Antidiabetic Activities of *Foeniculum Vulgare* Mill. Essential Oil in Streptozotocin-Induced Diabetic Rats

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Abstract

**Background:** *Foeniculum vulgare* Mill (F. Apiaceae) is an ancient common herb and spice known to the ancient Egyptians and Greeks, traditionally used as a carminative, a weak diuretic and lactation stimulant.

**Aim:** To evaluate the essential oil of *Foeniculum vulgare* Mill for its hypoglycaemic effect and antioxidant activity in addition to histopathological study in streptozotocin induced diabetic rats.

**Material and Methods:** Rats were divided into 3 groups; normal control, diabetic control, and diabetic group receiving orally *Foeniculum vulgare* Mill essential oil (30 mg/kg bw). The dose of essential oil was chosen according to its LD50. Serum glucose and whole blood glutathione peroxidase were measured in addition to histopathological study of rats kidney and pancreas.

**Results:** Ingestion of essential oil of *Foeniculum vulgare* Mill to diabetic rats corrected the hyperglycemia from (162.5 ± 3.19 mg/dl) to (81.97 ± 1.97 mg/dl) with *p*<0.05 and the activity of serum glutathione peroxidase from (59.72 ± 2.78 U/g Hb) to (99.60 ± 6.38 U/g Hb) with *p*<0.05. Also, improved the pathological changes noticed in their kidney and pancreas.

**Conclusion:** Essential oil of *Foeniculum vulgare* Mill corrected the hyperglycemia and pathological abnormalities in diabetic induced rats, which could be in part through its antioxidative effect and restoring of redox homeostasis. This makes the possibility of its inclusion in antidiabetic drug industry.

Introduction

Since ancient times, plants have been an exemplary source of medicine [1]. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed [2]. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs [3].

Diabetes is an example of a disease that has been treated with plant medicines [4]. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic properties [5, 6].

Diabetes mellitus is among the most common disorders in developed and developing countries. The disease is increasing rapidly in most parts of the world [7]. In 1995, the World Health Organization reported that approximately 150 million persons worldwide had diabetes mellitus, and this number may well be double by 2025.
Oxidative stress has been implicated in the development of many pathophysiological conditions including diabetes [9, 10].

Oxidative stress takes place due to the disturbance of the balance between the formation of reactive oxygen species (ROS) and the defense provided by cellular antioxidants [11]. Reduced glutathione (GSH), ubiquitously distributed in all mammalian cells, is a reducing sulfhydryl (-SH) tripeptide and plays important roles in the endogenous antioxidant system because it conjugates toxic substances [12].

Intracellular GSH and its related enzymes, such as glutathione peroxidase (GSHPx), glutathione-s-transferase (GSHT), glutathione reductase (GSHR), and glutamate cysteine ligase (GCL), constitute the cellular glutathione antioxidant system and represent a crucial defensive system to protect cells against ROS [13].

Numerous spices and aromatic herbs have been examined for their antioxidant/antiradical activity, *Foeniculum vulgare* Mill (fennel) is one of these herbs. Fennel is a plant belonging to the Kingdom: Plantae; Order: Apiales; Family: Apiaceae (Umbelliferae); Genus: Foeniculum; Species: *F. Vulgare*; Binomial name: *Foeniculum vulgare* Mill. It was known and used by humans since antiquity [14].

*Foeniculum vulgare* Mill. (fennel) is a typical aromatic plant of the Mediterranean area, long used as a medicinal and spice herb. Wild fennel was found to exhibit a radical scavenging activity, as well as a total phenolic and total flavonoid content, higher than those of both medicinal and edible fennels. Owing to commercial importance of its essential oil, fennel oil has been extensively studied for many years [15].

The herb fennel was known to the ancient Chinese, Indian, Egyptian and Greek civilizations [1]. The name foeniculum is from the Latin word for fragrant hay. Fennel was in great demand during the middle Ages. Wealthy people routinely added the seed to fish and vegetable dishes, while the poor reserved its use for fasting days as an appetite suppressant. As an herbal medicine, fennel is reputed to increase milk secretion, promote menstruation, facilitate birth, ease the male climacteric, and increase the libido. These supposed properties led to research on fennel for the development of synthetic estrogens during the 1930s [16].

