ABSTRACT
Garlic has been in use worldwide since ages, especially as food and for its health benefits. However, concern has been raised on its untoward effects on male reproductive functions. The present study examined the effects of aqueous garlic extract on some semen parameters and erythrocyte superoxide dismutase in Wistar rats. 21 male Wistar rats were grouped into 3, and aqueous extract of garlic was administered orally at different doses (Group B: 500 mg/kg/d; Group C: 1000 mg/kg/d) to the 2 treated groups, and distilled water given to the control group (Group A), for 28 days. Sperm concentration, motility and morphology were studied, and the activity of superoxide dismutase (SOD) was measured. The results of the semen analysis revealed reduction in all the parameters, which was dose-dependent. The percentage of morphologically normal spermatozoa was significantly reduced, as well as sperm concentration, compared with findings in the control animals. Garlic also caused a significant reduction in SOD activity in the blood, and this was dose-dependent, as the least activity was recorded among the high dose group. As people desire to enjoy the maximum beneficial health effects of garlic, its potential to adversely affect the reproductive functions, especially at higher doses, should be borne in mind.

KEYWORDS: garlic, aqueous extract, semen parameters, SOD.

INTRODUCTION
Return to use some plants recently which have been common in herb medicine, have attracted the attention of drug producers. Herb medicine has been known as scientific one among all nations for many centuries ago, and its therapeutic effects has been proved empirically. There is no exact statistics from people who consume herbal medicines in the Middle East up to now. However, about 15 million people consume herbal medicines and synthetic drugs simultaneously in America. Garlic aqueous is among the widely – used herbal medicines which is used as a relish. The studies being conducted in herb medicine area show that garlic aqueous consumption is very useful in disease treatment when body defense system becomes weak because of infection presence, and has no side effects contrary to antibiotics and causes body to fortify. Moreover, garlic aqueous not only fights against microbes existing in respiratory system but also causes mucus’s to open. Antioxidant and antigenic properties of garlic aqueous have been proved in studies conducted on animal and poultry. The important components, among others, include allin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) which is thought to be the chief bioactive compound present in aqueous garlic extract; allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate and γ-L-glutamyl-S-allyl-L-cysteine (Aql et al., 1991). Garlic preparations are known for their cardioprotective effects (Martin et al., 1994; Ried et al., 2008), ability to lower blood pressure (Milner, 2001) in people with high blood pressure, lower lipid levels, reduce the risk of atherosclerosis, increase blood levels of antioxidant enzymes, and also offer some benefits in conditions like diabetes mellitus and cancer (Hammami et al., 2009). Although many positive health effects have been attributed to the use of garlic, the effects tend to be undesirable on testicular functions, as many studies have observed some adverse effects on reproductive functions. Although the mechanisms of this action of garlic are not very clear, apoptosis has been suggested to be likelihood (Banerjee et al., 2001). Allium sativum is an antioxidant and a detoxifying agent, which scavenges the reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes, (such as superoxide dismutase, catalase, glutathione peroxidase, etc) thereby protecting the cells against disease-causing oxidative damage. Administration of garlic can alter the activities of endogenous antioxidants, depending on the dosage as well as the type of antioxidants (Hammami et al., 2008). Studies by Banerjee et al., (2008) showed that use of garlic at low doses could significantly increase superoxide dismutase (SOD), whereas at higher doses, the activity of SOD is reduced. Garlic causes a dose-dependent increase in the percentage of empty seminiferous tubules, thereby altering spermatogenesis and reducing testosterone secretion (Oi et al., 2001). Meanwhile, studies by Oi et al., (2001) found an increased testicular testosterone with garlic supplementation. Also associated with garlic use is the inhibition of Leydig steroidogenic enzyme expression and Sertoli cell markers, which are capable of inducing apoptosis in testicular germ cells (spermatocytes and spermatids), characterised by increased levels.
of active Caspase 3 (Banerjee et al., 2001; Khaki et al., 2013).

In the present study, the effect of orally administered aqueous extract of Allium sativum was studied on some semen parameters in wistar rats and its effect on erythrocyte superoxide dismutase (SOD).

