The Egyptian Journal of Hospital Medicine (2008) Vol., 30: 39– 50

Diabetes Mellitus and Some Egyptian's Volatile Oils

Fahmy Gad Elsaid
Zoology Department, Faculty of Science, Mansoura University, Egypt

Abstract

Background: Diabetes mellitus as a metabolic disorder is characterized by hyperglycemia, dyslipidemia and deflection in protein metabolism. Natural products as a fashion remedy was undertaken and this study was designed to search the role of anise, fennel, thyme volatile oils and γ-terpinene (a bioactive constituent extracted from caraway and cumin volatile oils) to deal with the biochemical changes in sera, liver and muscle of streptozotcin (STZ)-induced diabetic rats.

Material and methods: Thirty rats (*Sprague dawelly)* were divided into three groups: control group; diabetic group, STZ-induced diabetic rats and diabetic & volatile oils group. The STZ-induced diabetic & volatile oil group was orally administered with different volatile oils by gavage (2 ml/ kg body weight) and subdivided into four subgroups: diabetic & anise; diabetic & fennel; diabetic & thyme and diabetic & γ- terpinene.

Results: There were highly significant increase in sera glucose, total lipids, total cholesterol, and triglycerides in diabetic rats. Liver and muscle malondialdehyde (MDA) and protein carbonyl (PC), superoxide dismutase, catalase and glutathione-s-transferase activities were remodeling after administration of different volatile oils. Sera insulin, liver glucose-6-phosphate dehydrogenase, liver and muscle glycogen was highly significantly decreased in diabetic rats. On the other hand, the alleviation in these parameters was highly noticed in the different diabetic & volatile oil subgroups.

Conclusion: The counter effects of different volatile oils upon these changes reflect the antihyperglycemia and antioxidant roles of these volatile oils with a different range in STZ-induced diabetic rats.

Key words: *Diabetes mellitus, streptozotcin, volatile oils, oxidative stress, antioxidants, glucose metabolism.*

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and protein metabolism that results from defects in both insulin secretion and/or insulin action (Adisakwattana *et al*., 2005). Activation of multiple metabolic pathways in diabetes leads to increased of generation of superoxide and derivative reactive oxygen species (ROS). These include increased mitochondrial electron transport activity that induced by hyperglycemia and fatty acids and enhanced glucose auto-oxidation (Desco *et al.,* 2002). Insulin resistance includes decreased stimulation of muscle glycogen synthesis, defects in glycogen synthesis and hexokinase activity (Muller *et al*., 1973) and decrease glucose-6-phosphate dehydrogenase (G6PhD) (Wan *et al*., 2002).

In streptozotocin (STZ) induced type 1 diabetes, hyperglycemia and oxidative stress have been implicated in the etiology and pathology of disease complications (Baynes, 1991). Diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) or impaired and reduction of antioxidant enzyme (Pari and Latha 2004; Vincent *et al*., 2004; Rahimi *et al*., 2005). During pathogenesis of diabetes mellitus, oxidative and nitrosative stresses contribute to the destruction of insulin-producing β-cells (Denaly *et al*., 1997).

Lipid peroxidation (LPO) is a key marker of oxidative stress is a free radical-induced process causing oxidative

deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction. The significant extent of LPO products that was measured as thiobarbituric acid reactive substances has been reported in diabetes (Rajasekaran *et al*., 2005). An increased accumulation of the LPO product has been shown in the liver of diabetic rats (Traverso *et al*., 2002). ROS leading to protein oxidation include radical species such as superoxide ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$), peroxyl ($RO_2^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroperoxyl ($HO_2^{\cdot}$), and nonradical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (Berlett and Stadtman 1997). The usage of PC groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins (Dalle-Donne *et al*., 2003).

Oxidative stress, arising as a result of an imbalance between free radicals and antioxidant defenses, is associated with damage to lipids, proteins, and nucleic acids, which could contribute to cellular dysfunctions leading to the pathophysiology of various diseases including atherosclerosis, cancer, and diabetes mellitus (Wan *et al*., 2000). The formation of ROS was prevented by an antioxidant system that included non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and ROS–scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) (Sarkhail *et al*., 2007). SOD is one of the important enzymes and scavenges the superoxide radical by converting them to H_2O_2 and molecular oxygen (Vincent et al., 2004; Lin *et al*., 2005). The decreased activities of SOD, CAT and glutathione peroxidase (GPx) in liver during diabetes mellitus may be due to the production of ROS such as superoxide ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) (Vincent *et al*., 2004; Kaleem *et al*., 2006). Moreover, CAT as has been regarded as a major determinant of hepatic antioxidant status and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals (Kaleem *et al*., 2006).