There is evidence from two randomized, double-blind, placebo controlled trials suggesting that fennel is effective in reducing infantile colic [17]. Fennel has also been studied in human clinical trials for dysmenorrhea [18], constipation [19] and antihirsutism [20], but additional research is merited for its effect in diabetes as little is known about its hypoglycaemic effect and possible mode of action in diabetes.

The aim of the present study is to evaluate the essential oil of *Foeniculum vulgare* Mill for its hypoglycaemic effect and antioxidant activity in addition to histopathological study in streptozotocin induced diabetic rats.

**Methods**

**Materials**

Essential oil of *Foeniculum vulgare* Mill was purchased from Kato Aromatic Company-Egypt. Streptozotocin (STZ) was purchased from Sigma chemical company, St Louis, Missouri. USA. Chloroform, Methyl alcohol, ether were purchased from BHD, England.

**Animals tested**

Fifty male albino rats weighing 150-200 g 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.

**Induction of diabetes**

Rats were kept on fasting prior to streptozotocin injection. On the day of administration, STZ was freshly dissolved in 50 mM sodium citrate (pH 4.5) solution containing 150 mM NaCl and subcutaneous injection was given at the dosage of (60 mg/kg bw). Blood glucose concentration was measured by the spectrophotometric GOD-PAP method (glucose oxidase-peroxidase) [21]. After 3 days of STZ injection, the animals with glucose concentration exceeding 200 mg/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; and group III: diabetic rats received *Foeniculum vulgare* Mill essential oil. (30 mg/kg bw orally).

The dose of essential oil was chosen according to its LD₅₀ (the medium 50 lethal doses after acute toxicity) [22].
**Samples collection**

After 21 days from the beginning of the experiment, rats were fasted for 12 hours then blood samples were collected. Blood was collected retro-orbitally from the inner canthus of the eye under ether anaesthesia using capillary tubes [23]. Every blood sample was divided into three tubes, one of them contains EDTA Na to determine hemoglobin immediately and the other heparinized whole blood for the determination of glutathione peroxidase immediately, the rest of the sample was separated in centrifuge at 3000 rpm for 5 minutes to obtain serum that was used to measure glucose in different studied groups.

**Biochemical measurements**

Serum glucose was estimated using kit (glucose PAP enzymatic oxidase method purchased from Stanbio Laboratory, Inc. [21].

Glutathione peroxidase activity was determined in heparinised whole blood by colorimetric method using Randox Laboratories Kit, UK as described by Ammerman et al [24].

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

**Histopathological and Histochemical Studies**

The kidney and pancreas of different groups were removed and fixed in 10% formal saline. Paraffin sections 5 mm thick were stained with haematoxylin and eosin [25].

**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 at p < 0.05. Statistics were done using SPSS for windows version 10.

**Results**

**Biochemical results**

The characteristic abnormalities observed in the diabetic rats were shown in Table 1 where the blood glucose was significantly increased when compared to control animals. A reduction was observed in blood glucose in diabetic rats treated with the tested essential oil of *Foeniculum vulgare* Mill.

<table>
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<tr>
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<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + fennel oil</th>
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<tbody>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>73.07 ± 9.11</td>
<td>162.5 ± 3.19</td>
<td>81.97 ± 1.65*</td>
</tr>
<tr>
<td>Glutathione peroxidase activity (U/lg Hb)</td>
<td>62.68 ± 4.48</td>
<td>59.72 ± 2.78</td>
<td>99.60 ± 6.36</td>
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Values are means ± S.E.M; N = number of animals per group; *p<0.05 compared to diabetic group.

Table 1 showed also glutathione peroxidise activity in different groups studied of experimental animals 21 days after induction of diabetes. Glutathione peroxidise activity were significantly reduced in diabetic group in comparison to control group. Essential oils of *Foeniculum vulgare* Mill significantly corrected this reduction in levels of serum glutathione peroxidase in treated diabetic rats compared to diabetic control group.

