

## ORIGINAL ARTICLES

### Efficacy of *Coriandrum Sativum* L. Essential Oil As Antidiabetic.

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#### ABSTRACT

Aim of work: Assess *Coriandrum sativum* essential oil for its chemical composition, hypoglycemic effect, antioxidant activity on glutathione peroxidase and antidiabetic effects on pancreas and kidney in streptozotocin induced diabetic rats. Materials and Methods: Thirty male albino rats were divided into three groups where diabetes was induced in two groups of them. *Coriandrum sativum* essential oil was analysed using gas chromatography, and administered to a group of them (40 mg/kg b.w. orally) for 21 days according to its LD<sub>50</sub>. Serum glucose and glutathione were measured in addition to histological examination of kidney and pancreas. Results: The major components of coriander oil are linalool (40.9%), geranyl acetate (12.8%) and  $\gamma$ -terpinene (10.6%). In the diabetic group coriander essential oil significantly reduced serum glucose from 162.5 $\pm$ 3.19 (mg/dl) to 72.96 $\pm$ 1.73 (mg/dl); (p<0.05) and increased glutathione peroxidase level from 59.72 $\pm$ 2.78 (u/g Hb) to 124.83 $\pm$ 2.31 (u/g Hb); (p<0.05). The kidney and pancreas of the diabetic rats subjected to coriander showed some protective effects against diabetic pathological changes. Conclusion: Our findings confirm the hypoglycemic and antioxidant activity of *Coriandrum sativum* L. oil, besides improving kidney and pancreas pathological changes as a result of induction of diabetes. These could be contributed to the synergistic action of its bioactive compounds.

**Key words:** *Coriandrum sativum*; essential oil; diabetes ;glutathione peroxidase ;histological study.

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#### Introduction

The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries. Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes (Lipinski, 2001) and more importantly in the development of diabetic complications (Baynes, 1997).

Although several therapies are in use for treatment of diabetes, there are certain limitations due to high cost and side effects (Dey *et al.* 2002). Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. Medicinal plants are being looked up once again for the treatment of diabetes. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy (Gray & Flatt, 1997a).

One of these herbal spices is *Coriandrum sativum* L.; Apiaceae, Umbelliferae (coriander –cilantro-chinese parsley- dhani) that have been used through different traditions and considered to have cooling, stimulant, carminative, digestive properties, cystitis (Aboelsoud, 2010), headaches, measles, rectal prolapse, prevent cancer (Li and Ben, 1999), relief of anxiety and insomnia, and may have potential sedative, hypotensive, and muscle relaxant effects (Emamghoreishi *et al.*, 2005; Swanston-Flatt *et al.* 1990), diuretic, antipyretic, stomachic, aphrodisiac, laxative and anthelmintic properties (Deepa and Anuradha, 2011).

*Coriandrum sativum* has been advocated as an anti-diabetic remedy (Bailey and Day, 1989). More recent studies have confirmed the antihyperglycaemic effect of coriander in streptozotocin-diabetic mice (Swanston-Flatt *et al.* 1990). Anti-diabetic agents can exert beneficial effects in the diabetic environment by improving and/or mimicking insulin action and/or by enhancing insulin secretion (Gray & Flatt, 1999).

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Aim of work: coriander seed oil is promising oil with high levels of bioactive compounds. However, little information is available on the effect of administration of this oil on diabetes in experimental animals. To understand better its antidiabetic effect, the present study assesses the chemical composition of coriander essential oil, its hypoglycemic and antioxidant activity on glutathione peroxidase in streptozotocin-induced diabetic rats, in addition to a study of histological specimens of their pancreas and kidney.

## Materials And Methods

### Materials:

- Streptozotocin (STZ) was purchased from Sigma Chemical Company, St Louis, Missouri, USA.
- Chloroform, Methyl alcohol, ether were purchased from BHD, England.
- Plant materials: coriander seeds (*Coriandrum sativum* L.) was purchased from local markets and authenticated in the herbarium of Faculty of Science, Cairo University and National Research Center, Egypt.

### 2.2. Methods:

#### 2.2.1. Preparation of coriander essential oil:

One kg of coriander seeds was subjected to hydrodistillation. The volatile oil then collected and dried in desiccators over anhydrous  $\text{CaSO}_4$ . The volatile oil sample was kept in a dark bottle till used.

