

THERAPEUTIC EFFECTS OF VOLATILE OILS AND ALCOHOLIC AQUEOUS EXTRACTS OF BASIL AND THYME LEAVES ON DIABETIC RATS

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ABSTRACT

The objective of this work to study the therapeutic effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves in blood glucose and lipid profile of alloxan- induced in diabetic rats. volatile oils were extracted by hydrodistillation and alcoholic aqueous extracts which had been obtained by using methanol. In the experiment animals, fifty rats were divided into ten groups (n=5). Rats were injected (i.p) with alloxan- monohydrate (100mg/kg b.w.).Animals showing fasting blood glucose higher than 300mg/dl were selected and used as diabetic rats. volatile oils and alcoholic aqueous extracts of basil and thyme were administered at dose levels of 200 and 300 mg/kg body weight orally. Scavenging effect on DPPH radicals were determined .Hypoglycemic and hypolipidemic effects of the extracts were evaluated by the determination of blood glucose, plasma insulin, total cholesterol, triglyceride, LDL-cholesterol, ALT, AST, creatinine, uric acid and bilirubin. Also, evaluated some blood antioxidant parameters by the determination of MDA, SOD and GSH.

The results showed high scavenging activity with a concentration of 1000 ug/mg where alcoholic aqueous extracts of basil leaves were the best ones (96.97 ±0.58), followed by BHT (95.9 ±3.18) then by alcoholic aqueous extracts of thyme leaves (94.2 ± 58).The results revealed that the group treated with volatile oils and alcoholic aqueous extracts of basil and thyme exhibited high levels of insulin in comparing with diabetic control (4.8Uu/ml). The rats group treated with basil oil(200mg),thyme oil (200mg) and basil oil(300mg)significant increase $P < 0.01$ of plasma insulin (9.01Uu/ml),(9.4 Uu/ml) and (9.51Uu/ml) respectively. The results showed that the diabetic rats receiving volatile oils and alcoholic aqueous extracts of basil and thyme had significantly lower levels of serum total cholesterol, triglyceride and LDL-cholesterol levels than those of diabetic control group. All treated groups showed significant increase in superoxide dismutase compared with diabetic control group.

Keywords: Hypoglycemia, hypolipidemia, DPPH, basil oil, thyme oil

INTRODUCTION

According to WHO projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 millions diabetics worldwide and this is likely to increase to 300 million or more by the year 2025.Statistical projection about India suggests that the number of diabetic will rise from 15 million in 1995 to 57million in the year 2025 making it the country with the highest number of diabetics in the world (King *et al.*,1998; Boyle *et al.*, 2001,Mohammed *et al.*, 2007).

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world. It is responsible for many

complications affecting various organs in the body. DM results in hyperglycemia in comparison with absolute insulin deficiency which characterized as type I DM or type II in insulin resistance due to receptor insensitivity to endogenous insulin (EL-Hilaly *et al.*, 2007, Aly, 2010)).

Sweet basil (*Ocimum basilicum* L.) family of Labiatae is widely used in cooking for its culinary attributes. They the investigated the hypocholesterolemic and hypotriglyceridemic activities of the basil aqueous extract were illustrated in high fat diet-induced hyperlipemicrats. Hyperlipemia was developed by a high fat diet containing cholesterol, lard and cholic acid. Sweet basil caused a significant decrease on plasma and liver total cholesterol and triglyceride. Similar result was observed on plasma LDL-cholesterol concentrations. Furthermore, the basil extract shows a significant ameliorative action on elevated atherogenic index (AI) and LDL/HDL-C ratio levels(Harnafi *et al.*, (2009)).

The oils of *O. basilicum* and *O. gratissimum* from different locations showed chemical variation, antifungal activity, free radical scavenging capacity and antimycotoxicogenic property. These properties are attributed to the phenolic compound eugenol(Dambolena *et al.*, (2010)).

Nutraceutical properties of basil (*Ocimum basilicum* cv. *Genova*) grown in hydroponics in comparison with that grown in soil.Owing to the traditional phytotherapeutic use of basil and its importance as a basic component of the Mediterranean diet. The antioxidant activities of aqueous and lipid extracts of basil leaves were evaluated both by spectrophotometric detection with the 2, 20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS+) and by electron paramagnetic resonance (EPR) detection with the stable radicals peroxyaminedisulphonate (Fremy's salt, hydrophilic) and 1,1-diphenyl-2-picrylhydrazyl (DPPH, lipophilic). From EPR decay kinetics analysis, it was possible to distinguish (in the lipid extract) a fast rate constant and a slow rate constant, likely attributable to two different kinds of lipophilic antioxidants. Hydroponic cultivation improved antioxidant activity of both aqueous and lipid extracts, increasing the contents of vitamin C, vitamin E, lipoic acid, total phenols and rosmarinic acid (Sgherri *et al.*, 2010)).

Basil is believed to have significant health benefit. The leaves of African varieties are said to contain thymol oil which is regarded as highly antiseptic and it is also used to prevent mosquito bite(Agnaniet *et al.*, 2005).

Traditionally, basil has been used as a medicinal plant in the treatment of headache, diarrhea, wart, worms and kidney function (Seung-Jo-Lee *et al.*, 2004).

Thyme (*Thymus vulgaris* L.) belonging to the Lamiacea family is often added to meat, fish and food products and also used as herbal medicinal products. Thyme essential oil and its ingredients have been shown to exhibit a range of biological activities such as thymol and carvacrol can be used alone or in combination for the treatment of oral infectious diseases because of their inhibitory activity on oral bacteria. Also, thyme was found to exert antimycotic activity and thereby inhibit aflatoxin production European Medicines Agency (EMA), (2007); Faleiro *et al.*, (2005); Basch *et al.*, (2004); Kohlert *et al.*, (2002) and Ditry *et al.*, (1994).

