

In case of GPT liver enzyme of positive control rats group recorded the higher value when compared with negative control group with significant difference. The mean values were 19.70 and 5.50 U/L, respectively. While, the highest GPT liver enzyme of treated group recorded for group fed on 0.5 % cinnamon oil but, the lowest value recorded for group fed on 0.5% mixture oils with significant difference. The mean values were 9.93 and 5.0 U/L, respectively. These results are in agreement with, they reported that **Longe et al., (2015)**, they found that the extract of cinnamon oil caused significant decrease ($P<0.05$) in the activity of AST, ALT and ALP values.

The effect of cinnamon oil, cloves oils and their mixture on the serum lipid profiles of diabetic rats are shown in Table (4). The obtained results indicated that the triglyceride of positive control group recorded the higher value when compared with negative control group with significant difference. The mean values were 221.2 and 81.30 mg/dl, respectively. While, the lowest triglyceride recorded for group fed on 0.25 % cinnamon oils while the higher value recorded for 0.5% mixture oils with significant

difference. The mean values were 159.10 and 69.50 mg/dl, respectively. In the other hand, the cholesterol levels of positive control group recorded the higher value when compared with negative control group with significant difference. The mean values were 97.0 and 63.5 mg/dl, respectively. While, the lowest cholesterol levels recorded for group fed on 0.5 % mixture oils while the higher value recorded for 0.5% cinnamon oil with significant difference. The mean values were 64.6 and 82.6 mg/dl, respectively. These results are in agreement with **Blevins et al., (2007)**, they reported that cinnamon extract lowered plasma TC, TG, LDL-C levels and increase HDL-C concentrations in the treated rats, and this could account for its use in traditional medicine for the treatment of diabetes and hypertension.

Data presented in Table (5) show the effect of cinnamon oil, cloves oils and their mixture on the serum lipid profiles of diabetic rats. The results indicated that the HDL-c of negative control rats group recorded the higher value when compared with positive control group with significant difference. The mean values were 53.30 and 23.30 mg/dl, respectively. While, the higher HDL-c of treated group recorded for group fed on 0.5 % mixture oils but, the lowest value recorded for group fed on 0.25% cinnamon oil with significant difference. The mean values were 41.0 and 27.30 mg/dl, respectively.

On the other hand, the LDL-c of positive control rats group recorded the higher value when compared with negative control group with significant difference. The mean values were 29.06 and 13.94 mg/dl, respectively. While, the highest LDL-c of treated group recorded for group fed on 0.25%

0.5% mixture oils with significant difference. The mean values were 58.27 and 46.25 mg/dl, respectively.

On the other hand, the uric acid level of positive control rats group recorded the higher value when compared with negative control group with significant difference. The mean values were 4.97 and 2.11 mg/dl, respectively. While, the higher uric acid level of treated group recorded for group fed on 0.25% cinnamon oil but, the lower value recorded for group fed on 0.5% mixture oils with significant difference. The mean values were 3.77 and 1.95 mg/dl, respectively.

In case of creatinine, the level of positive control rats group recorded the higher value when compared with negative control group with significant difference. The mean values were 1.13 and 0.87 mg/dl, respectively. While, the highest creatinine level of treated group recorded for group fed on 0.25 % cloves oil but, the lower value recorded for group fed on 0.5% mixture oils with significant difference. The mean values were 0.99 and 0.89 mg/dl, respectively. These results are in agreement with **Hassanen, (2010)**, they reported that 300 and 600 mg/kg of clove oil showed significant decrease in the levels of serum creatinine and urea as compared to untreated diabetic rats. The mean values of serum creatinine and urea were decrease gradually with increasing the dose of clove oil.

Table (1): Effect of cinnamon and cloves oil and their mixture on body weight, feed intake and feed efficiency ratio of diabetic rats

Groups	BWG (g/28 day)	FI (g/day)	FER (%)
	Mean ± SD	Mean ± SD	Mean ± SD
G1 control (-)	23.60 ^c ±0.40	18.39 ^b ± 0.70	0.046 ^d ± 0.07
G2 Control (+)	22.31 ^d ±0.20	18.46 ^b ± 0.60	0.043 ^d ± 0.02
G3+ 0.25% cinnamon oil	27.37 ^a ±0.80	20.29 ^a ±1.00	0.048 ^c ± 0.05
G4 +0.5% cinnamon oil	25.78 ^b ± 0.60	16.21 ^c ±0.20	0.057 ^a ±0.03
G5 + 0.25% cloves oil	16.99 ^f ±0.90	16.07 ^c ± 0.50	0.038 ^e ±0.04
G6 + 0.5% cloves oil	20.46 ^c ±0.50	18.96 ^b ±0.70	0.039 ^e ±0.08
G7+ 0.25% Mixture oil	20.80 ^c ±0.30	19.29 ^a ±0.40	0.039 ^e ±0.05
G8+0.5% Mixture oil	20.50 ^c ±0.60	19.46 ^a ±0.50	0.038 ^e ±0.04
LSD P≤0.05	1.092	1.18	0.009

BWG=Body weight gain, FI=Feed intake, FER =Feed efficiency ratio.

