Remedial Effect of Cinnamon Zeylanicum on serum anti-oxidants levels in male diabetic Rat

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Abstract: Cinnamon is an antioxidant and it has been shown to reduce oxidative stress. Previous study confirmed antioxidants enzymes have imbalanced on diabetes by hyperglycemia role on reactive oxygen’s spaces and is one of the male infertility agents. Wistar male rat (n=40) were allocated into four groups, control group (n=10), Cinnamon group that received 75mg/kg by gavage method, diabetic group and diabetic group that treatment Cinnamon 75mg/kg daily for 30 days respectively, however the control group just received an equal volume of distilled water daily. In 30 day, 5 cc blood sample of each rat was taken for anti-oxidants measurement. serum catalase, Superoxide dismutase and Glutathione peroxidase levels significantly were increased in group that has received 75mg/kg Cinnamon in comparison to control and diabetic groups (P<0.05). Serum malondialdehyde level significantly was decreased in group that has received 75mg/kg Cinnamon in comparison to control and diabetic groups (P<0.05). Since in our study 75mg/kg Cinnamon has significantly increased serum anti-oxidants levels so it seems using it in diabetic male patients has beneficial effects.


Keywords: Antioxidant, CAT, Cinnamon, GPX, infertility, MDA, SOD.

1. Introduction

In most instances, simple lifestyle changes like weight loss through proper exercise and nutrition can help reverse the effects of infertility. However, diseases like diabetes can cause extra complications for both women and men when it comes to fertility. Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced (Evans et al., 2003). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia. Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Humans are capable of digesting some carbohydrates, in particular those most common in food; starch, and some disaccharides such as sucrose, are converted within a few hours to simpler forms, most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. Research has also shown a direct link between blood sugar levels and sperm quality. In patients with elevated blood sugar levels, the incidence of malformed or dead sperm being found is semen is greatly increased (khaki et al., 2010).

Antioxidants have become rather popular lately, and I thought a list of most potent antioxidant spices available in nowadays. Among dietary antioxidants, polyphenols have been linked with the hypothesis that their redox activities may confer specific health benefits (Scalbert et al., 2005). As we know oxidative stress has been described as an important factor in many diseases such as diabetes (Dandona et al., 2005) atherosclerosis (Furukawa et al., 2004; Govindarajan et al., 2005), infertility and inflammation (Dragsted, 2003). In people that are interface with oxidative stress, by increasing antioxidant dietary supplements intakes will be a possible method to reduce the incidence of these pathologies (khaki et al., 2011). Among dietary antioxidants, polyphenols have been linked with the hypothesis that their redox activities may confer specific health benefits (Scalbert et al., 2005). Cinnamon extract would improve oxidative stress in people that are overweight or obese with impaired fasting glucose, and consequently be a possible nutritional approach in reducing the risk of infertility, cardiovascular, inflammations diseases and oxidative stress related complications (Shobana et al., 2000). The flavonoids are a group of benzopyran derivatives which occur widely in plants. The flavonoids typically consist of a benzene ring fused with the heterocyclic six-membered ring containing an oxygen atom. Flavonoids are products of plant metabolism and have different phenolic structures, (Drobiova et al., 2011). The aim of this study was to seen the
2. Material and Methods

2.1. Animals
Forty adult wistar albino male rats were 8 weeks old and weighing 250±10g, they were obtained from animal facility of pasture institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz Medical University. All Rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the wistar male rats (n=20) were allocated into two groups, control group (n=10) and Cinnamon zeylanicum group that received 75mg/kg (n=10), by gavage method, daily for, 4 weeks, respectively; however, the control group just received an equal volume of distilled water daily.

2.2. Preparation of Cinnamon:
Cinnamon zeylanicum were bought in Istanbul province, Istanbul city of Turkey. By mixer 100 grams of Cinnamon zeylanicum were reduction to powder. Daily 75mg/kg of it was solve in 2cc distilled water and each rat was received it daily for 30 consequences days.

2.3. Induction of experimental type I diabetes
Experimental type I diabetes was induced in rats by intra peritoneal (I.P) injection of 55 mg/kg streptozotocin (STZ) in distilled water. Control rats were only received distilled water.