#### 2.2.2. Analysis of coriander essential oil:

The oil was analysed using the GC and GC/MS analysis.

##### 2.2.2.1. Gas chromatography (GC):

For analysis of the chemical constituents of the plant essential oil, a gas chromatograph (Perkin-Elmer model 8700), fitted with a flame ionization detector (FID) was used. An HP-5MS capillary column (30 m  $\times$  0.25 mm, film thickness 0.25  $\mu\text{m}$ ) was used for separation purposes. The initial column temperature was set at 80°C and then raised to 220°C by the rate of 4°C/min. The initial and final column temperatures were held for 3 and 10 min, respectively. The operating temperatures for detector and injector were 220 and 290°C, respectively. The mobile phase used was helium at a flow rate of 1.5 ml/min. A 1.0  $\mu\text{l}$  sample was injected using split mode (split ratio 100:1). All the quantitative measurements were made using a built-in data-handling program of the gas chromatograph (Perkin-Elmer, Norwalk, CT, USA). The composition of oil chemical constituents was reported as a relative percentage of the total peak area.

##### 2.2.2.2. Gas chromatography/mass spectrometry analysis (GC/MS):

The essential oil was also analysed and authenticated for chemical composition using an Agilent-Technologies 6890N network gas chromatographic (GC) system (Little Falls, California, USA), equipped with 5975 inert XL mass selective detector and 7683B series auto injector (Agilent-Technologies). A sample volume of 1.0  $\mu\text{l}$  was injected, applying split mode (split ratio 100:1), into HP-5 MS capillary column (30 m  $\times$  0.25 mm, film thickness 0.25  $\mu\text{m}$ ; Little Falls, CA, USA) using the same column temperature and gas flow rate as selected previously for GC analysis. An electron ionization (EI) system, with ionization energy (70 eV), was used for GC/MS detection. Mass scanning range was varied over 50 to 550 m/z. The injector and MS transfer line temperature were 220 and 290°C, respectively. The essential oil chemical compounds were identified on the basis of matching their retention indices in relation to *n*-alkanes (C9-C24) and moreover with those of authentic compounds or published data (Minica, *et al.*, 2003; Vagionas *et al.*, 2007).

Besides, the comparison of MS spectral data of the compounds with those from NIST mass spectral library was also applied to authenticate the compounds (Masada, 1976; Adams, 2001).

#### 2.2.3. Animals tested:

Thirty male albino rats weighing 150-200g were supplied by the Animal House of National Research Center, Cairo-Egypt. Rats were caged under controlled temperature 20-24°C and 12 h light/dark cycle. They were fed with standard laboratory chow and water ad libitum.

### 2.2.3.1. Induction of Diabetes:

Rats were kept on fasting prior to streptozotocin injection. On the day of administration, STZ was freshly dissolved in 50Mm sodium citrate (pH 4.5) solution containing 150 mMNaCl and subcutaneous injection was given at the dosage of (60mg/kg b.w.). Blood glucose concentration was checked by the glucose oxidase method (Trinder, 1969). After 3 days of STZ injection. The animals with glucose concentration exceeding 200mg /dl were considered diabetic.

Rats were divided into 3 groups 10 rats in each group.

Group I: normal control rats

Group II: diabetic control rats

Group III: diabetic rats received coriander essential oil. (40 mg/kg b.w. orally).

The dose of essential oil was chosen according to its LD<sub>50</sub> (the medium 50 lethal doses after acute toxicity).

### 2.2.3.2. Samples Collection:

After 21 days from the beginning of the experiment, rats were fasted for 12 hours then blood samples were collected retro-orbitally from the inner canthus of the eye under ether anaesthesia using capillary tubes containing EDTA sodium (Madway *et al.*, 1969) , Blood samples were divided into two tubes, one of them heparinized whole blood for the determination of glutathione peroxidase immediately, the rest of the sample separated in centrifuge at 3000 rpm for 5 minutes to obtain the serum for measurement of glucose in different studied groups.

### 2.2.4. Biochemical Measurements:

-Glucose was estimated using kit (glucose PAP enzymatic oxidase method purchased from Stanbio Laboratory, Inc. (Trinder, 1969).

-Glutathione peroxidase was determined in heparinised whole blood by colorimetric method using Randox Laboratories Kit,UK as described by Ammerman *et al.*1980.