Leafy parts of thyme and its essential oil have been used in foods for the flavour, aroma, preservation and also in folk medicines. The genotoxicity of thymol and carvacrol was examined using comet assay. Thymol and carvacrol displayed a concentration dependent antioxidant capacity. There was a lack of clastogenic activity for thymol and carvacrol at biologically relevant concentrations, and a moderate antioxidant activity in vitro Undeger *et al.*, (2009).

According, this study was carried out to determine the therapeutic effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on plasma glucose and lipids patterns of diabetic rats.

MATERIALS AND METHODS

Samples preparation:

Green plant leaves of basil (*OcimumBasilicum*), and thyme (*Thyme Vulgaris*) were obtained from Agriculture Research Center, Giza, Egypt. They were cleaned, air dried and grinded in a blender. The powder of each sample was kept in a polyethylene bags and preserved in deep freezer until use.

Gross chemical composition of basil and thyme.

Extraction of experimental essential oils

The dry leaves of basil and thyme were ground by domestic model electronic mixer. Each sample was subjected to hydrodistillation apparatus in a Clevenger type apparatus for 6 hours according to the method recommended by European pharmacopeia procedure, (1983). Oils (yield 1% and 3%, respectively) with characteristic odor and sharp taste were obtained. The oils were dried over anhydrous sodium sulphate to remove traces of moisture and stored in refrigerator in dark at 4°C until use.

Extraction of the alcoholic aqueous extracts:

The extraction procedure for the hydro-alcoholic extract was carried out according to Charles *et al.*, 1993. About 250 grams of each milled plant samples were macerated in 500 ml of methanol over night at room temperature, then filtered and the methanolic crude extract was collected. Another portion of 500 ml of methanol were added to the plant residue and boiled for two hours reflux condenser in a water bath and then filtered. The filtered was collected to the previous crude extract. In the same manner 500 ml portion of water were added to the residue plant and left at room temperature overnight, then filtered.

The filtrate was added to the previous crude extract. Another volume of water was added to the residue, boiled for two hours under reflux condenser and filtered. The hot water filtrate and the methanolic crude extract obtained previously were gathered to form the hydro-alcoholic crude extract. The solvents were evaporated under vacuum using rotary evaporator. The crude extract was obtained, kept in dark bottles and stored in a deep freezer until use.

Experimental, Biological Evaluation:

Animals:

Adult male white rats weighing (69-74) were used in this study. All animal were kept under standardized conditions (12h light/ dark cycle, 22°C) and were provided free access to standard diet (Table1) and water according to (NRC,1995). Half of the rats were subjected to alloxan monohydrate to be diabetic.

Table (1): Composition of the standard diet.

Ingredients	g/kg diet
Casein	200
Corn starch	497
Sucrose	100
Vitamin mixture	020
Mineral mixture	100
Corn oil	050
Cellulose	030
Methionine	003

Induction of Diabetes:

Rats were injected (i.p) with alloxan- monohydrate (BDH) (100mg/kg b.w.) dissolved in normal saline. Seven days after alloxan administration, blood was collected from the rat eye by means of Haematocrit tubes in EDTA tubes. Plasma was separated by centrifugation and analysed for blood glucose. Animals showing fasting blood glucose higher than 300mg/dl were selected and used as diabetic rats.

Preliminary phytochemical screening of crude extracts of volatile oils and alcoholic aqueous extracts of leaves basil and thyme.

Detection of tannins and resins:

Tannins and resins were detected in the plant sample according to the method of El-Badrawy (1996).

Detection of saponins:

Saponins substances were detected in different crude extracts under investigation according to the method of Trease(1961).

Detection of terpenes:

Terpenes substances were detected in different crude extracts under investigation according to the method of Finar (1968).

Detection of flavonoids:

Flavonoids substances were detected in extracts of different samples using the method of Geissman (1962).

Detection of carbohydrates and glycosides:

Carbohydrates and glycosides were treated by Molish test according to the method of Blabaa *et al.*, (1976).

Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

The effect of basil and thyme on DPPH radical was studied, employing the modified method described earlier by Yamaguchi, *et al.*, (1998). Briefly, 1.5 ml of DPPH solution (0.1 mM, in 95% Ethanol)was incubated with varying concentrations of the extract (potato and apple peels, 0.75 - 5.0 mg).The

reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:
Scavenging effect % = $1 - \frac{A \text{ Sample (517nm)}}{A \text{ Control (517nm)}} \times 100$

Experimental design:

Experiment:

This experiment was designated to study the therapeutic effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on plasma glucose, insulin and lipids profile levels in diabetic rats. Also, evaluated some blood antioxidant parameters by the determination of superoxide dismutase (SOD), malondialdehyde (MDA), and Glutathione (GSH). Also, for studying its effect on body weight, contained normal rats, while the test groups were diabetic as follows:

In this experiment fifty rats were divided into ten groups (n=5), one of them Group1: Normal untreated rats, received distilled water (2.5ml/kg)

Group2: Diabetic rats control.

Group3: Diabetic rats received of basil oil(200mg/kg).

Group4: Diabetic rats received of basil oil(300mg/kg).

Group5: Diabetic rats received of thyme oil(200mg/kg).

Group6: Diabetic rats received of thyme oil(300mg/kg).

Group7:Diabetic rats received alcoholic aqueous extracts of basil (200mg/kg) dissolved in distilled water 1 ml/kg.

Group8: Diabetic rats received alcoholic aqueous extracts of basil (300mg/kg) dissolved in distilled water 1 ml/kg.

Group9: Diabetic rats received alcoholic aqueous extracts of thyme (200mg/kg) dissolved in distilled water 1 ml/kg.

Group10: Diabetic rats received alcoholic aqueous extracts of thyme(300mg/kg) dissolved in distilled water 1 ml/kg.

At the end of 4 weeks, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes, one with anticoagulant (potassium oxalate and sodium fluoride) for plasma and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation.

Body weights of the rats were measured three times a week during four weeks. Daily changes in body weights as percentages were recorded. The percentage of daily changes in body weights was calculated according to the following formula:

Change in body weights (%) = $100 \times \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}}$

Food efficiency ratio (FER) was calculated at the end of experiment as following: FER= Body weight gain (gm) / Food intake (gm)

Biochemical Analysis:

Fasting blood glucose was estimated by an enzymatic colorimetric method according to (Siest *et al.*, 1981).