2.4. Blood glucose determination
Blood samples were collected from the tail’s vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL). Sample collections were then made 48 h after STZ injection, and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dl were declared diabetic and used in the experimental group. One week after the induction of experimental diabetes, protocol was started.

2.5. Measurement of Serum MDA
Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard.

2.6. Glutathione peroxidase (GPx) activity measurement in serum
GPx activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H2O2 in the presence of reduced glutathione (10 mM), NADPH, (4 mM), and 1 U enzymatic activity of GR.

2.7. Super oxide dismutase (SOD) activity measurement in serum
The activity of superoxide dismutase (SOD) was measured by following the method of Beyer and Fridovich.

2.8. Catalase (CAT) activity measurement in serum
Serum catalase activity was determined according to the method of Beers and Sizer as described by (Usoh et al., 2005) by measuring the decrease in absorbance at 240nm due to the decomposition of H2O in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50mM, pH 7.0) and 2.9ml of 30mM H2O2 in phosphate buffer pH 7.0. An extinction coefficient for H2O2 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2 reduced per minute per mg protein at 240nm of 40.0M-1 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2O2 reduced per minute per mg protein.

2.9. Statistical analysis
Statistical analysis was done using the ANOVA and test for comparison of data in the control Group with the experimental groups. The results were expressed as mean ± S.E.M (standard Error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

3. Results
3.1. Results of blood Super oxide dismutase, Catalase, Glutathione peroxidase and malondialdehyde level:
Administration of 75mg/rat Cinnamon zeylanicum for thirty consecutive days significantly increased Super oxide dismutase, Catalase, Glutathione peroxidase level in experimental (diabetic and non-herb) groups as compared with the control group(P<0.05). also, 75mg/rat Cinnamon
zeylanicum could significantly decreased level of MDA in experimental (diabetic and non-herb) groups as compared with the control group (P<0.05)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=10)</th>
<th>Cinnamon, (75mg/kg/rat) (n=10)</th>
<th>STZ (55mg/kg/IP) (n=10)</th>
<th>Cinnamon, (75mg/kg/rat) + STZ (55mg/kg/IP) (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase, u/mg Hb</td>
<td>296±0.05</td>
<td>451±3.05*</td>
<td>100±0.05*</td>
<td>254±0.05*</td>
</tr>
<tr>
<td>Super oxide dismutase, u/g Hb</td>
<td>987±0.55</td>
<td>1436±0.55*</td>
<td>651±0.55*</td>
<td>873±0.55*</td>
</tr>
<tr>
<td>GPX, u/mg Hb</td>
<td>141±0.5</td>
<td>186±0.5*</td>
<td>100±0.5*</td>
<td>121±0.5*</td>
</tr>
<tr>
<td>MDA, mmol/ml</td>
<td>2.8±0.05</td>
<td>2.0±0.05*</td>
<td>6.2±0.05*</td>
<td>3.1±0.05*</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>122.3±0.05</td>
<td>109.3±0.05</td>
<td>387.1±0.05*</td>
<td>287.3±0.05*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.
*Significant different at P<0.05 level, (compared with the control group).