**MATERIALS AND METHODS**

2.1. Preparation of Garlic Aqueous Extract

The raw garlic plants were collected from market and authenticated at the Department of Plant Biology, Tabriz research center. The raw garlic cloves were peeled, chopped into small pieces and blended. It was then dissolved in distilled water and kept in the Refrigerator for 12 hours. The solution was thereafter filtered, and the filtrate was concentrated in a water bath at a temperature of 40°C, into the paste form, from which the required dosages (500 mg/kg/d and 1000 mg/kg/d) were prepared.

2.2. Experimental Animals

In this study, 21 male Wistar rats (220–250 g and 2-3 month age) were selected for the study and were purchased from Animal House. Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Tabriz research center was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum.

2.3. Experimental Protocol

Animals randomly divided into 3 equal groups: group1; healthy control rats received standard diet; Group 2 Treated Group with aqueous extract of garlic in a dose of 500 mg/kg/d; Group 3, Treated Group with aqueous extract of garlic in a dose of 1000 mg/kg/d. 24 hour after the last day of administration, the animals were weighed and thereafter sacrificed by cervical dislocation. Blood samples of animals were collected into lithium heparinized bottles. Using a midline abdominal incision, the abdominal cavity was opened to access the reproductive organs. The testes were excised and weighed using an electronic sensitive analytical scale. An aliquot of this solution was diluted with Tris buffer solution (Saalu et al., 2010) to 0.5 ml. An aliquot of this solution was observed under the light microscope at a magnification of x400. The mean motility estimation was reported as the final motility score for each sample. The morphology of the spermatozoa was determined using the original dilution for motility, diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). The sperm cells were categorised based on the presence of one or more abnormal features such as tail defects (short, irregular, coiled or multiple tails); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (round head, small or large size, double or detached head). Findings were expressed as percentage of morphologically normal sperm (Pal et al., 2006).

2.4. Statistical analysis

Data were analysed statistically by application of student’s t-test, using the SPSS version 19 software, presented as mean and standard error mean (SEM), and values of p <0.05 were considered to be statistically significant.

**RESULTS**

Semen parameters in all the treatment groups (Groups 2 and 3) decreased when compared with the control group. Only a minimal decrease was observed in the percentage sperm motility of the treated groups, and in the percentage of morphologically normal spermatozoa of the low dose group (Table 2). The percentage of normal spermatozoa was markedly reduced in the animals exposed to 1000 mg/kg/d (high dose) of garlic (65.33±1.22) compared with animals that received a lower dose of 500 mg/kg/d (84.29±1.42) and the control animals (87.36±0.80) given distilled water. There was a decrease in sperm motility in the treatment groups (Control: 89.58±0.79; Low dose group: 87.46±1.51; High dose group: 85.34±1.12), but this was not statistically significant (p>0.05). Sperm concentration decreased in the treated groups with the animals treated with 1000 mg/kg/d aqueous garlic extract having the lowest value (57.26±1.87) compared with those that received...
500 mg/kg/d of the extract (63.48±1.24) and the control group (70.13±0.61) that received distilled water. Superoxide dismutase enzyme was markedly reduced in the low dose group (255.47±4.69 IU/ml; p<0.05) compared with the control animals (500.44±15.36 IU/ml), with a much more decreased activity in the group that received a higher dose of 1000 mg/kg/d (190.98±3.82 IU/ml; p<0.05).

Table 1: Weight Of Testes Following Administration Of different doses of extract

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Testis weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Normal Control)</td>
<td>100.36±1.24</td>
<td>161.32±1.45</td>
<td>1.24±0.10</td>
</tr>
<tr>
<td>2 (Low dose)</td>
<td>114.13±1.46</td>
<td>170.78±2.16</td>
<td>1.69±0.41</td>
</tr>
<tr>
<td>3 (High dose)</td>
<td>126.64±2.18</td>
<td>183.12±2.23</td>
<td>1.91±0.84</td>
</tr>
</tbody>
</table>