Plasma insulin level was assayed by Enzymatic Linked Immuno sorbent Assay (ELISA) Kit as described by Nakagawa *et al.*, (1973).

Total cholesterol, HDL-cholesterol and triglyceride content were determined by enzymatic colorimetric method according to Allian *et al.*, (1974); Richmond (1973) and Fossati and Principe (1982), respectively.

LDL-cholesterol and VLDL-cholesterol were calculated by the Friedewald Formula according to Friedewald (1972).

Bilirubin, Plasma alanine and aspartate aminotransferase enzymes activities (ALT and AST) were also determined according to the method of Reitman and Frankel (1957).

Plasma total protein was determined an enzymatic method according to Henry (1964).

Plasma uric acid was estimated an enzymatic method according to Trinder (1969).

Plasma creatinine was determined according to Henry (1974).

Determination of some antioxidant parameters

Superoxide dismutase (SOD) activity according to (Dechatelet *et al.*, 1974). Determination of malondialdehyde (MDA) in red blood cells RBCs by the method described by Stocks and Donnandy (1971). Glutathione (GSH) according to (Beutler ., 1984).

Statistical analysis:

Results of the biochemical estimations of the rats are reported as mean \pm S.E.M. (Standard Error of Mean). The total variation was analysed by performing one-way analysis of variance. "LSD (Least Significant Difference) test" was used for determining significance (Sümbüloğlu *et al.* 1998). Probability levels of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of volatile oils and alcoholic aqueous extracts of basil and thyme leaves.

The phytochemical screening of tannins, saponins, resins, terpenes, flavonoids and carbohydrates of volatile oils and alcoholic aqueous extracts of basil and thyme leaves were detected and recorded in Table (2).

It was noticed that tannins, saponins, resins, terpenes, flavonoids and carbohydrates were found in the volatile oil of basil, alcoholic aqueous extracts of basil and thyme.

On the other hand, data in Table (2), revealed that, terpenes, flavonoids and carbohydrates were found in the volatile oil of thyme. Basil and thyme are a rich source of phenolic phytochemicals having high antioxidant activity Dragland *et al.*, (2003) and Naghibi *et al.*, (2005) .

Table (2) : Preliminary phytochemical screening of volatile oils and alcoholic aqueous extracts of leaves basil and thyme.

Type of extract	Tannins	Saponins	Resins	Terpenes	Flavonoids	Carbohydrates
Basil oil	+	+	+	+	+	+
Thyme oil	-	-	-	+	+	+
Basil extract	+	+	+	+	+	+
Thyme extract	+	+	+	+	+	+

+ Present

- Absent

Scavenging effect (%) of alcoholic aqueous extracts of basil and thyme leaves on (DPPH) radical.

Antioxidative properties of aqueous plant extracts were evaluated using common methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method. The antiradical activity of the extracts against the DPPH radical expressed as the antiradical power , it reveals the order of oregano , lemon balm , thyme Heilerova *et al.*, (2003).

The free-radical scavenging and ferric-reducing antioxidant properties of acetonic and methanolic extracts of (*O. basilicum*) basil, (*O. onites*) origanum and (*T. vulgaris*) thyme (Crete, Greece). Its 2,2 -diphenyl-1-picrylhydrazyl radical scavenging activity was (1.16 mg/mL) Lagouri and Nisteropoulout (2009).

The radical scavenging activity was assessed with DPPH test (Conforti, *et al.*, 2008) are shown in Table (3) extracts showed high scavenging activity with a concentration of 1000 ug/mg where alcoholic aqueous extracts of basil leaves were the best ones (96.97 ±0.58), followed by BHT (95.9 ±3.18) followed by aqueous extracts of thyme leaves(94.2 ± 58).The DPPH radical scavenging capacity of the tested extracts might be explained; partly, due to the presence of phenolic components Siddhuraju,(2002)and(Al-Bishri and Danial (2013)).

Table (3) : Scavenging effect (%) of alcoholic aqueous extracts of basil and thyme leaves on (DPPH) radical.

Type of extract	1000ug/ml	500 ug /ml	250 ug /ml
BHT	95.9 ± 3.18	89.0 ± 2.56	75.6 ± 2.94
Extract of basil	96.97 ± 0.58	90.14 ± 2.90	70.87 ± 0.58
Extract of thyme	94.2 ± 58	86.35 ± 0.59	68.04 ± 59

Scavenging effect (%)of volatile oils of basil and thyme leaves(DPPH).

The radical scavenging activity of oils on samples under study is shown in Table (4), the scavenging activity of basil oil at concentration of 20 ul, 15 ul and 10 ul were (82.97%),(76.14 %) and (70.87%) respectively. On the other hand, the scavenging activity of thyme oil at concentration of 20 ul, 15 ul and 10 ul were (79.2%), (69.3 %) and (55.4%) respectively. The essential oil extracted from the basil cultivars was the highest antioxidant

activity which found in the Sweet basil essential oils Juliani and Simon (2002).

Table (4): Scavenging effect (%)of volatile oils of basil and thyme leaves(DPPH).

Type of oil	20 ul	15 ul	10 ul
Basil oil	82.97 ± 0.2.6	76.14 ± 2.7	70.87 ± 2.0
Thyme oil	79.2 ±1.7	69.3 ± 0.9	55.4 ±0 9

Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on body weight gain, food intake and food efficiency ratio of diabetic rats.

Data in table (5) shows that the final weight of all the rats groups increased at the end the experimental. Among the treated group, the highest weight gain% was noticed in the diabetic groups received basil oil (200mg), basil oil(300mg) and thyme oil (300mg)were (89.9%), (89.8%), (85.9%) respectively.