The glutathione-S-transferases (GSTs) constitute one of the major components of the phase II drug-metabolizing enzyme and antioxidant systems. It appears that GSTs are also regulated both in vivo and in vitro by reactive oxygen species (ROS) such as superoxides, H_2O_2 and by the products of membrane lipid peroxidation (Mari and Cederbaum, 2001). The GSTs catalyze the conjugation of glutathione to a wide range of electrophiles and represent a protective mechanism against oxidative stress (Ketterer, 1998). In STZ-induced diabetic rats, it has also been shown to cause a reduction in the glutathione (GSH)-dependent antioxidant potentials of tissues, thereby making them more susceptible to increased oxidative damage (Baynes and Thorpe, 1999). Tissue-specific altered expression of GST isoenzymes and related catalytic activities in STZ-induced diabetic rats were proved by (Raza *et al*., 2000). Saito-Yamanaka *et al*. (1993) found decreased GST activity in the liver of streptozotocin-induced diabetic rats as compared with normal rat livers.

The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Four major classes of oral hypoglycemic agents have been used extensively: insulin secretagogues, biguanides, thiazolidinediones and aglucosidase inhibitors (Charpentier, 2002). Each drug class works on different mechanism of actions, including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increase in insulin receptor sensitivity and delay of digestion and absorption of carbohydrate, respectively. Unfortunately, these agents could produce severe hypoglycemia, weight gain and gastrointestinal disturbances.

Many traditional folk medicinal herb extracts have been used for the treatment of diabetes mellitus. Many extracts showed good free radical scavenging activity. However, most of them have been shown to exert little or no effect on glycemic control in experimental studies, although some herbs possess hypoglycemic properties (Bailey and Day, 1989). Currently, there is growing interest in herbal remedies due to the side effects associated with the therap-

eutic agents (oral hypoglycemic agents and insulin) for the treatment of diabetes mellitus (Kameswara Rao *et al*., 1997). Natural plant drugs are frequently considered to be less toxic with lower side effects than synthetic ones (Hu *et al*., 2003; Aliahmadi *et al*., 2006). Recently, natural products have been explored for antidiabetic activity.

There is an extensive use of essential oils in various domains of human activities such as, aromatherapy, food flavoring fragrances, cosmetics and pharmacy. Anise and fennel essential oils (*Pimpinella anisum* L., *Foeniculum vulgarius* M., family *Apiaceae*) are commonly used as a natural remedy and as ingredients of cosmetic and pharmaceutical products for their balsamic, cardiotonic, digestive, lactogogue and tonic properties (Damjanovic *et al*., 2005). Hypoglycemic effects have been reported for some plants that contain terpenoids, iridoid glycosides, flavonoids, and other phenolic compounds (Li *et al.*, 2004). In addition, a number of secondary metabolites like flavonoids, phenolic acids, phenylpropanoids, and terpenoids have shown significant antioxidant properties (Harput *et al*., 2006; Topu *et al*., 2007).

Material and methods

Chemicals:

Anise (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgaris* M.) essential oils are commercially available and were purchased from Harraz Drug stores, Bab El-Khalk, Cairo, Egypt. They were analyzed by (GC) and GC-MS spectrometry (Dawidar *et al.*, 2007). The main components of anise oil were; *trans*-anethole (61.7%), α-longipinene (10.1%), *cis*-anethole (7.3%), cyclosativene (5.2%), isoledene (3.4%) and safrol (2.4.%), while those of fennel essential oil (EO) were; *d*-limonene (34.6%), *trans*-anethole (13.6%), d-carvone (11%), *cis*- sabinene hydrate (5.9%), fenchone (3.9%), β-longipinene (2.5%), 1,8-cineol (2.0%) and apiol (1.2%). Thyme (*Thymus vulgaris* L.) caraway (*Carum carvi*) and cumin (*Cuminum cyminum*) essential oils are commercially available in the local Egyptian market. They were also analyzed by (GC) and GC/MS spectrometry and one component of caraway and cumin oils (γ-terpinene, 9.77%) was only used. The chemicals used for chemical analysis were obtained from Sigma, BDH chemicals LTD.

Induction of diabetes:

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St Louis, MO, USA) into the tail vein at a dose of 65 mg/kg body weight. STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5 (Eddoucks et al., 2004). The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the 3rd day after STZ injection. The treatment was started on the 4th day after STZ injection and this was considered as 1st day of treatment. The treatment was continued for 7 days.

Animal grouping:

Male albino rats (*Sprague dawelly)*, with an average body weight of 130 to150 g, were purchased from National Research Center, Giza, Egypt. Animals were divided into three groups of five rats per group: control group; diabetic group, STZ-induced diabetic rats and diabetic & volatile oils group. The later one was subdivided into four subgroups: diabetic & anise; diabetic & fennel; diabetic & thyme and diabetic & γ-terpinene. The STZ-induced diabetic & volatile oil subgroups were individually administered with oils by gavage (2 ml/ kg body weight). During four hours of treatment, the animals were housed in steel mesh cage and were given free access to water and a powdered diet. Blood samples were collected from the medial acanthus of the eye for measuring blood glucose levels by One Touch blood glucose meter from life scan. With the end of seventh day, the animals were sacrificed and blood collected and centrifuged at 4000 rpm for 15 min. Sera collected and stored at -20°C for later analysis. The liver and gastrocnemius muscle specimens were cut and homogenized by electrical homogenizer and diluted to 10% in phosphate buffer (pH 7.4).

