Renoprotective effects of Caraway (Carum carvi L.) essential oil in streptozotocin induced diabetic rats

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Abstract

Aim of the study was to assess the renoprotective effect of Carum carvi essential oil. Thirty male albino rats were divided into three groups: normal control (group A), diabetic positive control (group B), and experimental (group C) receiving Carum carvi oil as a renoprotective agent at 10 mg/kg of body weight orally. Group B and C rats received STZ (60 mg/kg) for induction of diabetes. Rats with fasting blood glucose levels over 200 mg/dl were considered diabetic. Blood samples were collected on the 22nd day for the determination of glutathione peroxidase and glucose. Kidneys were also extracted for examination. Carum carvi oil was analysed using the GC and GC/MS analysis. The major components of caraway essential oil were Carvone (70.1%) followed by γ-Terpinene (12.6%) and Limonene (5.5%) in addition to some minor compounds including Linalool (0.7%), Thymol (0.5%), γ-Cadinene (0.5%), α-Farnesene (0.4%), δ-Cadinene (0.4%). The diabetic group rats showed an increase in the serum level of glucose, and decrease in glutathione peroxidase. 10 mg/kg body weight dose of Carum carvi oil significantly corrected these parameters. The morphological examination of group B rats kidneys showed glomerular and tubular degeneration with massive cellular infiltration, hemorrhage in interstitial tissue and deformed renal tissue architecture. Whereas the kidney rats in group C showed marked improvement with minor pathological changes. Conclusion: Carum carvi oil showed renoprotection against diabetic nephropathy. This could be contributed to Carum carvi constituents, especially carvone, γ-Terpinene and Limonene which have strong anti-oxidant activity.

Introduction

Diabetes mellitus is a metabolic disorder of the endocrine system that is found in all parts of the world and is rapidly increasing worldwide. In spite of all the advances in therapeutics, diabetes still remains a major cause of morbidity and mortality. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge (Jung et al., 2006).

The search for a cure for diabetes mellitus continues along traditional and alternative medicine. Many herbal supplements have been used for the treatment of diabetes, but not all of them have scientific bases to support their effectiveness (Morelli & Zoorob, 2000). This led us to perform this study on caraway (Carum carvi L.) oil as possible treatment for diabetes mellitus. Caraway (Carum carvi L.) is a member of the group of aromatic Apiaceae (umbelliferous plants). Caraway is one of the oldest spices cultivated in Egypt. It is naturally found in North Africa, Turkey, Iran, India, Siberia, and Northern and Central Europe. The fruit of caraway is a schizocarp, which at harvest splits into two halves called seeds (De Carvalho & Da Fonseca, 2006). Caraway has been used since ancient times, especially for the treatment of digestive disorders. For ancient Egyptians, caraway soothes flatulence, digestive, breath freshener and antihelminthic (Abou El-Soud, 2010).
Caraway is known to have antihyperglycemic effect. Eddouks et al., 2004 showed that aqueous extract of caraway has antihyperglycemic effect in STZ induced diabetic rats. The plant extract and volatile oils from Carum carvi have also been used as antiulcerogenic agents and anti-flatulent colic in infants (Khayyal et al., 2001). Caraway has been commonly used in phytomedicine as antibacterial and laxative agent (Singh et al., 2002; Matev et al., 1981). Caraway seed oil is promising oil with high levels of bioactive compounds. The major constituents of its seeds are carvone, flavonoids and limonene. In addition, myrcene, beta caryophyllene, thujaone, anethole, and pinene are present as minor components (De Carvalho & Da Fonseca, 2006). The flavonoid constituents of caraway have been separated by means of chromatography on cellulose columns and constituents such as quercetin-3-glucuronides, isoquercitrin, quercetin 3-0 caffeoylglicoside, and kaempferol 3-glucoside were obtained (Kunzemmann & Hermann, 1977). However, little information is available on the effect of administration of caraway oil on kidney of diabetic experimental animals. To understand better its renoprotective effects, the present study assess the chemical composition of caraway essential oil and its hypoglycemic and antioxidant activity on glutathione peroxidase, in addition to study of histological specimens of kidney in streptozotocin induced diabetic rats.

MATERIALS & 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: caraway seeds (Carum carvi L.) was purchased from local markets and authenticated in the herbarium of Faculty of Science, Cairo University and National Research Center, Egypt.