### 2.2.5. Histological study:

The kidney and pancreas of different groups were removed and fixed in 10% formal saline. Paraffin sections 5 µm thick were stained with haematoxylin and eosin (Drury and Wallington, 1980).

*The protocol of the study was reviewed and approved by Ethical Committee of National Research Center.:*

*Authors declare that there is no actual or potential conflict of interest for this work.:*

### 2.2.6. Statistical Analysis:

The data for various biochemical parameters were expressed as mean ± SEM and compared using one way analysis of variance (ANOVA) test. Values were considered statistically significant when  $p < 0.05$ . Statistics were done using SPSS for windows version 10.

## Results:

### 3.1. Analysis of coriander oil:

The essential oil content of the dried seeds of coriander was 0.27 %. The corresponding qualitative and quantitative chemical compositional data is given in Table 1. A total of 41 compounds were identified in the essential oil, accounting for 94.6% of the total oil. The essential oil tested contained high amount of linalool (40.9%) followed by geranyl acetate (12.8%),  $\gamma$ -terpinene (10.6%),  $\beta$ -Sesquiphellandrene(3.0%),  $\alpha$ - Thujene (2.8%), Limonene (2.5%), Citronellal (2.1%),  $\beta$ - Pinene (2%), m-Cymene (2%), Geraniol (2%), Citronellyl acetate (2%). Some minor compounds including Eugenol (1%),  $\beta$  – Myrecene (0.7%), Camphene (0.4%), Verbenol (0.4%), Sabinene (0.3%), Borneol (0.3%), Z- Ocimene (0.2%), Thujol (0.2%),  $\alpha$ -Campholenal (0.2%),  $\alpha$  – Terpinene (0.1%),  $\beta$ -Farnesene (0.1%).

### 3.2. Chemical results:

The characteristic abnormalities observed in the studied groups were shown in table 2&3. In diabetic rats, the blood glucose was significantly increased while serum glutathione peroxidase was significantly decreased when compared to normal control group. Treatment with coriander essential oil led to reduction in blood glucose level back to normal and increase in serum glutathione peroxidase even above the control group.

### 3.3. Histological Results:

#### 3.3.1. Kidney:

The normal histological structure of the kidney was observed in (Fig. 1, a). The kidney of streptozotocin induced diabetic rats showed vacuolar degeneration in some tubular epithelial cells and cell debris scattered in tubular lumina. Increase in thickness of tubular epithelial cells with narrowing of lumen, signs of degeneration in the form of karyolysis and karyorrhexis. Massive cellular infiltration, areas of hemorrhage in interstitial tissue and deformed renal tissue architecture were seen. Some glomeruli showed complete degeneration with thickening of Bowman's capsule, while others showed lobulation with wide urinary space (Fig. 1, b, c & d).

The kidney of the streptozotocin induced diabetic rats subjected to coriander showed some protective effects as compared to the control diabetic group. Examination of kidney sections showed some glomerular degeneration, thickening of Bowman's capsule, cell debris in some tubular lumina and mild cellular infiltration in interstitial tissue (Fig. 2).

#### 3.3.2. Pancreas:

The normal architecture of pancreatic tissue was noticed in (Fig. 3,a). Pancreatic sections stained with HE showed that streptozotocin caused severe necrotic changes of pancreatic islets, especially in the center of the islets. Nuclear changes, karyolysis, disappearing of nucleus and in some places residue of destructed cells were visible. The relative reduction of the size of islets, dilatation and congestion of large vessel and marked increase in connective tissue component at the expense of functioning tissue were obvious. The exocrine part of the gland (serous acini) showed flattening of their nuclei that were pushed to the bottom of the cells (Fig. 3, b & c).

The pancreas of streptozotocin induced diabetic rats, subjected to coriander showed a mild protective effect as compared to group of rats treated with streptozotocin only. Examination of pancreatic sections of this group showed that necrotic changes were still observed in both endocrine and exocrine parts of the gland. Blood vessels were still dilated and congested (Fig. 4).