Methodology:

The concentration of serum glucose was estimated according to the method of Trinder (1969). The concentration of serum Insulin was estimated by the method of Yallow and Berson (1959). Liver glycogen

content was determined as described by Nicholas *et al.,* (1956). Glucose-6-phosphate dehdrogenase activity was measured as described by Chan *et al.,* (1965). Protien carbonyl content was measured by first forming labeled protein hydrazone derivatives using 2, 4- dinitrophenyl hydrazine (Smith *et al.,* 1991). The SOD activity of Pancreas and liver homogenates was assayed by the procedure of Niskikimi *et al*. (1972). Catalase (CAT) activity was measured according to the method of Aebi (1980). Glutathione-s-transferase (GST) enzyme activity was determined according to the method of Habig and Jakoby (1981). Lipid peroxidation (MDA) was measured in liver homogenate according to the modification of the method of Ohkawa *et al.* (1979). Liver total lipids level was estimated according to the method of Frings *et al.* (1972). The concentration of total cholesterol in serum was estimated method of Young (2001). Serum triglycerides concentration was estimated by method of Fossati and Prencipe (1982).

Results

Table (1), serum insulin shows very highly decrease in diabetic rats compared to their control but there is a highly significant hyperglycemia which reflected by highly significant decrease in liver and muscle uptaking of glucose from the blood as there is a decrease in the liver and muscle glycogen content. γ-Terpinene is more efficient than anise, fennel and thyme in alleviation of these changes.

Table (2), shows very highly significant increase in malondialdehyde in both liver and muscle tissues in diabetic rats, but the improvements are highly significant in the active component γ-terpinene than the other volatile oils. On the other hand, liver and muscle total lipids showed very highly significant decrease in diabetic rats but in sera there are highly significant increases in total lipids, total cholesterol and triglycerides levels in diabetic rats. The improvement is pronounced in all treated groups.

In table (3): there is a highly significant increase in liver and muscle protein carbonyl in diabetic group in comparing with control group. There is more alleviation in diabetic & thyme group than in others treated groups.

Table (4): liver and muscle SOD, CAT and GST activities are very highly significant decrease in diabetic rats and all volatile oils and γ-terpinene show high efficient as antioxidant agents.

Table (1): Serum glucose and insulin level (mg/dl); liver glucose-6-phosphate dehydrogenase (U/g); liver and muscle glycogen content (mg/100g) in different groups:

	Control	**Diabetes**	**Diabetes & Anise**	**Diabetes & Fennel**	**Diabetes & Thyme**	**Diabetes & γ-Terpinene**
Insulin	0.85±0.03	0.24±0.02***	0.52±0.01***	0.73±0.16***	0.45±0.05***	0.87±0.01
Glucose	107.4±5.4	366.6±6.1***	203.0±2.5***	228.2±3.4***	225.8±1.1***	112.0±1.6
Liver glycogen	661.9±16.7	252.3±2.3***	261.9±4.1***	329.9±9.5***	350.9±4.4***	388.6±6.5***
Muscle glycogen	284.6±5.1	97.1±1.6***	133.1±2.0***	184.1±2.8***	193.4±2.7***	198.2±1.3***
G6phD	3.7±0.05	1.1±0.03***	2.1±0.12***	2.7±0.08***	1.4±0.05***	3.5±0.23**

Mean±SD, P< 0.5 *, P< 0.01 ** and P< 0.001 ***

Table (2) Liver and muscle MDA (nmole/g) and total lipids (mg/g) and serum total lipids (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) in different groups:

	Control	Diabetes	Diabetes & Anise	Diabetes & Fennel	Diabetes & Thyme	Diabetes & γ-Terpinene
Liver MDA	108.3±6.3	396.9±3.6***	310.4±6.5***	234.5±3.6***	287.3±5.3***	125.3±3.1***
Muscle MDA	85.9±4.2	189.6±2.1***	118.6±1.8***	139.6±1.6***	79.8±2.1***	62.5±1.4***
Liver total lipids	591.1±1.5	355.2±1.6***	442.2±2.1***	486.8±2.2***	344.2±1.5***	445.3±7.4***
Muscle total lipids	551.1±0.9	361.8±1.7***	481.2±2.2***	488.2±1.8***	464.9±2.5***	435.5±1.5***
Serum total lipids	444.9±3.8	524.7±4.2***	372.7±1.9***	365.9±8.8***	342.2±3.3***	377.4±2.3***
Serum total cholesterol	71.3±1.2	176.3±1.5***	71.96±0.92	100.4±1.1***	105.2±1.7***	83.2±1.5***
Serum triglycerides	205.6±1.5	265.2±4.5***	257.6±2.2***	247.2±1.5***	246.8±2.5***	234.3±2.6***