Each value is represented as mean ± standard deviation (n = 3).

Mean with the same letters in the same horizontal column are not significantly different at P≤0.05.

Table (2): Effect of Effect of cinnamon and cloves oil and their mixture on glucose level of diabetic rats

Groups	Glucose (mg/dl)
G1 control (-)	108 ^d ± 0.70
G2 Control (+)	230 ^a ± 1.10
G3+ 0.25% cinnamon oil	111.7 ^d ± 0.50
G4 +0.5% cinnamon oil	115.2 ^c ± 0.80
G5 + 0.25% cloves oil	124.5 ^b ± 0.90
G6 + 0.5% cloves oil	119.0 ^c ± 0.40
G7+ 0.25% Mixture oil	109.21 ^d ± 0.80
G8+0.5% Mixture oil	97.53 ^e ± 0.90
LSD P≤0.05	4.53

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Table (3): Effect of cinnamon and cloves oil and their mixture on (ALP), (GOT) and (GPT) of diabetic rats

Groups	(ALT) U/L	(GOT) U/L	(GPT) U/L
G1 control (-)	93 ^f ± 1.70	8.22 ^g ± 1.10	5.50 ^f ± 0.80
G2 Control (+)	175 ^a ± 0.90	54.82 ^a ± 1.35	19.70 ^a ± 0.40
G3+ 0.25% cinnamon oil	133 ^b ± 2.10	38.4 ^b ± 2.05	8.20 ^c ± 1.20
G4 +0.5% cinnamon oil	121 ^c ± 0.50	30.0 ^c ± 0.60	9.93 ^b ± 0.90
G5 + 0.25% cloves oil	103 ^d ± 1.10	26.15 ^d ± 1.25	7.81 ^c ± 0.50
G6 + 0.5% cloves oil	97 ^e ± 0.80	16.21 ^e ± 0.90	7.4 ^d ± 0.60
G7+ 0.25% Mixture oil	95 ^e ± 0.30	15.21 ^e ± 0.90	7.0 ^e ± 0.60
G8+0.5% Mixture oil	87 ^g ± 0.50	11.21 ^f ± 0.90	5.0 ^f ± 0.60
LSD P≤0.05	2.3	2.23	1.15

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Table (4): Effect of cinnamon and cloves oil and their mixture on serum cholesterol and triglyceride of diabetic rats

Groups	TG (mg/dl)	TC (mg/dl)
	Mean ± SD	Mean ± SD
G1 control (-)	81.3±2.61 ^h	63.5±2.28 ^f
G2 Control (+)	223.2±2.83 ^a	97.0±3.69 ^a
G3+ 0.25% cinnamon oil	169.1±2.83 ^b	82.6±3.41 ^b
G4 +0.5% cinnamon oil	153.5±3.16 ^c	75.6±3.35 ^c
G5 + 0.25% cloves oil	145.9±3.22 ^d	74.6±3.35 ^c
G6 + 0.5% cloves oil	135.9±3.30 ^e	70.0±3.35 ^d
G7+ 0.25% Mixture oil	115.9±3.15 ^f	67.5±2.61 ^e
G8+0.5% Mixture oil	95.5±3.23 ^g	64.6±1.30 ^f
LSD P≤0.05	3.67	1.27

Each value is represented as mean ± standard deviation (n = 3).

TG= Triglyceride. TC= Total Cholesterol,

Mean with the same letters in the same horizontal column are not significantly different at P≤0.05

Table (5): Effect of cinnamon and cloves oil and their mixture on the serum lipid profiles of diabetic rats