Table 2: Semen Parameters and SOD activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>1 (Normal Control)</th>
<th>2 (Low dose)</th>
<th>3 (High dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration</td>
<td>70.13±0.61</td>
<td>63.48±1.24</td>
<td>57.26±1.87</td>
<td></td>
</tr>
<tr>
<td>Sperm motility</td>
<td>89.58±0.79</td>
<td>87.46±1.51</td>
<td>85.34±1.12</td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>87.36±0.83</td>
<td>84.29±1.42</td>
<td>65.33±1.22</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>500.44±15.36</td>
<td>255.47±4.69</td>
<td>190.98±3.82</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

To protect molecules against toxic free radicals and other ROS, cells have developed antioxidant defenses that include the enzymes superoxide dismutase (SOD), which dismutates superoxide; catalase and glutathione peroxidase, which destroy toxic peroxides, and small molecules including glutathione. External sources of antioxidant nutrients that are essential for antioxidant protection include antioxidant vitamins C and E, vitamin A/provitamin A and the mineral selenium, a component of selenium-dependent glutathione peroxidase (Borek 1993). Phytochemicals from plant-rich diets, including garlic, provide important additional protection against oxidant damage (Borek 1997). The variety of antioxidant phytochemicals in AGE, which protect against disease-causing oxidative damage (Horie et al. 1992; Fatihazad et al., 2013), may act in single and combined fashion (Amagase et al. 1996, Borek 1993 and 1997). The antioxidative actions of AGE and its components are determined by their ability to scavenge ROS and inhibit the formation of lipid peroxides. These effects are determined by measuring the decrease in ROS-induced chemiluminescence, inhibition of thiobarbituric acid reactive substances (lipid peroxides) (TBARS assay), and in vitro inhibition of the release of pentane, a product of oxidized lipids, in the breath of an animal exposed to oxidative stress (Horie et al. 1989). Oxidized LDL promotes vascular dysfunction, which contributes to atherosclerosis, in part by its cytotoxic effects on endothelial cells. Using an in vitro system of endothelial cells exposed to oxidant copper ions, AGE and SAC were shown to scavenge ROS, inhibit oxidation of LDL and inhibit endothelial cells injury by oxidized LDL (Ide and Lau 1997). AGE has been shown to inhibit lipid peroxide formation in several studies (Wei and Lau 1998). In one study, TBARS induced by hydrogen peroxide were inhibited 31–89% by AGE and 33–67% by SAC in a concentration-dependent manner (Yamasaki et al. 1994), thus mitigating oxidation events, which are implicated in the formation of atherogenic lesions.

An additional assay, the 1,1-diphenyl-2-picrylhydrazine assay, showed the antioxidant effects of allixin, SAC, SMAC and diallyl polysulfides, whose radical-scavenging action increased with the number of sulfur atoms (Imai et al. 1994). More recently, other components of AGE, N-fructosyl arginine and N-fructosyl glutamate, showed antioxidant effects by spin resonance spectroscopy. The effects of garlic on male gonads, and by extension, the male reproductive function, may appear somewhat different from the usual cytoprotective activities observed by various workers in other tissues and organs of the body; although, the amount of garlic administered, to some extent, could be a factor (Hammami et al., 2008). Administration of garlic augments the activities of SOD and also protects the cells against the damaging effects of free radicals, as given by some biochemical and histopathological evidences. However, on the testes, use of garlic has been noted to compromise some male reproductive functions, as it affects spermatogenesis and testosterone levels, which are vital to reproduction (Banerjee et al., 2001). Both at lower and higher doses of garlic extract, the activity of SOD was noted in this study to decrease with increasing dosage. The endogenous antioxidative functions of the enzyme was compromised, thereby predisposing the cells of the testes to the detrimental effects of various oxygen radicals and other chemically-induced oxidative stress. In this study, both the percentage morphology and sperm count were significantly reduced, although sperm motility was only slightly reduced. Irrespective of the motility, in the event of increasing number of abnormal spermatozoa morphologically, spermatogenesis and other reproductive capability of an intact spermatozoon, could still be compromised. This is because previous studies have shown that use of Allium sativum is associated with increased percentage of empty seminiferous tubules and consequent reduction in testosterone secretion and altered spermatogenesis. The effects of garlic on semen parameters may not be unconnected to the suggested mechanism of apoptosis (Banerjee et al., 2001).

REFERENCES


Yokoi K, Mayi ZK. Organ apoptosis with cytotoxic drugs. Toxicology 2004;290:78-85.