Mean±SD, P< 0.5 *, P< 0.01 ** and P< 0.001 *

Table (3) Liver and muscle protein carbonyl (µmol/g) and total protein contents (mg/g) in different groups:

	Control	Diabetes	Diabetes & Anise	Diabetes & Fennel	Diabetes & Thyme	Diabetes & γ-Terpinene
Liver PC	60.3±1.11	146.1±1.5***	124.8±2.3***	107.5±2.5***	75.1±1.5***	120.3±3.2***
Muscle PC	91.2±1.6	204.8±3.6***	154.8±1.9***	128.7±3.9***	112.3±2.22***	123.0±6.19***
Liver total protein	10.1±0.78	8.4±0.44**	13.2±1.9***	9.0±0.1	9.8±0.47	10.6±0.45
Muscle total protein	10.7±0.66	7.7±0.16***	8.2±0.12***	7.5±0.15***	8.4±0.26***	7.3±0.16***

Mean±SD, P< 0.5 *, P< 0.01 ** and P< 0.001 *

Table (4): Liver and muscle SOD (U/mg), CAT (U/mg) and GST (U/sec/mg) activities in different groups:

	Control	Diabetes	Diabetes & Anise	Diabetes & Fennel	Diabetes & Thyme	Diabetes & γ-Terpinene
Liver SOD	16.5±0.3	14.3±0.2***	16.6±0.3	11.4±0.3***	15.2±0.2***	15.5±0.2***
Muscle SOD	13.9±0.2	8.6±0.2***	12.7±0.2***	11.7±0.5***	10.6±0.3	11.9±0.4***
Liver CAT	1.4±0.1	0.35±0.02***	0.48±0.02***	0.89±0.01***	0.67±0.02***	1.3±0.1
Muscle CAT	0.77±0.02	0.37±0.02***	0.82±0.01**	0.63±0.4***	0.48±0.01***	0.72±0.4*
Liver GST	1.3±0.07	0.19±0.02***	0.31±0.02***	0.44±0.02***	0.95±0.03***	0.36±0.2***
Muscle GST	1.8±0.04	0.36±0.02***	0.3±0.02***	0.25±0.2***	0.93±0.04***	0.19±0.01***

Mean±SD, P< 0.5 *, P< 0.01 ** and P< 0.001 *

Discussion

Reactive oxygen and nitrogen species are highly reactive and potentially damaging transient species (characterized by the presence of unpaired electrons) formed in all tissues during normal aerobic cellular metabolism (e.g. via leakage of electrons from mitochondria) (Cheeseman and Slater, 1993) and are formed also in higher fluxes under pathological conditions such as diabetes. In STZ-induced diabetic rats there was an increase in lipid peroxidation and an indirect evidence of intensified free radical production (Maritim *et al*., 2003). Most of the tissue damage is consider to be mediated by the free radicals and ROS by attacking membranes through peroxidation of unsaturated fatty acids (Stringer *et al.,* 1989). Also, an observed increase in the pancreas level of thiobarbituric acid reactive substances (MDA) may be due to increased susceptibility of the tissue of diabetic rats to lipid peroxidation (Matkovics *et al.,* 1998). In STZ-induced diabetes, oxidative stress contributes to the destruction of insulin-producing β-cells, (Denaly *et al.,* 1997) and this logically explains the acute shortage of plasma insulin level and a significant hyperglycemia in diabetic rats. It is well documented that there is an elevation of serum lipids concentration in diabetics (Chase and Glasgow, 1976). The increase in serum total lipids, triglycerides, total cholesterol levels, due to the increase of liver and muscle lipid peroxidation, decrease insulin-stimulated glucose uptake in the whole body, mainly in the muscle and liver which impair insulin mediated suppression of endogenous glucose production, mainly from the liver (Nakahara *et al*., 2004), decrease liver G6PhD level (Wan *et al*., 2002) and impairs hepatic glucose uptake that can lead to metabolic derangement in diabetic rats. A significant hyperglycemia; enhanced glucose auto-oxidation (Desco *et al.,* 2002) and insulin resistance includes decreased stimulation of muscle glycogen synthesis, all of these defect glycogen synthesis (Muller *et al.,* 1973). The decrease in liver and muscle glycogen contents may be interpreted by a decrease in liver glucose-6-dehydrogenase(Wan *et al*., 2002) which attacked and inhibited by ROS in diabetic rats. The present findings indicate significantly increased lipid peroxidation in liver and muscle of STZ induced diabetic rats and its attenuation by different volatile oils treatment especially with γ-terpinene and fennel. This suggests a protective role of volatile oils and bioactive γ-terpinene which act as strong superoxide radical and singlet oxygen catchers.