On the other hand, data in table (5) showed that food efficiency ratio of diabetic control was(2.89), decreased significantly with all diabetic rats, the highest significant were basil oil(300mg) was (5.43), basil oil (200mg) was(5.35) and thyme alcoholic aqueous extract. (200mg/kg) was (5.1). These results agreed with (Ozlem *et al.*, 2005) who reported that, body weight was significant lower in rats with streptozotocin diabetes than in the control group during the experiment, administration of glibornuide for 28 days caused an increase in body weights in the diabetic groups.

Table (5): Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on body weight gain, food intake and food efficiency ratio of diabetic rats.

Groups	Initial weight(g)	Final weight(g)	weight gain(g)	weight gain %	Daily food intake	food efficiency ratio
Control -	70.3±2.7	149.40±20.7	79.1.0±8.27	112.53	13.66±3.63	5.75±1.81***
Diabete control	72.1±3.49	103.94±15.3	31.8±5.32*	44.14	10.28±2.97	2.89±0.80
Basil oil(200mg/kg)	71.5±2.9	135.8±18.2	64.3±7.2***	89.9	12.0±3.6	5.35±1.3***
Thyme oil (200mg/kg)	71.8±2.7	131.7±21.7	59.99±7.31*	83.55	12.0±4.1	4.9±1.8**
Basil oil(300mg/kg)	69.9±3.5	132.7±19.7	62.8±6.1**	89.8	11.55±2.3	5.43±1.0***
Thyme oil (300mg/kg)	70.1±2.1	130.3±19.8	60.23±6.2**	85.9	12.3±3.8	4.8±1.8**
Basil ex. (200mg/kg)	70.4±2.3	125.11±17.7	54.7±6.7	77.71	11.12±3.39	4.9±1.7**
Thyme ex. (200mg/kg)	72.4±3.1	129.7±18.5	57.3±8.1*	79.15.	11.4±2.6	5.1±1.9***
Basil ex. (300mg/kg)	73.8±4.4	119.8±20.3	46.07±7.11	62.42	11.2±4.2	4.1±1.1**
Thyme ex (300mg/kg).	70.8±2.5	120.7±17.9	50.07±0.9	70.8	11.7±2.7	4.2±1.2**

Each value is the mean+ SD of 5 rat.

Significant with control group

*p< 0.05 ** P< 0.01

***P< 0.001.

Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on serum lipids pattern of diabetic rats.

Administration of alloxan to normal rats resulted in a significant increase in serum total cholesterol, triglyceride and LDL-cholesterol levels as shown in diabetic control Table (6), the results recorded in the Table (6) showed that the diabetic groups receiving volatile oils and alcoholic aqueous extracts of basil and thyme parts had significantly lower levels of serum total cholesterol, triglyceride and LDL-cholesterol levels than those of diabetic control group.

Concerning total cholesterol, data presents in Table (6) revealed that the groups treated with volatile oils and alcoholic aqueous extracts of basil and thyme decrease significantly of total cholesterol levels in comparing with diabetic control (110.0mg/dl). The highest significant $P < 0.01$ in rats treated with basil oil(300mg), thyme oil (300mg), basil oil(200mg) and basil alcoholic aqueous extracts (300mg) were (68.4mg/dl, 75.0mg/dl, 76.80mg/dl and 77.0mg/dl) respectively. While diabetic rats received alcoholic aqueous extract of basil. (200mg) was(80.8mg/dl)($p < 0.05$). Results in Table (6), indicated that all the test groups diabetic receiving volatile oils and alcoholic aqueous extracts of basil and thyme revealed significant decreases in triglyceride and LDL-cholesterol levels in comparing with that of diabetic control the reduction percentage were basil oil(300mg)46.3%, basil alcoholic aqueous extracts. (300mg) 44.9 % and basil oil (200mg) 43.1% for triglyceride, and basil oil(300mg) 34.5% and basil oil(200mg)30.9% for LDL-cholesterol, from these percentages values, it was clear that the difference between the highest and lowest percentage is low, so it is advised by giving volatile oils of basil and thyme to the patients with hyperlipidemia or those exposed to atherosclerosis.

These results agreed with Harnafi *et al.*, (2009) who found that sweet basil caused a significant decrease on total cholesterol and triglyceride. Similar result was observed on plasma LDL-cholesterol concentrations. On the other hand, significant increase in HDL-cholesterol for diabetic rats receiving basil oil (300 and 200mg) were 34.0 and 33.3 mg/dl respectively, thyme oil (300mg) was 32.7mg/dl and basil alcoholic aqueous extracts. (300mg) was 32.0 mg/dl. when compared with diabetic control (23.0mg/dl). The hypolipidaemic activity of basil (*Ocimum basilicum* L.) family in mice could be attributed to the presence of valuable polyphenolic compounds.

Data in Table (6) revealed no significant differences in VLDL-c of the diabetic groups. Results in Table (6), indicated that all the test diabetic groups receiving volatile oils and alcoholic aqueous extracts of basil and thyme showed decrease in atherogenic indexes (CHO / HDLc and LDLc / HDLc) than those of diabetic control group. These results are in accordance with those found by Harnafi *et al.*, (2008), who demonstrated that basilicum may contain polar products able to lower plasma lipid concentrations and might be beneficial in treatment of hyperlipidemia and atherosclerosis

Ocimum basilicum aqueous extract displayed a very high antioxidant power and hypolipidaemic Amrani *et al.*, (2006). *Ocimum basilicum* extract

Concerning creatinine, data presented in Table (7) revealed that significant decrease ($P < 0.01$), in alloxan diabetic rats receiving basil oil (300 and 200mg) were 1.7 and 1.86 respectively, than diabetic control 2.64.

Data in Table (7) revealed a significant decrease ($P < 0.01$) and ($p < 0.05$) in uric acid levels of alloxan diabetic groups treated with volatile oils and alcoholic aqueous extracts of basil and thyme when compared with diabetic control.