**Table 1:** *Coriandrum Sativum* Essential Oil Components

Component	Composition (%)
1. $\gamma$ - Terpinene	10.6
2. Camphene	0.4
3. Verbenol	0.4
4. Sabinene	0.3
5. $\beta$ - Pinene	2.0
6. $\beta$ - Myrecene	0.7
7. Cyclooctanol	0.1
8. $\alpha$ - Thujene	2.8
9. m-Cymene	2.0
10. Limonene	2.5
11. E-Ocimene	0.1
12. Z- Ocimene	0.2
13. $\alpha$ - Terpinene	0.1
14. Verbenol	0.1
15. Linalool	40.9
16. Thujol	0.2
17. $\alpha$ -Campholenal	0.2
18. Citronellal	2.1
19. Borneol	0.3
20. 4-Terpineol	0.1
21. Decanal	0.2
22. Z-Verbenone	0.1
23. Citronellol	1.5
24. Citral	1.8
25. Geraniol	2.0
26. Eugenol	1.0

27.	Carveol	0.3
28.	Methyl geranate	0.2
29.	Citronellyl acetate	2.0
30.	Geranyl acetate	12.8
31.	β-Elemene	0.1
32.	Dodecenal	0.2
33.	Caryophyllene	0.4
34.	β-Farnesene	0.1
35.	2- Dodecenal	0.2
36.	Curcumene	1.1
37.	α-Cubebene	0.2
38.	β-Bisabolene	0.9
39.	β-Sesquiphellandrene	3.0
40.	E-Nerolidol	0.2
41.	α-Bisabolol	0.2
Total %		94.6

**Table 2:** Fasting Blood Glucose Levels In Different Groups Studied Of Experimental Animals 21 Days After Induction Of Diabetes

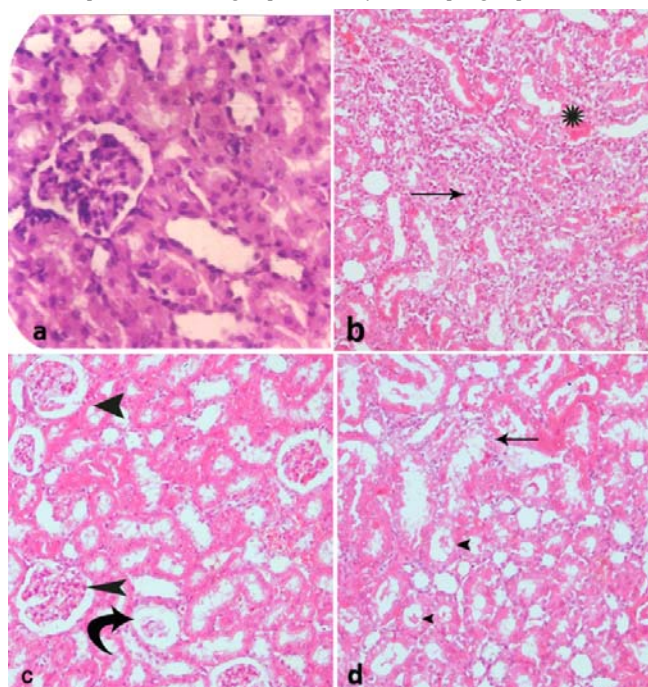
	Control	diabetic	diabetic + coriander oil
Fasting blood sugar (mg/dl)	73.07±9.11*	162.5±3.19	72.96±1.73*

Values are Means ± S.E.M; \**p*<0.05 compared to diabetic group. Number of animals per group = 10

**Table 3:** Glutathione Peroxidase In Different Groups Studied Of Experimental Animals 21 Days After Induction Of Diabetes

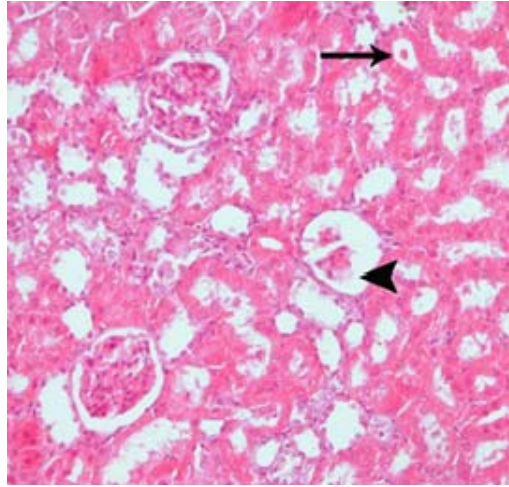
	Control	diabetic	diabetic + coriander oil
Glutathione peroxidase (u/g Hb)	82.68±4.48*	59.72±2.78	124.83±2.31*

Values are Means ± S.E.M; \**p*<0.05 compared to diabetic group. Number of animals per group = 10