4. Discussion and conclusion

Cinnamon, a natural product with a long history of safety, is rich in polyphenolic components that have been shown to improve the action of insulin in vitro (Anderson et al., 2004), in animal studies (Qin et al., 2003, 2004) and to possess in vitro antioxidant activity (Shobana et al., 2000). Phytotherapy has recently gained popularity in Europe and the United States. Many fruits contain compounds called phytochemicals that can be included into three major groups: the flavonoids, limonoids and carotenoids. Diabetes is one of the pathological processes known to be related to an unbalanced production of ROS, such as hydroxyl radicals (HO), superoxide anions (O2) and H2O2. Therefore, cells must be protected from this oxidative injury by antioxidant enzymes. Previous study showing impairment by streptozotocin (STZ) of antioxidant enzymes, may contribute to STZ-induced experimental diabetes (Winkler and Moser, 1992). Studies have shown that antioxidants have a widespread effect in andrology, protect spermatozoa from ROS producing abnormal spermatozoa, scavenge ROS produced by leucocytes, prevent from DNA fragmentation, improve semen quality in smokers, reduce cryodamage to spermatozoa, and finally, block premature sperm maturation. It has been postulated that oxidants interfere with normal sperm function via membrane lipid peroxidation and fragmentation of nucleic acids, which result in sperm dysfunction (Hesham et al., 2008). Seminal plasma is considered to be the central source of antioxidants that protect sperm cells against oxidative damages. The most studied antioxidants are the SOD, GPX and catalase enzymes. Decreased antioxidants concentrations in seminal plasma of infertile men were accompanied by increased levels of seminal MDA of the same patients. In this respect, the diminution of antioxidants can explain the elevated lipid peroxidation in infertile patients. GPX plays a crucial role in the antioxidant defenses of the epididymis and the ejaculated spermatozoa (Sunde, 1984). Human spermatozoa are known to possess all of the major antioxidant defensive systems' including catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GRD), their effectiveness is impaired by their limited concentrations and distribution. There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways. Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate 'OH radicals (Turko et al., 2005). ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of 'O2'. Nonenzymatic antioxidants include vitamins A, C and E; glutathione; α-lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q10 (CoQ10); and cofactors like folic acid, uric acid, albumin, and vitamins B6, B12. Alterations in the antioxidant defense system in diabetes have recently been reviewed (Vega-Lopez et al., 2004). Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi and Wanasundara, 1992; Kelen et al., 2007) and prevent lipid auto oxidation (Brand-Williams et al., 1995; Bondet et al., 1997). Worldwide studies have been done to make use of herbal medicine in different fields of medicine. Base on ancient Persians traditional books Use of herbal medicine has positive
effect on treatment of different diseases especially on diabetes mellitus. Onion contains A, B, C vitamins, flavonoids and selenium which their antioxidant role has been proved (Jedlinska – Krakowska et al., 2006). Make use of onion and Quercetin in diabetic patient treatment has been experimented (Khaki et al., 2010; Khaki et al., 2009). Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect sperm DNA from free radicals and increase blood-testis barrier stability (Jedlinska-crakowska et al., 2006). Evidence suggests that certain phytochemicals found in citrus sources, such as flavonoids and limonoids, play a major role in treating or retarding chronic diseases, including anti-oxidative, anti-carcinogenic, cardiovascular protective, neuro-protective, bone health promotion and anti-inflammatory diseases (khaki et al., 2011).

In this study results showed administration 75mg/kg Cinnamon zeylanicum cause to significantly enhance in serum SOD, CAT, GPX and decreasing MDA levels in all diabetic groups that received this herb. This results due to its flavonoids and related compounds. Researches confirmed that flavonoid especially Phytoestrogens have the potential to affect steroid biosynthesis and metabolism through a number of pathways (Whitten and Patisaul, 2001). A number of phytoestrogens also inhibit 17βhydroyxysteroid dehydrogenase (Makela et al., 1995). Research by on male rat after feeding with phytoestrogens showed no change in hypotalamic aromatase activity, even though isoflavone levels were 8 times higher in the brains of these animals than in the controls (Leffart et al., 2000). In other side results in experimental group showed Cinnamon zeylanicum could increase sperm parameters such as population, viability and motility, as before confirmed herbal Antioxidants and antioxidant enzyme are main detoxifying systems for peroxides like CAT and GSH, this compounds which dispose, scavenge, and suppress the formation of ROS and lipid peroxidation. So ROS excessive levels decreasing is important factor for sperm cells generation and improve spermatogenesis (khaki et al., 2010; Henkel, 2005) and increase chance of fertility. When the blood has excess sugar in it, the sperm produced in the testes are more likely to have defects that prevent fertilization. There are also other symptoms of diabetes that can severely impede the ability to reproduce. Therefore suggested, increased use of herbal medicine, fruit, vegetables, onion, tea and black burgundy grape which are full of flavonoids and Cinnamon zeylanicum such as herbal antioxidant can improve blood total anti-oxidants and prevent body tissue from Reactive oxygen species (ROS) levels and increase chances in diabetic male patients to get new life.

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References

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