On the other hand, it was found that significant increase in bilirubin for control diabetic rats caused this ratio, by about 2 folds than that the group treated with volatile oils and aqueous extracts of basil and thyme. It was likely that the antioxidant activity of the extracts produced better response in such stressful conditions compared to normal (non-diabetic) condition (Sushruta *et al.*, 2006). These results of treatment effects of basil and thyme leaves oils or alcoholic aqueous extracts on some renal function represented in creatinine, uric acid, bilirubin. It is also possible to suggest that these extracts might directly improve the structural and functional integrities of cells of the blood, liver and kidney.

Table (7): Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on serum ALT, AST, urea, creatinine, uric acid and bilirubin of diabetic rats.

Groups	ALT	AST	Urea	Creatinine	Uric Acid	Bilirubin
Control -	12.6 ±5.85	28.40±4.8	12.2±2.9	0.8±0.10	2.8±0.38	0.45±0.03
Diabetic control	30.8±4.29	45.4±3.50	29.70±3.2	2.64±0.15	4.04±0.58	1.0±0.03
Basil oil(200mg\kg)	26.2±7.2	29.4±2.96*	24.2±3.32*	1.86±0.43**	2.8±0.5**	0.55±0.09**
Thyme oil (200mg\kg)	29.9±5.0	41.2±7.3	26.1±3.6	2.2±0.17	3.1±0.5	0.62±0.05
Basil oil(300mg\kg)	21.4±7.4**	26.0±3.93**	23.7±2.6**	1.7±0.11**	2.54±0.6**	0.44±0.06**
Thyme oil (300mg\kg)	28.1±7.8	39.6±7.2	25.2±3.4	1.92±0.21	3.2±0.4	0.57±0.03*
Basil ex. (200mg\kg)	25.1±6.2	38.9±6.1	25.1±3.7	2.1±0.18	2.94±0.35*	0.53±0.08**
Thyme ex. (200mg\kg)	29.3±4.9	38.2±5.4	27.1±2.8	2.3±0.2	3.4±0.3	0.7±0.04
Basil ex. (300mg\kg)	24.8±3.1*	34.7±8.2	23.25±2.4**	1.9±0.08	2.7±0.7**	0.47±0.07**
Thyme ex (300mg\kg).	27.8±6.7	34.6±4.9	25.4±3.7	2.02±0.2	3.0±0.8	0.56±0.07*

ALT:alanine aminotransferase enzymes AST:aspartate aminotransferase enzymes
Each value is the mean+ SD of 5 rats. Significant with control group * $p < 0.05$ ** $P < 0.01$

Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on blood glucose, plasma insulin of diabetic rats.

Data in table (8) revealed a significant elevation in fasting blood glucose and significant decrease in plasma insulin level of diabetic control when compared with all the rats

Concerning plasma insulin, data presents in Table (8) revealed that the groups treated with volatile oils and alcoholic aqueous extracts of basil

and thyme exhibited high levels of insulin in comparing with diabetic control (4.8Uu/ml). The groups treated with basil oil(200mg),thyme oil (200mg) and basil oil(300mg)significant increase $P < 0.01$ of plasma insulin (9.01Uu/ml),(9.4 Uu/ml) and (9.51Uu/ml) respectively.

As evident from Table (8) significant decrease in fasting blood glucose was observed in the diabetic groups treated with volatile oils and alcoholic aqueous extracts of basil and thyme when compared with diabetic control.

Among the test groups blood glucose was lowest in diabetic rats receiving basil oil(300mg)was(209.4±42.69mg/dl) followed by diabetic rats receiving basil oil(200mg)was (228.14±35.7mg/dl), which differed significantly from diabetic control (329.4±63.50mg/dl).

Rats with diabetes induced by streptozotocin (Heibashy, 2005) or alloxan(Ye *et al.*, 2002), reduced fasting blood glucose and HbA1C when rats fed on the tested therapeutic plant origins (Lima *et al.*, 2006; Eidi and Eidi, 2009). Free radicals and the associated oxidative stress play an important role in the cause and subsequent complication of diabetes mellitus. While administration of free radical scavenging activity of basil and thyme may be useful in controlling glucose levels in diabetic rats.

Table (8): Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on blood glucose, plasma insulin of diabetic rats.

Groups	Insulin(µl l)	Fasting blood glucose (mg/dl)
Control -	12.6 ±1.85	113.40±15.8
Diabetic control	4.8±0.99	329.4±63.50
Basil oil(200mg/kg)	9.01±1.2**	228.14±35.7*
Thyme oil (200mg/kg)	9.4±1.8**	299.2±67.3
Basil oil(300mg/kg)	9.51±1.1**	209.4±42.96**
Thyme oil (300mg/kg)	8.9±2.1*	269.6±47.2
Basil ex. (200mg/kg)	7.33±2.2*	268.9±46.1
Thyme ex. (200mg/kg)	8.3±4.9*	278.2±35.4
Basil ex. (300mg/kg)	7.22±2.4*	255.7±48.2
Thyme ex (300mg/kg).	7.8±6.7*	267.6±24.9

Each value is the mean+ SD of 5 rats.

Significant with control group

* $p < 0.05$ ** $P < 0.01$

Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on some blood antioxidant parameters.

Data in table (9) revealed decrease significantly in malondialdehyde (MDA) and a significant elevation in superoxide dismutase (SOD) and glutathione (GSH) were observed in the diabetic groups treated with volatile oils and alcoholic aqueous extracts of basil and thyme leaves when compared with diabetic control.

As evident from Table (9) significant decrease $p < 0.05$ in malondialdehyde (MDA) were observed in basil oil (300mg) was12.5±1.7and thyme oil (300mg) was13.4±1.09when compared with diabetic control was19.2±0.8. Data present in table (9) revealed increase significantly $P < 0.001$ in superoxide dismutase (SOD) was observed in basil oil(300mg)was0.75, thyme oil (300mg)was0.69, basil oil (200mg)was0.67,

basil alcoholic aqueous extract (300mg) was 0.66 and thyme oil (200mg) was 0.65 when compared with diabetic control was 0.26.