Methods

Preparation of caraway essential oil

One kg of caraway seeds was subjected to hydrodistillation. The volatile oil then collected and dried in desiccators over anhydrous Ca SO₄. The volatile oil sample was kept in dark bottle till used.

Analysis of caraway essential oil

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

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 x 0.25 mm, film thickness 0.25 μm) 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 μl 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.

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 μl was injected, applying split mode (split ratio 100:1), into HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm; 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 alkane standards (C9-C24) and moreover with those of authentic compounds or published data (Minica, et al., 2004; 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).

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.

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 mM NaCl 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 caraway essential oil. (10 mg/kg b.w. orally).

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

**Biochemical Measurements**

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

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

**Histological study**

The kidney 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).

**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**

**Analysis of caraway oil**

The essential oil content of the dried seeds of caraway was 0.29%. The corresponding qualitative and quantitative chemical compositional data is given in Table 1. A total of 32 compounds were identified in the essential oil, accounting for 94.1% of the total oil.

The essential oil tested contained high amount of Carvone (70.1%) followed by γ –Terpinene (12.6%) and Limonene (5.5%). Some minor compounds including Linalool (0.7%), Thymol (0.5%), γ –Cadinene (0.5%), α- Farnesene (0.4%), δ- Cadinene (0.4%) α-Pine (0.3%), Linalyl acetate (0.3%), Germacrene-D (0.3%), Spathulenol (0.3%) Camphene (0.2%), Camphor (0.2%), β-Caryophyllene (0.2%), β- Selinene (0.2%), Eugenol (0.2%), Carvacrol (0.2%).

**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 caraway essential oil led to reduction in blood glucose level back to normal and increase in serum glutathione peroxidase even above the control group.

**Table. 1: Caraway (Carum carvi L.) seeds essential oil components.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>0.3</td>
</tr>
<tr>
<td>Camphene</td>
<td>0.2</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>0.1</td>
</tr>
<tr>
<td>β-Mycene</td>
<td>0.1</td>
</tr>
<tr>
<td>Limonene</td>
<td>5.1</td>
</tr>
<tr>
<td>γ – Terpinene</td>
<td>12.6</td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>0.1</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>0.1</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>0.1</td>
</tr>
<tr>
<td>limonene oxide</td>
<td>0.1</td>
</tr>
<tr>
<td>Camphor</td>
<td>0.2</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.7</td>
</tr>
<tr>
<td>Linalyl acetate</td>
<td>0.3</td>
</tr>
<tr>
<td>Terpinene-4-ol</td>
<td>0.1</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>0.2</td>
</tr>
<tr>
<td>Dihydrocarvone</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>0.1</td>
</tr>
<tr>
<td>Germacrene-D</td>
<td>0.3</td>
</tr>
<tr>
<td>Carvone</td>
<td>7.01</td>
</tr>
<tr>
<td>β- Selinene</td>
<td>0.2</td>
</tr>
<tr>
<td>α- Farnesene</td>
<td>0.4</td>
</tr>
<tr>
<td>Citronellol</td>
<td>0.1</td>
</tr>
<tr>
<td>δ - Cadinene</td>
<td>0.3</td>
</tr>
<tr>
<td>γ –Cadinene</td>
<td>0.5</td>
</tr>
<tr>
<td>Caminaldehyde</td>
<td>0.1</td>
</tr>
<tr>
<td>Nerol</td>
<td>0.2</td>
</tr>
<tr>
<td>Trans-carveol</td>
<td>0.1</td>
</tr>
<tr>
<td>Nonadecane</td>
<td>0.1</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>0.3</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.2</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.5</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>94.1</td>
</tr>
</tbody>
</table>

**Table. 2: Fasting blood glucose levels in different groups studied of experimental animals 21 days after induction of diabetes.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>diabetic</th>
<th>diabetic + caraway oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>73.07±9.11</td>
<td>162.5±3.19</td>
<td>61.76±3.73</td>
</tr>
</tbody>
</table>

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

**Table. 3: Glutathione peroxidise in different groups studied of experimental animals 21 days after induction of diabetes.**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>diabetic</th>
<th>diabetic + caraway oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (μg Hb)</td>
<td>82.68±4.48</td>
<td>59.72±2.78</td>
<td>99.93±5.86</td>
</tr>
</tbody>
</table>

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

**Histological Results**

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.
Fig. 1: Section of the kidney of a rat (a): control (Hx. & E. X 200). (b): Section of the kidney of streptozocin induced diabetic rat showing massive cellular infiltration (arrow) and areas of hemorrhage 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 kidney from streptozocin induced diabetic rat subjected to caraway showing only mild cellular infiltrate in the interstitial tissue (star), some glomeruli showed lobulation (arrow head) and others showed degeneration with wide urinary space (arrow).
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 caraway showed marked improvement with minor pathological changes in the form of some glomerular lobulation and some degeneration with wide urinary space. Mild cellular infiltration in interstitial tissue was also seen. (Fig. 2).