**Histological and histochemical results**

**Kidneys:**

The normal histological structure of the kidney was shown in (Fig. 1, a). The kidney of the diabetic rats showed vacular degeneration in some tubular epithe-
lial 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 diabetic rats treated with *Foeniculum vulgare* essential oil showed more protective effects as compared to diabetic rats not treated with *Foeniculum vulgare* essential oil in the form of diminution of cellular infiltration, hemorrhage in interstitial tissue, glomerular degeneration and cell debris in tubular lumina, while vacuolar degeneration in some tubular epithelial cells still could be noticed (Fig. 2, c).

Pancreas:

The normal architecture of pancreatic tissue was shown in (Fig. 3,a). Pancreatic sections stained with Hx & E 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).
A protective effect was obtained by using *Foeniculum vulgare* Mill essential oil to diabetic rats. Examination of pancreas sections of this group showed that the pancreatic tissue retained its normal architecture (Fig. 4, e).

**Discussion**

Diabetes mellitus, an endocrine and metabolic disorder characterized by chronic hyperglycemia produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidation that play role in the progression of the symptoms of diabetes [26]. Several hypotheses have been posulated to explain the development of free radicals in diabetes which include auto oxidation of glucose, enzymatic and non-enzymatic glycation of proteins with increased formation of glucose derived advanced glycosylation end products (AGEs), enhanced glucose flux through polyol pathway [27] and reduction of antioxidant defence [28].

Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run. So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern [3].

Spices are dried herbs they are known to exert several beneficial physiological effects including the antidiabetic influence [29]. Among the spices, fenugreek seeds (*Trigonella foenumgraecum*), garlic (*Allium sativum*), onion (*Allium cepa*), and turmeric (*Curcuma longa*) have been experimentally documented to possess antidiabetic potential. In a limited number of studies, cumin seeds (*Cuminum cyminum*), mustard (*Brassica nigra*), ginger (*Zingiber officinale*), curry leaves (*Murraya koenigii*) and coriander (*Coriandrum sativum*) have been reported to be hypoglycaemic [30].

Most bioactive spices constituents are collectively called phytochemicals. The large majority of these phytochemicals are redox active molecules and therefore defined as antioxidants [14]. *Foeniculum vulgare* Mill (Fennel) is another spice that has been used for centuries in the Mediterranean area as an aromatic herb and also in folk medicine, due to the pharmacological properties of its essential oil. Typically, fennel and its preparations are used to cure various disorders, acting as a carminative, digestive, lactogoge and diuretic agent [31].

Fennel seeds contain between 3% and 6% of an essential oil and approximately 20% of a fixed oil composed of petroselinic acid, oleic acid, and tocopherols. The essential oils of sweet and bitter fennel contain up to 90% trans-anethole, up to 20% fenchone and small amounts of limonene, camphor, alpha-pinene, and about 6 additional minor volatile compounds [32]. It was reported that fennel oil possessed antiinflammatory, antioxidant and pro-oxidant activities [33].

In this study we used streptozotocin (STZ) to induce diabetes in rats. STZ is well known for its selective pancreatic B-cell toxicity and has been extensively used in induction of diabetes mellitus in animals [34]. Experimental evidence has demonstrated that some of its deleterious effects are attributable to induction of metabolic processes, which lead on to an increase in the generation of reactive oxygen species (ROS) [35]. Apart from production of ROS, STZ also inhibits free radical scavenger-enzymes [36].

Antioxidant defense mechanisms are important for the protection of cells and tissues against oxidative damage. The major endogenous antioxidant enzyme-systems in mammalian body include superoxide dismutase (SOD), catalase (CAT), selenium-dependent glutathione peroxidase (GSHPx-Se), glutathione peroxidase (GSHPx), and glutathione reductase (GSHR). The major non-enzymatic endogenous antioxidants include glutathione (GSH) and vitamin E [37]. Reduced antioxidant levels, as a result of increased free radical production in experimental diabetes, have been previously reported [38, 39].