Concerning glutathione (GSH), Data presents in Table (9) revealed that increase significantly ($P < 0.01$) in glutathione (GSH) was observed in basil oil (300mg) was 7.7 in comparing with diabetic control 4.22, also a significant increase $p < 0.05$ were observed in basil alcoholic aqueous extract (300mg) was 6.66, basil oil (200mg) was 6.25, thyme alcoholic aqueous extract (300mg) was 6.17, basil alcoholic aqueous extract (200mg) was 6.16 and thyme oil (300mg) was 6.07 in comparing with diabetic control 4.22.

Basil leaf extract was very effective in elevating antioxidant enzyme response by increasing significantly the hepatic glutathione reductase (GR), superoxide dismutase (SOD), and catalase activities. Reduced glutathione (GSH), the major intracellular antioxidant, showed a significant elevation in the liver and also in all the extrahepatic organs Dasgupta *et al.*, (2004). Thyme was reflected on marked decrease of MDA and increase of GSH and HDL-C El-Sheikh (2008). Blood glutathione (GSH) levels increased more so in test group compared with control group on stress induction.

The activity of SOD increased significantly in both test and control group on stress induction, whereas activities of Px and CAT decreased following NDEA treatment, and the effects were of lower degree in test group Rana and Soni (2008). Rats fed with thyme essential oil showed higher activities of liver endogenous enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as an increase of total antioxidant status compared to the control group Vitaglione *et al.*, (2004).

Table (9): Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on some blood antioxidant parameters.

Groups	MDA U/mL	SODmg/L	GSH U/mL
Control -	11.26±1.4	0.54±0.12	9.27±0.6
Diabetic control	19.2±0.8	0.26±0.09	4.22±1.54
Basil oil (200mg/kg)	14.0±1.2	0.67±0.05***	6.25±0.03*
Thyme oil (200mg/kg)	14.45±2.5	0.65±0.17***	5.66±0.79
Basil oil (300mg/kg)	12.5±1.7*	0.75±0.2***	7.7±0.88**
Thyme oil (300mg/kg)	13.4±1.09*	0.69±0.07***	6.07±1.5*
Basil ex. (200mg/kg)	15.6±0.9	0.63±0.08***	6.16±0.69*
Thyme ex. (200mg/kg)	14.57±1.2	0.53±0.2**	5.58±1.84*
Basil ex. (300mg/kg)	14.5±1.2	0.66±0.12***	6.66±0.77*
Thyme ex (300mg/kg).	14.2±2.5	0.58±0.6**	6.17±0.85*

MDA: malondialdehyde SOD: superoxide dismutase GSH: glutathione

Each value is the mean ± SD of 5 rats.

Significant with control group * $p < 0.05$

** $P < 0.01$ *** $P < 0.001$.

Increased concentration of MDA level was observed in liver and kidney of diabetic control group when compared to diabetic groups treated with extracts or standard drug and with normal control group. (Wilson *et al.*, (2001), Ugwu *et al.*, (2013)) have reported that the concentration of lipid peroxides, increases in the kidney of diabetic rats. This present work shows that administration of *Ocimum basilicum* leaf extracts tends to bring the kidney and liver MDA back to normal. The results of SOD and CAT activities

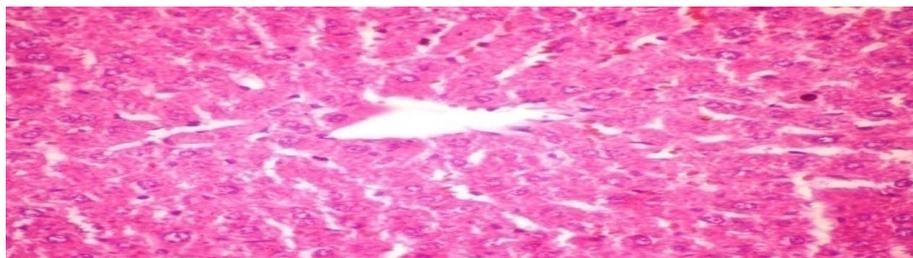
clearly showed that *Ocimum basilicum* leaf extracts contain a free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of $\cdot\text{O}_2$ -and OH^* . This action, predominantly due to the extract, could involve mechanism related to scavenging activity. *Ocimum* species has been extensively reported for its essential oil content (Roberto *et al.*, 2003).

Effect of volatile oils and alcoholic aqueous extracts of basil and thyme leaves on liver in diabetic rats .

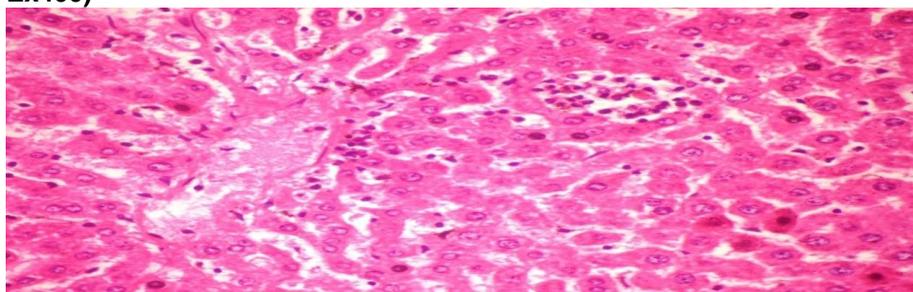
Pathological examination of rats liver showed that alloxan-mono hydrate (BDH) induced focal hepatic necrosis associated with inflammatory cells infiltration, of diabetic control (+ve) group Pic. (2). The liver of the group 1, normal control- rats showing the normal histological structure of hepatic lobule Pic. (1). The liver of the group 3, diabetic rat treated with basil oil (200mg/kg) showing few leucocytes in hepatic sinusoids Pic. (3). The liver of the group 4, diabetic rat treated with thyme oil (200mg/kg) showing slight activation of kupffer cells Pic. (4). The liver of the group 5, diabetic rat treated of basil oil (300mg/kg) showing no histopathological changes Pic. (5). The liver of the group 6, diabetic rat treated with thyme oil (300mg/kg) showing cytoplasmic vacuolization of hepatocytes, hepatoportal blood vessel Pic. (6). The present work also showed that injection induces diabetes caused hepatocellular damage treatment with volatile oils.