Groups	HDL-C (mg/dl)	LDL-C (mg/dl)	(VLDL _C) (mg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD
G1 control (-)	53.3±2.61 ^a	13.94±3.16 ^b	16.26 ^a ± 0.69
G2 Control (+)	23.3±3.16 ^f	29.06±2.28 ^a	44.64 ^a ± 1.20
G3+ 0.25% cinnamon oil	27.3±2.99 ^e	11.48±2.98 ^b	33.82 ^b ± 1.72
G4 +0.5% cinnamon oil	28.5±3.42 ^e	8.30±2.28 ^c	30.70 ^b ± 0.90
G5 + 0.25% cloves oil	35.0±2.20 ^d	5.72±2.28 ^c	29.18 ^c ±2.20
G6 + 0.5% cloves oil	38.5±2.31 ^c	2.32±2.13 ^d	27.18 ^c ±4.50
G7+ 0.25% Mixture oil	34.1±2.42 ^d	10.22±1.16 ^b	23.18 ^c ±2.10
G8+0.5% Mixture oil	41.0±2.53 ^b	4.50±1.10 ^c	19.10 ^f ±2.10
LSD P≤0.05	2.63	3.02	3.01

HDL-C= High density lipoprotein Cholesterol. LDL =Low density lipoprotein Cholesterol

Each value is represented as mean ± standard deviation (n = 3).

Mean with the same letters in the same horizontal column are not significantly different at P≤0.05

Table (6): Effect of cinnamon and cloves oil and their mixture on kidney functions of diabetic rats

Treatments Groups	Urea (mg/dl)	Uric acid (mg/dl)	Serum creatinine (mg/dl)
G1 control (-)	42.20 ^f ± 2.10	2.11 ^c ± 0.20	0.87 ^a ± 0.577
G2 Control (+)	73.65 ^a ± 3.20	4.97 ^a ± 0.90	1.13 ^a ± 0.115
G3+ 0.25% cinnamon oil	58.27 ^b ± 0.90	3.77 ^b ± 0.20	0.99 ^a ± 0.025
G4 +0.5% cinnamon oil	50.96 ^c ± 1.60	2.60 ^c ± 0.30	0.94 ^a ± 0.177
G5 + 0.25% cloves oil	52.27 ^c ± 0.20	3.50 ^b ± 0.20	1.03 ^a ± 0.105
G6 + 0.5% cloves oil	48.10 ^d ± 0.50	2.40 ^c ± 0.30	0.96 ^a ± 0.020
G7+ 0.25% Mixture oil	46.25 ^d ± 0.50 3.24	2.27 ^c ± 0.60 1.26	0.92 ^a ± 0.010 2.14
G8+0.5% Mixture oil	44.20 ^e ± 0.20	1.95 ^c ± 1.10	0.89 ^a ± 0.030
LSD P≤0.05	3.36	1.08	0.31

Each value is represented as mean ± standard deviation (n = 3).

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

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التأثير المضاد للسكر لزيت القرفة والقرنفل في الفئران المصابة بالسكر بتأثير الألوكسان

رهام البدراوى عبد الوهاب زهران

أخصائى تغذية بمستشفى نور الشروق – القاهرة

الملخص العربى

مرض السكر هو أكبر مرض في الغدد الصماء في العالم مرتبط بزيادة معدل المراضة والوفيات. يحتوي مرض السكري (DM) على مجموعة من الاضطرابات الأيضية الشائعة التي تتميز بارتفاع السكر في الدم. تم تقييم تأثير تركيزات مختلفة (٠,٢٥ , ٠,٥ ، %) من زيت القرفة ، زيت القرنفل ومزيجها على الفئران المصابة بمرض السكر. تم استخدام ٤٨ من ذكور الفئران البيضاء في هذه الدراسة وتم تقسيمها إلى ٨ مجموعات ، كل مجموعة تحتوي على ٦ فئران. تم إصابة الفئران بواسطة مادة الألوكسان بتركيز ١٥٠ مجم /كجم من وزن الفأر. تم تقدير وظائف الكبد (ALT ، GOT ، GPT)، الكوليسترول الكلى ، الدهون الثلاثية ، (HDL-c ، LDL-c ، VLDL-c)، مستوى الجلوكوز ، وظائف الكلى (اليوريا ، حمض اليوريك والكرياتينين) ، اظهرت النتائج المتحصل عليها من الفئران المصابة بالسكر أن مخلوط الزيوت بتركيز ٠,٥ % (القرفة والقرنفل) أظهرت تغييرات كبيرة في التحاليل الكيميائية الحيوية. كما استتجت الفئران المصابة بمرض السكر التي تمت معالجتها بمخلوط الزيوت بنسبة ٠.٥ إلى تحسن ملحوظ في مستوى الجلوكوز ، وصورة دهون الدم ، وظائف الكبد والكلى مقارنة بالتركيزات الأخرى.

الكلمات الكاشفة: زيوت التوابل - الفئران . التأثير المضاد للسكر . التحاليل الكيميائية الحيوية.