**DISCUSSION**

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 (Oberlay, 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). The series of auto-oxidative reactions that results in the formation and accumulation of advanced glyco-sylation end-products (AGE) in tissue proteins (Hunt et al., 1993), has oxidizing potential and can promote tissue damage by free radicals. (Baynes, 1991). The activation of hyperglycemia-induced secondary mediators, such as protein kinase C (PKC) and mitogen-activated protein kinase (MAPK), and cytokine production are also responsible for oxidative stress induced renal injury in the diabetic conditions. (Cooper, 2001).

Nephropathy is one of the important microangiopathic complications of diabetes mellitus. Recent studies indicate that reactive oxygen species play a key intermediate role in the pathophysiology of diabetic nephropathy (Ha & Kim, 1995). Hyperglycemia enhances the non-enzymatic glycosylation of proteins and form advanced glycosylation end-products (AGE), which are stable and re-sistant to degradation by enzymes and injure cells by the structural rearrangement of proteins. Increased serum levels of AGE seem to predict changes in renal morphology such as expansion of mesangial cell matrix and glomerular basement membrane (GBM) thickening (Berg et al., 1997). STZ induces diabetes mellitus (DM) in rodents and results in the development of nephropathy similar to early stage clinical diabetic nephropathy.

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). *Carum curvi* is one of these spices. Beside the antibacterial activity of *Carum curvi* (Iacobellis et al., 2005), nematicidal effect (Oka et al., 2000), cancer chemopreventive effect (Zheng et al., 1992) specially on colon cancer (Deeptha et al., 2006), hypolipidemic effect (Saghir et al., 2012), It was found that caraway may have potential antihyperglycemic properties (Haidari et al., 2011; Eddouks et al., 2004; Modu et al., 1997).

In trial to understand the mechanism of action of caraway oil and its effect on renal tissues in streptozotocin induced diabetic rats, we did quantitative and qualitative composition analysis of the used oil in addition to histopathological study of renal tissues. The present study confirmed the antihyperglycaemic action of *carum curvi* oil where the elevated glucose level in streptozotocin induced diabetic rats returned to normal level after treatment with caraway oil. In previous studies, the major constituents of caraway seed oil were carvone, flavonoids and limonene. In addition, myrcene, beta Caryophyllene, thujone, anethole, and pinene were present as minor components. (De Carvalho & Da Fonseca, 2006).

In the present study, the major component of caraway oil was Carvone (70.1%). Carvone is a monoterpen. Monoterpenes are 10-carbon members of the isoprenoid family of natural products (Gershenzon and Croteau, 1993). They are widespread in the plant kingdom and are often responsible for the characteristic odors of plants (Banthorpe and Charlwood, 1980). Elmastas et al., 2006 reported that carvone had a strong antioxidant activity. The next major components of caraway oil are monoterpenes namely γ–Terpinene (12.6%) and Limonene (5.5%), which are strong antioxidants. γ-Terpine is a major component of essential oils made from citrus fruits and shows strong antioxidant activity in various assay systems (Amiri, 2012), 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 selenocystein. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of selenocystein (Yrsini 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 caraway oil. The reduction of the enzyme level due to diabetes coupe 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 caraway oil
treatment could be related to antioxidative effect of caraway oil that decrease the oxygen free radical formation therefore increase the enzyme activity.

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 caraway oil but still with minimal residual effects in the form of glomerular lobulation and some degeneration with wide urinary space and mild cellular infiltration in interstitial tissue. A previous study had also reported similar histological findings (Sadiq et al., 2010).

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 caraway 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 renoprotective effects of Caraway (Carum carvi L.) essential oil in streptozotocin induced diabetic rats. These could be contributed to the synergistic action of its bioactive compounds namely carvon, γ-terpinene and Limonene.

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