In this study we noticed the reduction of glutathione peroxidase (GSHPx) activity in the diabetic induced rats which can be due to the oxidative stress as part of diabetes pathogenesis. Cytosolic GSHPx is an enzyme containing four selenium-cofactors that protects tissues from damage by catalyzing the breakdown of hydrogen peroxide and organic hydroperoxides [13]. The current results showed that GSHPx activity largely decreased in diabetic induced rats compared to non-diabetic control rats, and this decrease was reversed by administration of fennel essential oil.

Glutathione peroxidase (GSHPx) is reported to have a broader protective spectrum than other enzymes as catalase for example because in addition to H$_2$O$_2$, it also metabolizes other hydroperoxides including lipid hydroperoxides [40]. The accumulation of H$_2$O$_2$ and other hydroperoxides might have induced the activity of...
GSHPx leading to its up-regulation in the diabetic rats.

The observation that rodent and human islets have reduced expression of GSHPx has led to the conclusion that a normal approach for protection of B-cells against oxidative stress would involve over-expression of GSHPx [41]. Thus, the over-expression of GSHPx could be a protective mechanism against oxidative stress.

Administration of fennel oil down-regulated the activity of GSHPx, thus indicating that fennel oil attenuated the changes in the pancreatic antioxidant enzymes in response to generation of oxidants. The antioxidant properties of this oil have been recognized in previous studies [42, 43] which could be attributed to the rich content of phenolic compounds [44].

The current results indicated that the pathological effects in rats kidney and pancreas tissues induced by diabetes due to oxidative stress were reversible and corrected by ingestion of fennel oil this could be attributed to its antioxidative effects.

The kidney histopathology data of STZ induced diabetic rats showed marked tubular degeneration, haemorrhage, thickening in the Bowman’s space due to glomerular damage. The results indicated a primary and a secondary effect of the diabetic state on the kidney of the rat. The primary effect, the diabetes factor was associated with hyperglycaemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes [45]. Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus [46].

The recovery of renal function with treatment of fennel oil can be explained by the regenerative capability of the renal tubules. Similar results have been observed with the treatment of STZ induced diabetic rats with other herbal extracts as fenugreek alkaloid extract [5] and onion oil [6]. The role of fennel oil in reversing the diabetic state at the cellular level besides the metabolic normalization further proves its potential as an antidiabetic assert.

The present study revealed that the immediate action of STZ induced diabetes on pancreatic tissue was severe necrotic changes of pancreatic islets, especially in the center of the islets as well as nuclear changes, karyolysis, disappearing of nucleus and in some places residue of destructed cells. The ultra structure of STZ diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets. These are in agreement with earlier reports using other herbal extracts [5, 6]. The diabetic rats treated with fennel oil showed pancreatic islet regeneration. The excellent recovery of pancreatic tissue with treatment of fennel oil can be explained by the regenerative effect of exocrine cells of pancreas which may enlighten the positive effects of this agent on the production of insulin.

Fennel was traditionally reported to be highly recommended for diabetics. Our results demonstrated the marked improvements of hyperglycaemia and pathological changes induced by streptozotocin after fennel ingestion which can prove its effect as antidiabetic in folk medicine. Research studies on effect of fennel oil on blood glucose are not numerous. A single study by Barros et al [44] reported that fennel can improve rat glucose tolerance obviously.

The antioxidant potential of this herb, might explain some of its empirical uses in folk medicine for diabetes. This can be true as in diabetic subjects, altered glycaemia and lipaemia are closely correlated with markers of systemic oxidative stress [47]. In addition, fennel reported to suppress the nuclear factor-kappaB activation pathway which is linked to several inflammatory diseases as diabetes [48].

Based on our findings, we conclude that ingestion of essential oil of Foeniculum vulgare Mill to diabetic induced rats corrected the hyperglycaemia and pathological abnormalities which could be in part through its antioxidative effect and restoring of redox homeostasis. This makes the possibility of its inclusion in antidiabetic drug industry.

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