On the other hand, Reactive oxygen and nitrogen species cause cellular damage, an important part of which is the oxidation of amino acid residues on proteins, forming protein carbonyls (Chevion *et al*., 2000). Moreover, The PC may be introduced into proteins by secondary reaction of the nucleophilic side chains of cystiene, histidine, and lysine residues, with malondialdehyde (Dalle-Donne *et al*., 2003). In diabetic rats, liver and muscle PC contents (arguably of equal importance as lipid peroxidation) were significantly increased and which reflect the decrease in total proteins in both investigated tissues. These changes were alleviated after administration of different VOs especially in thyme volatile oil and γ-terpinene groups. Administration of medicinal plant extract to mildly STZ-diabetic rats resulted in activation of β-cells and returns to normal giving insulinogenic effect (Padmini and Chakrabarti, 1982). Volatile oils may bring about its hypoglycemic action through stimulation of surviving β-cells of islets of langerhans to release more insulin. A number of other plants have also been observed to exert hypoglycemic activity through insulin-release stimulatory effects (Pari and Uma Maheswari, 2000; Prince and Menon, 2000). Some bioactive compounds isolated from plants like terpenoids and flavonoids were reported to affect pancreatic β-cells and stimulate insulin secretion with numerous mechanisms such as exertion distal to K^+-ATP channels and L-type Ca^{2+} channels (Hoa *et al.,* 2007), activation of the cAMP/PKA signaling (Liu *et al*., 2006), and antioxidant activities (Waltner *et al*., 2002; Elmali *et al*., 2004). Since oxidative stress and free radicals injure or destroy pancreatic β-cells in

diabetes, so volatile oils are able to increase the secretion of insulin via its antioxidant actions.

Cells are protected from ROS induced damage by a variety of endogenous ROS scavenging proteins, enzymes and chemical compounds (Cheeseman and Slater 1993). Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes mellitus. Insulin deficiency promotes the β-oxidation of fatty acids with resulting H_2O_2 formation (Horie et al., 1981). Antioxidant enzymes (SOD, CAT) have been shown an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to STZ (Pari and Latha 2004).The present data well indicate that STZ-induced diabetes disrupts actions of hepatic and muscular antioxidant enzymes (Zhang and Tan 2002). These observations emphasis the critical importance of maintaining the antioxidant potential of pancreatic β-cells in order to ensure both its survival and insulin secretion capacity during times of increased oxidation stress. The decreased activities of SOD and CAT in liver during diabetes mellitus may be due to the production of ROS (Vincent *et al.,* 2004; Kaleem *et al.,* 2006). The observed decrease in SOD activity in diabetic control rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme, which have been reported to occur in diabetes (Soon and Tan 2002; Ravi *et al.,* 2004). CAT is involved in the elimination of high concentration of H_2O_2 and it has been regarded as a major determinant of hepatic antioxidant status and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals. Decrease in CAT activity could result from inactivation by superoxide radical and glycation of the enzyme (Rajasekaran *et al.,* 2005).

A reduced GST dependent detoxification of toxic metabolites accompanied by an increased production of ROS, during aerobic glycolysis and increased membrane LPO would, therefore, have serious consequences for cellular functions in the tissues in diabetes, so GSTs may offer protection against diabetes mellitus. Raza *et al.,* (2004) have reported hyperglycemia and glutathione-associated oxidative stress in rats one week after treatment with STZ. In this study lower levels of hepatic antioxidant enzymes and increased LPO in diabetic groups were observed when compared with normal control meanwhile oral administration with volatile oils partially reduced the imbalance between the generations of ROS and scavenging enzymes activity in diabetic rats. As mentioned above, this effect of VOs also backs to existence of terpenoids, iridoid glycosides, flavonoids and related phenolic compounds in it (Li *et al.,* 2004)., as well-known antioxidants (Rahimi *et al.,* 2005; Harput *et al.,* 2006; Topu *et al.,* 2007), which scavenge the free radicals and other ROS generated during diabetes.

Conclusion

In conclusion, the present study showed that anise, thyme and fennel volatile oils and γ-terpinene have double effects which are beneficial in the controlling of diabetes disorders because they have insulinogenic action on β-cells and reduce the insulin resistance of cells especially for liver and muscle cells, also combating oxidative stress by scavenging the ROS and activation of hepatic and muscular antioxidant enzymes.

References

1. **Adisakwattana S, Roengsamran S, Hsu W H and Yibchok-anun S (2005):** Mechanisms of antihyperglycemic effect of p-methoxycinnamic acid in normal and streptozotocin-induced diabetic rats. Life Sciences, 78: 406 -412.
2. **Aebi, H. (1984):** Catalase in vitro. Methods Enzymol., 105:121-126.
3. **Aliahmadi A, Rahmani N and Abdollahi M (2006):** Plant-derived human vaccines; an overview. Int J Pharmacol, 2: 268-279.
4. **Bailey CJ and Day C (1989):** Traditional plant medicines as treatments fordiabetes. Diabetes Care, 12: 553-564.