Indicating the induction was successful and there were no reversions. Lipid peroxide-mediated tissue damage has been observed in the development of both types 1 and 2 Diabetes (Stanely *et al.*, 1998).

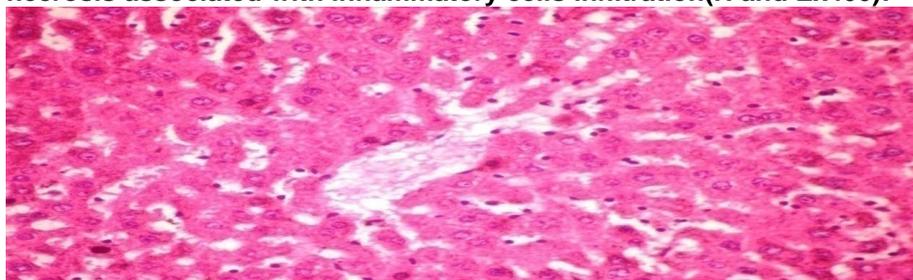
The liver of the group 7, diabetic rat treated with basil alcoholic aqueous extracts (200mg/kg) showing kupffer cells activation Pic. (7). The liver of the group 8, diabetic rat treated with thyme alcoholic aqueous extract. (200mg/kg) showing dilatation of hepatic sinusoids and cytoplasmic vacuolization of hepatocyte Pic. (8). The liver of the group 9, diabetic rat treated of basil alcoholic aqueous extract. (300mg/kg) showing normal hepatocytes Pic. (9). The liver of the group 10, diabetic rat treated with thyme alcoholic aqueous extract (300mg/kg) showing slight vacuolation of hepatocytes and slight cytoplasmic vacuolations of hepatocytes Pic. (10). Alcoholic aqueous extracts of basil and thyme leaves also showed that treated of injection induces diabetes caused hepatocellular damage. However, the antioxidant capacity of the plant extracts is mainly dependent on phenolic compounds (Ramarathnam *et al.*, 1997; Pitchersky and Gang, 2000). Antiradical activity of phenolic compounds seen in *Ocimum* species depend on their molecular structure; that is, on the availability of phenolic hydrogens, which result in the formation of phenoxy radicals due to hydrogen donation (Ramarathnam *et al.*, 1997).



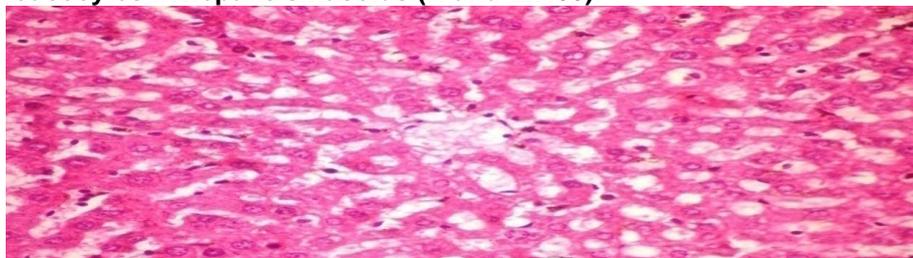
Pic. (1): Liver of rat from control- group showing the normal histological structure of hepatic lobule (H and Ex400)



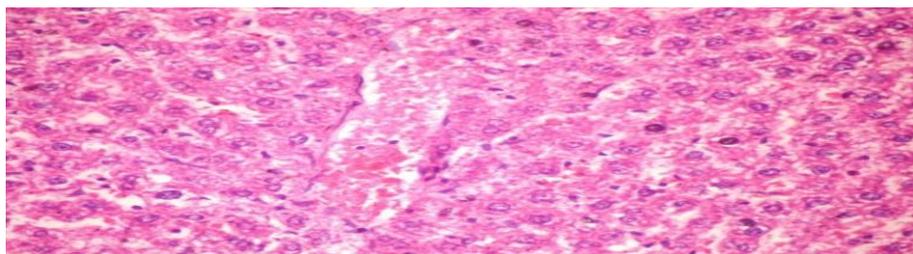
Pic. (2): Liver of diabetic control (+ve) group showing focal hepatic necrosis associated with inflammatory cells infiltration(H and Ex400).



Pic. (3): Liver of diabetic rat treated of basil oil (200mg/kg) showing few leucocytes in hepatic sinusoids (H and Ex400).

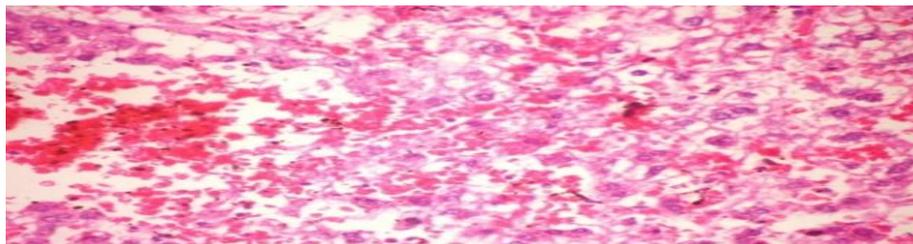


Pic. (4): Liver of diabetic rat treated of thyme oil (200mg/kg) showing slight activation of kupffer cells (H and Ex400).

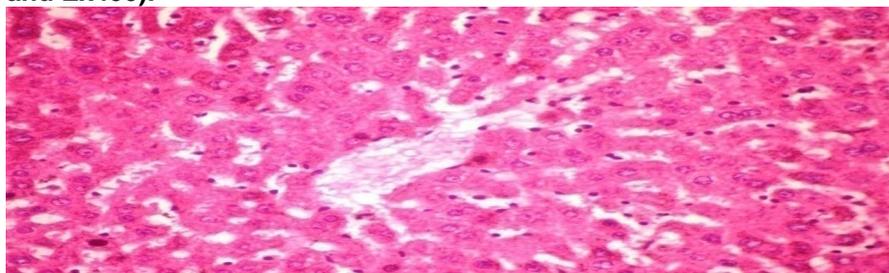


Pic. (5): Liver of diabetic rat treated of basil oil (300mg/kg) showing no histopathological changes(H and Ex400).