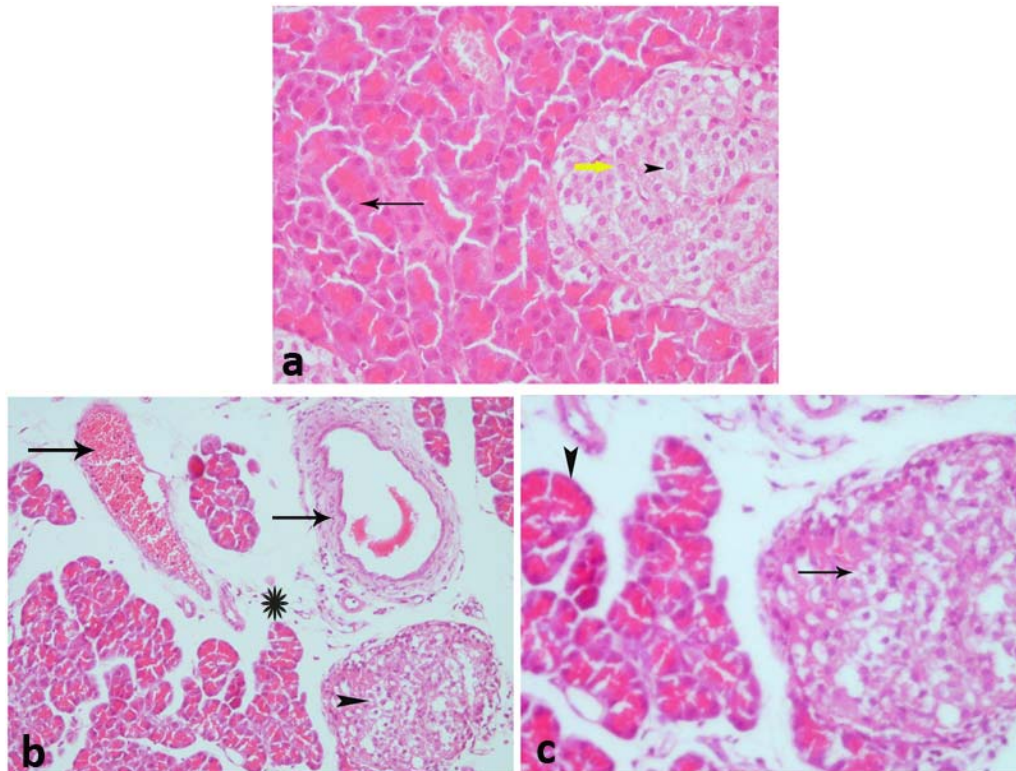


**Fig. 1:** Section of the kidney of a rat (a): control (Hx. & E. X 200). (b): Section of the kidney of streptozotocin diabetic rat showing massive cellular infiltration (arrow) and areas of haemorrhage in interstitial tissue (star). (Hx. & E. X 50) (c): Another field of the kidney of the same group showing some completely degenerated glomeruli with thickening of Bowman’s capsule (curved arrow), while others showed

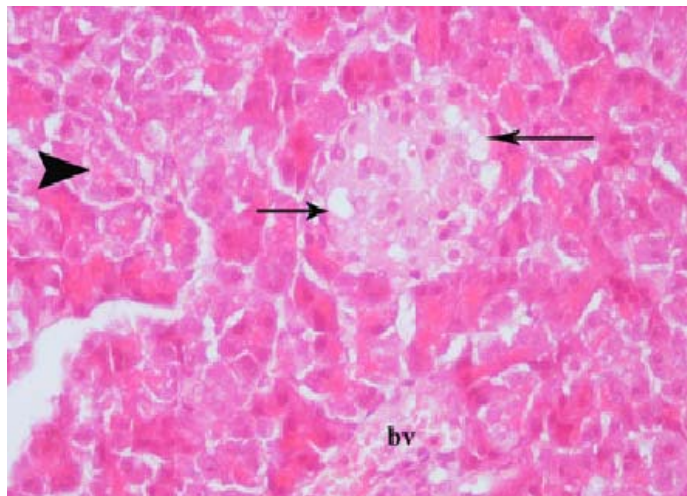
lobulation with wide urinary space (arrow head). (d): Another section of the same group showing vacuolar degeneration in some tubular epithelial cells (arrow), and cell debris scattered in tubular lumina (arrow head). (Hx. & E. X 100)