5. **Baynes J (1991):** Role of oxidative stress in development of complications in diabetes. Diabetes, 40, 405-412.
6. **Baynes JW and Thorpe SR (1999):** Role of oxidative stress in diabetic complications: a new perspective on an old paradigm.Diabetes, 48, 1–9.
7. **Berlett BS and Stadtman ER (1997):** Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.*, 272, 20313–20316.
8. **Chan TK, Todd D and Wong CC (1965):** "Colorimetric determination of glucose-6-phosphate dehydrogenase (G6PD) activity". J. Lab Clin. Med., 66: (6): 936-940.
9. **Charpentier G (2002):** Oral combination therapy for type 2 diabetes. Diabetes/Metabolism Research and Reviews 18 (Suppl 3), S70– S76.
10. **Chase PH and Glasgow AM (1976):** Juvenile diabetes mellitus and serum lipids and lipoprotein levels. American Journal of Diseases of Child, 130, 1113–1117.
11. **Cheeseman K H and Slater T F (1993):** An introduction to free radical biochemistry. Br Med Bull,. 49:481–493.
12. **Chevion M, Berenshtein E and Stadtman ER (2000):** Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic. Res.,* **33**: 99-108,.
13. **Dalle-Donne I, Rossi R, Giustarini D, Milzani A and Colombo R (2003):** Protein carbonyl groups as biomarkers of oxidative stress. Clinica Chimica Acta**,** 329, (2): 23-38.
14. **Damjanovic, B., Lepojević, Z., Živković, V. and Tolić, A. (2005):** Food Chemistry, 92: 143-149.
15. **Dawidar AM, Abdel-Mogeb M, El-Ghorab A H, Mahfouz M and Hussien Kh (2007):** Chemical composition and effect of photo-oxygenation on biological activities of Egyptian commercial essential oils. Ph.D., Chemistry Department, Faculty of Science, Mansoura University, mansoura, Egypt.
16. **Delaney CA, Paviovic D, Hoorens A, Pipeleers DG and Eizirik DL (1997):** Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells, Endocrinology 138: 2610–2614.
17. **Desco M, Asensi C and Marquez R (2002):** Xanthine oxidase is involved in free radical production in type 1 diabetes. Diabetes, 51:1118-24.
18. **Eddouks M, Lemhadri A and Michel JB (2004):** Caraway and caper: potential anti-hyperglycaemic plants in diabetic rats. J Ethnopharmacol, 94(1): 143-148.
19. **Elmali E, Altan N and Bukan N (2004):** Effect of the sulphonylurea glibenclamide on liver and kidney antiox-idant enzymes in streptozocin-induced diabetic rats. Drugs R D; 5: 203-208.
20. **Fossati P and Prencipe L (1982):** "Serum Triglycerides determined colourimetrically with an enzyme that produces hydrogen peroxide". Clin. Chem., 28(10):2077.
21. **Frings CS, Fendley TW, Dunn RT and Queen CA (1972):** Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. Clin Chem., 18: 673.
22. **Habig WH and Jakoby WB (1981):** Assays for differentiation of glutathione S-transferases. Methods Enzymol., 77:398-405.
23. **Harput US, Calis I, Saracoglu I, Donmez AA and Nagatsu A (2006):** Secondary metabolites from *Phlomis syriaca* and their antioxidant activities. Turk. J. Chem., 30: 383–390.
24. **Hoa NK, Norberg A, Sillard R, Van Phan D, Thuan ND, Dzung DT, Journvall H and Ostenson CG (2007):** The possible mechanism by which phansonide stimulates insulin secretion from rat islets. Endocrinol J.,192: 389-394.
25. **Horie S Ishii H and Suga T (1981):** Changes in peroxisomal fatty acid oxidation in the diabetic rat liver. Journal of Biochemistry 90,:1691-1696.