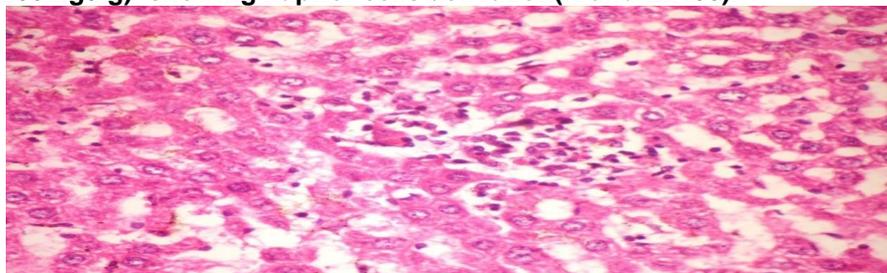
Pic. (6): Liver of diabetic rat treated of thyme oil (300mg/kg) showing



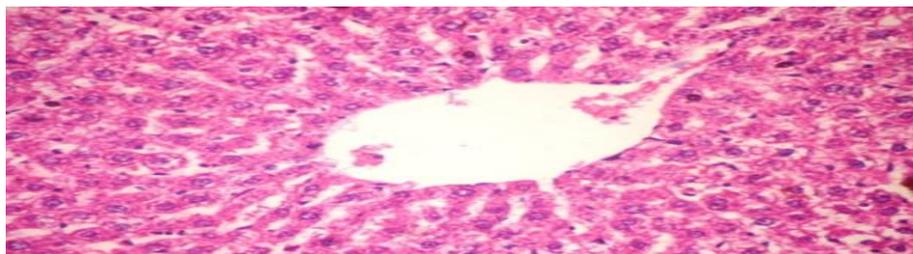
cytoplasmic vacuolization of hepatocytes, hepatoportal blood vessel (H and Ex400).



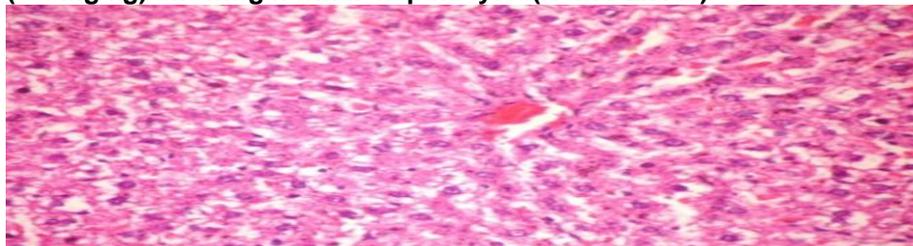
Pic. (7): Liver of diabeticrat treated of basil alcoholic aqueous extract. (200mg/kg) showing kupffer cells activation(H and Ex400).



Pic. (8): Liver of diabetic rat treated of thyme alcoholic aqueous extract (200mg/kg) showing dilatation of hepatic sinusoids and cytoplasmic vacuolization of hepatocyte(H and Ex400).



Pic. (9): Liver of diabetic rat treated of basil alcoholic aqueous extract (300mg/kg) showing normal hepatocytes(H and Ex400)



Pic. (10): Liver of diabetic rat treated of thyme alcoholic aqueous extract (300mg/kg) showing slight vacuolation of hepatocytes and slight cytoplasmic vacuolations of hepatocytes (H and Ex400).

In conclusion, the study showed that treatment with volatile oils and alcoholic aqueous extracts of basil and thyme leaves, it exhibited free radical scavenging effect. These results support its traditional use in the management of diabetes and cardiovascular diseases.

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التأثيرات العلاجية للزيوت الطيارة و المستخلص المائي الكحولي لكل من اوراق الريحان والزعر على فئران التجارب المصابة بالسكر

لبنى احمد شلباية- امانى احمد عبد القادر سلوو نانيس يوسف المتولى
قسم الاقتصاد المنزلى- كلية التربية النوعية- جامعة المنصورة- مصر

الهدف من هذا البحث هو دراسة التأثير العلاجى للزيوت العطرية و المستخلص المائى الكحولى لكل من اوراق الريحان والزعر على مستوى جلوكوز وليبيدات الدم فى فئران التجارب المصابة بالسكر عن طريق الحقن بالالوكسان حيث اجريت هذه التجربة على ٥٠ فار قسمت الى عشر مجموعات تم حقن تسع مجموعات بالالوكسان حتى اصبح مستوى جلوكوز الدم ٣٠٠مللج/دل وتم استخدام جرعات ٢٠٠ و ٣٠٠مجم/كجم من وزن الفار من الزيوت العطرية و المستخلص المائى الكحولى لكل من اوراق الريحان والزعر وايضا تم اختبار قدرة الزيوت العطرية و المستخلص المائى الكحولى على ازاحة الشقوق الحرة حيث اشارت النتائج ان المستخلص المائى الكحولى للريحان عند تركيز ١٠٠٠ميكروجرام/ملجم حقق افضل النتائج فى ازاحة الشقوق الحرة عند ٩٦,٩ يليه مضاد الاكسدة الصناعى عند ٩٥,٩ ثم المستخلص المائى الكحولى للزعر عند ٩٤,٢

فى حين ان الفئران المصابة بالسكر و المعالجة بالزيوت العطرية و المستخلص المائى الكحولى لكل من اوراق الريحان و الزعر حققت مستويات مرتفعة من الانسولين مقارنة بالفئران المصابة بالسكر كذلك حققت انخفاض معنوى فى مستوى الكوليستيرول و الترايجليسيريدات و الليوبروتينات منخفضة الكثافة بينما حققت ارتفاع معنوى فى الانزيمات المضادة للاكسدة

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
كلية التكنولوجيا و التنمية – جامعة الزقازيق

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