**Fig. 2:** Section of the kidney of streptozotocin diabetic rat treated with coriander oil showing some glomerular degeneration (arrow head) with thickening of Bowman's capsule and cell debris in some tubular lumina (arrow). (Hx. & E. X 100)



**Fig. 3:** Section of pancreas of a rat (a): control. (Hx. & E. X 100). (b): Section of pancreatic tissue of streptozotocin diabetic rat showing marked increase in the connective tissue component of the gland (interlobular and intralobular) (star), blood vessels are markedly dilated and congested (arrow). (Hx. & E. X 50) (c): Higher magnification of the previous group shows vacuolar degeneration in the cells of islets of Langerhan specially in the center (arrow), while the nuclei of the serous acini cells appeared flattened and pushed towards the bottom of the cells (arrow head). (Hx. & E. X 100)



**Fig. 4:** Section of pancreatic tissue of streptozotocin diabetic rat treated with coriander oil showing vacuolar degeneration is still observed in some cells of islets of Langerhan (arrows), also some of the acini cells show signs of degeneration (arrow head). The blood vessels are still slightly dilated and congested (bv). (Hx. & E. X 100)

#### Discussion:

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Ceriello, 2000). Diabetes is usually accompanied by increased production of free radicals (Baynes & Thorpe, 1999) or impaired antioxidant defences (Saxena *et al.* 1993).

Hyperglycemia is a hallmark of both non-insulin-dependent (type 2) and insulin dependent diabetes mellitus (type 1). Elevated glucose levels are associated with increased production of reactive oxygen species (ROS) by several different mechanisms (Oberley, 1988). Hyperglycemia was shown to cause increased ROS production at the mitochondrial complex II (Nishikawa *et al.* 2000). In addition, superoxide is generated by the process of glucose auto-oxidation that is associated with the formation of glycated proteins in the plasma of diabetic patients (Sakurai & Tsuchiya, 1988).

Many plant extracts and plant products have been shown to have significant antioxidant activity (Anjali & Manoj, 1995). There is a strong evidence of the preventive effect of spices and natural flavors for counter acting oxidative damages (Saleh *et al.* 2010). It was found that coriander may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes (Wangensteen *et al.* 2004).

Coriander was reported as a traditional treatment for diabetes. Early experiments involving administration of coriander fruit as a decoction did not reveal effects on fasting blood sugar levels of normal and alloxan diabetic rats, but demonstrated

alleviation of adrenaline-induced hyperglycaemia (Sharaf *et al.* 1963). Subsequent studies involving longer term administration of coriander seed in the diet showed that the plant purported as a traditional treatment for diabetes indeed decreased the hyperglycaemia of streptozotocin-diabetic mice (Swanston-Flatt *et al.* 1990). The present study confirmed this antihyperglycaemic action where the elevated glucose level in streptozotocin induced diabetic rats returned to normal level after treatment with coriander oil.

In trial to understand the mechanism of action of coriander oil we did quantitative and qualitative composition analysis of the used oil. In the present study, the major component of coriander oil was linalool (40.9%). Linalool is a monoterpene, and major volatile component of the essential oils of several aromatic plant species including *Coriandrum sativum* Linn. (Letizia *et al.* 2003). Beside the antibacterial, antiviral, antiinflammatory, analgesic and local anesthetic activities (Do Socorro *et al.* 2003; Peana *et al.* 2003), the hypoglycemic effect of Linalool in diabetic rats has already been reported (Afifi *et al.* 1998).

It was found that linalool reduced the plasma glucose level by increasing insulin levels. The antihyperglycemic effect of Linalool could be due to the stimulation of  $\beta$ -cells and subsequent release of insulin and activation of insulin receptors or directly potentiated pancreatic secretion of insulin. In addition, it was observed that Linalool could promote glucose utilization by cells (Deepa and Anuradha, 2011).

In our study the other major components of coriander oil are monoterpenes namely Geranyl acetate (12.8%) and  $\gamma$ -Terpinene (10.6%) which are strong antioxidants.

$\gamma$ -Terpinene is a major component of essential oils made from citrus fruits and shows strong antioxidant activity in various assay systems (Amiri, 2011), hypoglycaemic activity and an anticholinesterase effect (Conforti *et al.* 2007).

In diabetes, oxidative stress is associated with a pro-oxidative shift of the glutathione redox state in the blood (De Mattia *et al.* 1998). To test the effect of used oil on antioxidant state, we measured the enzyme glutathione peroxidase in blood.

Glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxides including hydrogen peroxide, by reduced glutathione and function to protect the cell from oxidative damage (Arthur, 2000). Glutathione is a monomer that contains selenocysteine. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of selenocysteine (Ursini *et al.* 1985).

In the present study, the reduced level of the enzyme glutathione peroxidase as a result of induction of diabetes was significantly elevated above the normal control level ( $p < 0.05$ ) after treatment with coriander oil.

The reduction of the enzyme level due to diabetes couple with previous results (Saxena *et al.* 1993). Glutathione peroxidase activity depends on the presence of its active center, selenium (Arthur *et al.* 2003), also its substrates, reduced glutathione and oxygen free radicals produced due to presence of diabetes state (Németh *et al.* 2004). These oxygen free radicals first increase and at continuously high level decrease the enzyme activity (Holovska *et al.* 1996). The reduction in glutathione peroxidase in diabetic group could be attributed to increased utilization to counteract the oxidative stress, or may be due to the interaction of advanced glycation end products with corresponding cell surface receptors that stimulates ROS production and decreases intracellular glutathione levels (Yan *et al.* 1994). The increase in this enzyme after coriander oil treatment could be related to antioxidant effect of coriander oil that decrease the oxygen free radical formation therefore increase the enzyme activity but it could also act as a substrate for the peroxidase system. There are some reports about the possible pro-oxidant effects of coriander oil (Samojlik *et al.* 2010). Previous studies confirmed activation of antioxidant enzymes (catalase, glutathione peroxidase) in rats treated by *Coriandrumsativum* (Chithra & Leelamma, 1999).

Streptozotocin (STZ) selectively destroys the pancreatic  $\beta$ -cells, which causes the inhibition of synthesis and release of insulin thereby leading to the onset of diabetes (BalkisBudin *et al.* 2009; Qiu *et al.* 2007). STZ is a nitric oxide (NO) donor; and NO had been reported to mediate the destruction of pancreatic islet cells, probably via DNA damage (Kroncke *et al.*, 1995). In addition to NO, STZ also generates ROS (from the action of this drug on the mitochondria and from increased xanthine oxidase activity) (Szkudelski, 2001). Thus, scavengers of NO and ROS will possess beneficial effects against DNA damage and  $\beta$ -cell toxicity induced by these substances.

In this work, there is marked tissue damage of pancreas of diabetic rats. This indicates increased production of ROS in this organ. Besides, amelioration of the pancreatic pathology in the coriander oil treated diabetic rats could be due to possible role of *Coriandrumsativum* phytochemicals (antioxidants), the prompt reversal of hyperglycemia in *Coriandrumsativum* treated diabetic rats may also be a contributory factor.

Diabetic nephropathy has been considered an important cause of mortality and morbidity and many of the end stage renal failure results due to diabetic nephropathy (Boon *et al.* 2006). STZ-induced diabetic rodents result in development of nephropathy similar to the early stage of human diabetic nephropathy (Rasch & Mogensen, 1980).

This histological study performed on the kidneys of diabetic rats showed damage to the glomerulus, thickened basement membrane and edematous proximal convoluted tubule with areas of hemorrhage in interstitial tissue and deformed renal tissue architecture which were found to be much improved in the diabetic kidneys treated with coriander oil but still with minimal residual effects in the form of glomerular degeneration, thickening of Bowman's capsule, cell debris in some tubular lumina and mild cellular infiltration in interstitial tissue. A previous study had also reported similar histological findings (Sun *et al.* 2002; Sugano *et al.* 2006).

Susceptibility of the kidney to oxidative stress during diabetes is an important factor to develop diabetic nephropathy where ROS activates inflammatory pathways leading to glomerular damage. Thus increasing the antioxidant enzymes (e.g glutathione peroxidase) and getting rid of free radicals could be implicated in utility of coriander oil to improve the pathology of diabetic nephropathy although with no complete reversal of all the abnormalities, which could be due to the need of longer duration of treatment.

#### Conclusion:

Our findings confirm the hypoglycemic and antioxidant activity of *Coriandrumsativum* L. essential oil, besides improving kidney and pancreas pathological changes as a result of induction of diabetes. These could be contributed to the synergistic action of its bioactive compounds namely linalool, geranyl acetate and  $\gamma$ -terpinene.



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