26. **Hu X, Sato J, Oshida Y, Yu M, Bajotto G and Sato Y (2003):** Effect of Gosha-jinki-gan (Chinese herbal medicine: Niu-Che-Sen-Qi-Wan) on insulin resistance in STZ induced diabetic rats. Diab Res Clin Pract., 59: 103-11.
27. **Kaleem M, Asif M, Ahmed QU and Bano B (2006):** Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J., 47 :670–5.
28. **Kameswara Rao B, Giri R, Kesavulu MM and Apparao C (1997):** Herbal medicine: in the management of diabetes mellitus. Manphar Vaidhya Patrika I, pp. 33–35.
29. **Ketterer B (1998):** Glutathione S-transfe-rases and prevention of cellular free radical damage, Free Radic. Res., 28: 647–658.
30. **Li W.L, Zheng HC, Bukuru J and De Kimpeb N (2004):** Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol., 9, (2): 1-21.
31. **Lin YF, Tsai HL, Lee YC and Chang S J (2005):** Maternal vitamin E supplement-ation affects the antioxidant capability and oxidative status of hatching chicks. J. Nutr., 135: 2457-2461.
32. **Liu D, Zhen W, Yang Z, Carter JD, Si H and Reynolds K A (2006):** Genistein acutely stimulates insulin secretion in pancreatic β-cells through a cAMPdep-endent protein kinase pathway. Diabetes., 55:1043–50.
33. **Mari M and Cederbaum AI. (2001):** Induction of catalase alpha and microsomal glutathione S-transferase in CYP 2E1 overexpressing HepG2 cells and protection against short-term oxidative stress. Hepatology 33: 652–661.
34. **Maritim A C, Sanders R A and Watkins JB (2003):** Diabetes, Oxidative Stress and antioxidants. Journal of Biochemical and Molecular Toxicology 17 (1): 24–38.
35. **Matkovics B (1977):** Effect of plant and animal lesions on superoxide dismutase activities. In: McCord, Michelson A.M., Fridovich, I. (Eds.), Superoxide and superoxide dismutase. Academic press, New York, pp. 501–515.
36. **Muller WA, Faloona GR and Unger RH, (1973):** Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. The American Journal of Chinese Medicine, 54: 52–57.
37. **Nakahara I, Matsuhisa M, Shiba Y, Akio Kuroda A, Nakatani Y, Hatazaki M, Kajimoto Y, Kubota M, Yamasaki Y and Hori M (2004):** Acute elevation of free fatty acids impairs hepatic glucose uptake in conscious rats. Diabetes Research and Clinical. Practice, 66: 109-118.
38. **Nicholas V, Robert W and Joseph H R (1956):** The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem, 220(2): 583-593.
39. **Niskikimi M, Rao A and Yagi K (1972):** "The occurrence of superoxide anion in the reaction of reduced phenazine methosu-lphate and molecular oxygen."Biochem. Biophys. Res. Communications, 46: 849–854.
40. **Ohkawa H, Oshishi N and Yagi K (1979):** Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351–358.
41. **Padmini K and Chakrabarti CH (1982):** Effects of Bittergourd (Momordica charantia) seed and glibenclamide in streptozotocin induced diabetes mellitus. Indian Journal of Experimental Biology, 20: 1232–1235.
42. **Pari L and Latha, M. (2004):** Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid perox-idation in STZ diabetic male Wistar rats. BMC Complement Altern Med; 4:16–24..,
43. **Pari L and Uma Maheswari J (2000):** Antihyperglycemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan diabetic rats. Journal of Ethnopharmacology, 14:136-138.

44. **Prince SM and Menon VP (2000):** Hypoglycemic and other related actions of Tinospora cordifolia in alloxan induced diabetic rats. Journal of Ethnopharmacology 70: 9-15.
45. **Rahimi R, Nikfar S, Larijani B and Abdollahi M (2005):** A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother; 59: 365-73.
46. **Rajasekaran S, Sivagnanam K and Subramanian S (2005):** Antioxidant effect of *Aloevera* gel extract in streptozotocin-induced diabetes in rats. Pharmacol Rep., 57:90-96.
47. **Ravi K, Ramachandran B and Subramanian S (2004):** Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. Biol Pharm Bull., 27: 1212–1217.
48. **Raza H Ahmad J and John A (2004):** Tissue specific expression and immunohi-stochemical localization of glutathione S-transferase in streptozotocin induced diabetic rats: Modulation by Momordica charantia (karela) extract. Life Sciences, 74: 1503–1511.
49. **Raza H, Ahmed I., John A and Sharma AK (2000):** Modulation of xenobiotic metabolism and oxidative stress in chronic streptozotocin-induced diabetic rats fed with Momordica charantia fruit extract. Journal of Biochemical and Molecular Toxicology 14: 131–139.
50. **Saito-Yamanaka N, Yamanaka H. and Nagasawa S (1993):** Glutathione related detoxification functions in streptozotocin-induced diabetic rats, J. Vet. Med. Sci., 55: 991-994.
51. **Sarkhail P, Rahmanipour S, Fadyevatan S, Mohammadirad A, Dehghan G, Amin G, Shafiee A, Abdollahi M (2007):** Antidiabetic effect of *Phlomis anisodonta*: Effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Pharmacological Research 56: 261-266
52. **Sekar N, Kanthasamy S, William S, Subramanian S and Govindasamy S (1990):** Insulinic actions of vanadate in diabetic rats. Pharmacological Research 22, 207-217.
53. **Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA and Markesbery W R (1991):** ″Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease″. Proc. Natl. acad. Sci. USA., 88:105540-105543.
54. **Soon YY and Tan BK (2002):** Evaluation of the hypoglycemic and anti-oxidant activities of *Morinda officinalis* in streptozotocin-induced diabetic rats. Singapore Med J ., 43: 077–85.
55. **Stringer MD, Gorgo PG, Freeman A and Kakkar VV (1989):** Lipid peroxides and atherosclerosis. Brazilian Medical Journal 298: 281-284.
56. **Topu G, Ertas A, Kolak U, Ozturk M and Ulubelen A (2007):** Antioxidant activity tests on novel triterpenoids from *Salvia macrochlamys*. ARKIVOC., 7: 195–208.
57. **Traverso N, Menini S, Odetti P, Pranzato MA, Cattalosso D and Marinari UM (2002):** Diabetes impairs the enzymatic disposal of 4-hydroxynonenal in rat liver. Free Radicals in Biology and Medicine 32: 350-359.
58. **Trinder P (1969):** Colourimetric method for the serum glucose. Ann. Clin. Biochem., 6: 24.
59. **Vincent AM, Russell JW, Low P and Feldman EL (2004):** Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev., 25: 612-628.
60. **Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M and Granner DK (2002):** Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem., 277: 34933-34940.
61. **Wan G H, Tsai S C and Chiu DT (2002):** "Decreased blood activity of glucose-6-phosphate dehydrogenase associates with

increased risk for diabetes mellitus". Endocrine, 19: 191-195.

62. **West JC (2000):** Radicals and oxidative stress in diabetes, Diabet. Med. 17: 171-180.
63. **Yallow R S and Berson S A (1959):** "Assay of plasma insulin in human subjects by immunological method". Nature (London), 148:1648.
64. **Young D S (2001):** "Effects of disease on clinical lab Tests".4th ed. AACC.
65. **Zhang X F and Tan B K (2000):** Anti-diabetic property of ethanolic extract of andrographis paniculata in streptozotcin-diabetic rats. Acta Pharmacol Sin, 21, 12: 1157-1164.

تأثير بعض الزيوت الطيارة المصرية على مرض السكر المستحث بمادة الستربتوزوتسين فى الجرذان

فهمى جاد السعيد

قسم علم الحيوان - كلية العلوم – جامعة المنصورة- مصر

يهدف البحث لدراسة تأثير بعض الزيوت الطيارة المصرية مثل الينسون و الزعتر و الشمر و أيضا مادة الجاما- تيربينين المستخلصة من زيتي الكمون و

الكراوية العطريين. و قد استخدمت الدراسة ذكور الجرذان البالغة و التى تزن فى المتوسط 140 جم للفأر. و قسمت المجموعات كالتالى:

1 -المجموعة الضابطة: لم يتم حقنها بأى مادة.

2 -المجموعة المصابة بالسكر: تم حقنها فى الوريد الذيلى بجرعة واحدة من الستريبتوزوتسين (55مجم/كجم) و فى اليوم الثالث اعتبرت الجرذان مصابة بالمرض عند وصول مستوى سكر الدم إلى 250 مجم/100 ملى دم.

3 -المجموعة المصابة بالسكر و المعالجة بالزيوت الطيارة: و قد إستحث المرض فيها بنفس الجرعة السابقة ثم تم الحقن الفمى للزيوت الطيارة بجرعة (2ملى/كجم) و ذلك من اليوم الرابع و لمدة سبعة أيام على التوالي و قسمت كالتالي:

أ - المجموعة المصابة بالسكر و المعالجة بزيت الينسون العطرى:
تم حقن الجرذان المصابة بالسكر بزيت الينسون العطرى (2مجم/كجم).

ب- المجموعة المصابة بالسكر و المعالجة بزيت الزعتر العطرى:
تم حقن الجرذان المصابة بالسكر بزيت الزعتر العطرى (2مجم/كجم).

ت- المجموعة المصابة بالسكر و المعالجة بزيت الشمر العطرى:
تم حقن الجرذان المصابة بالسكر بزيت الشمر العطرى (2مجم/كجم).

ث- المجموعة المصابة بالسكر و المعالجة بمادة الجاما- تيربينين :
تم حقن الجرذان المصابة بالسكر بالجاما تيربينين (2مجم/كجم).

و قد استنبطت الدراسة أن ما يزيد من الاصابة بمرض السكر هو الزيادة المعنوية للضغط التأكسدى فى أنسجة الجسم عامة وخاصة الكبد و العضلات محل الدراسة متمثلة بالارتفاع المعنوى فى مستوى المالون داى ألدهيد و محتوى الكربونيل بروتين أيضا بسبب النقص الحاد فى مستوى إنزيمات الأكسدة مثل SOD و CAT وكذلك فى إنزيم GST للمجموعة المصابة بالسكر.

و كان لاستخدام الزيوت الطيارة الينسون و الزعتر و الشمر تأثيرا معنويا فى تحسن الاختلالات المصاحبة لمرض السكر و إن كان التأثير أكثر وضوحا فى المجموعة المعالجة بالجاما تيربينين المستخلص من زيتى الكمون و الكراوية العطريين.

وبناءا عليه توصى الدراسة باستخدام الزيوت الطيارة و مستخلاصاتها لما لها من تأثيرات مختلفة حيث تعمل على تقليل سكر الدم و ذلك لأنها تعمل على زيادة إفراز الانسيولين من خلايا بيتا فى البنكرياس و تقلل مقاومة خلايا الكبد و العضلات للانسيولين كما أنها تعتبر من مضادات الأكسدة لما تحتويه على فلافينويدات و تيربينويدات و غيرها و التى تعمل على زيادة نشاط إنزيمات الأكسدة SOD و CAT و غيرها مثل GST كما تعمل أيضا على التخلص من الشوارد الحرة بأنواعها المختلفة لذلك فهى تقلل الضغط التأكسدى فى الأنسجة.