EFFECTS OF PARSLEY AND PUMPKIN ON ALCOHOL INDUCED TESTICULAR DAMAGE IN RAT MODEL

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ABSTRACT

In this study, we intended to determine the possible preventive effects of pumpkin and parsley on oxidative stress due to ethanol usage in rat testes. The animals were divided into four groups as follows: Group 1 was control. Group 2 received ethanol. Group 3 received ethanol and pumpkin oil for four weeks. Group 4 received ethanol and parsley oil for four weeks. Following sacrifice, the testes were treated for morphological and biochemical (superoxide, glutathione transferase, glutathione peroxidase, catalase, malondialdehyde) analyses. In Group 2, congestion was observed in the intertubular blood vessels. In Groups 1, 2 and 3 no histopathological alterations were noted. Pumpkin and parsley rich diet may have a preventive role on histopathological changes caused by alcohol in rat testes.

KEYWORDS: Parsley, Pumpkin, Alcohol, Testes, Infertility.

INTRODUCTION

It was demonstrated that use of ethanol might cause gonadal disorders, including structural testicular changes and a decrease in testicular and serum levels of testosterone (T) (Van Thiel et al. 1983).

Ethanol-induced oxidative stress is not restricted to the liver where the ethanol is actively oxidized, but can affect various extrahepatic tissues such as the central nervous system, the heart and the testes (Van Thiel et al., 1983).

Pumpkin seeds are excellent sources of protein (25.2–37%), vitamins and oil (37.8–45.4%) (Makni et al., 2008), especially Omega 6 fatty acids which have a number of biological applications along with significant antioxidant activity, in addition to anti-inflammatory and hypolipidemic effects.

Phytochemical screening of parsley has revealed the presence of flavonoids (apiin, luteolin, and apigenin-glycosides), ascorbic acid, tocopherol, volatile compounds (myristicin,
apiole), coumarines (bergapten, imperatorin, phthalides, furanocoumarins, and sesquiterpenes (Sacan et al., 2006, Gadi et al., 2009).

It is imperative to study the responses of the testis antioxidant defense system in rats chronically exposed to ethanol. In this study, we intended to determine the possible preventive effects of dietary pumpkin and parsley on oxidative stress caused by chronic ethanol usage in rat testis.

MATERIALS AND METHODS

Chemicals:
Parsley and pumpkin oils were purchased from El Kaptin pharmaceutical company (Alexandria, Egypt), All other chemicals used for the investigation were of analytical grade.

Experimental animals:
White male albino rats (Rattus norvegicus) weighing about 140-180g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25±5°C) as well as 12 hours daily normal light periods. Moreover, they were given access of water and supplied daily with standard diet of known composition and consisting of not less than 20% proteins, 5.5% fibers, 3.5% fats and 6.5% ash and were also supplied with vitamins and mineral mixtures. The rats were divided into four groups and treated as described below.

Group I. Normal control (NC): Six rats were received normal (0.9%) saline orally via orogastric tube for equivalent handling.

Group II. Ethanol treatment (Et): Six rats were received absolute EtOH orally (10%) in drinking water for 15 days before the experiment (Hekmatpanah et al., 1994).

Group III. Ethanol plus Pumpkin oil (Et+P): Rats were given orally both EtOH and Pumpkin oil (50 mg/ kg) (Sacan et al., 2008) for 4 weeks.

Group IV. Ethanol plus Parsley oil (Et+Pa). This group of rats received both EtOH and Parsley oil (50mg/kg) (Al-zuhair et al., 2000) for a period of 4 weeks.
**Induction of alcoholism:**

Alcoholism was experimentally induced in animals by giving absolute ethyl alcohol (10%) in drinking water for 15 days before the experiment (Hekmatpanah *et al.*, 1994).

**Determination of biochemical assays:**

Testosterone concentration in serum was determined in Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive medicine, New Children Hospital, Faculty of Medicine, Cairo University according to the method of Andreyko *et al.* (1986) using reagent kits purchased from Biosource Company, (Belgium). Serum LH was determined according to the method of Braunstein *et al.* (1976) using reagent kits purchased from Monobind, INC (USA). FSH concentration in serum was determined according to the method of Odell *et al.* (1968) using reagent kits purchased from Monobind, INC (USA). Testis glutathione (GSH) was determined according to the method of Beulter *et al.* (1963). Testis lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Preuss *et al.* (1998). Glutathione-S-transferase activity was determined according to Mannervik and Gutenberg (1981). Catalase activity was assayed using the method of Aebi (1984).

**Statistical analysis of the results:**

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD test to compare various groups with each other. Results were expressed as mean ± standard error (SE) and values of P>0.05 were considered non-significantly different, while those of P<0.05 and P<0.01 were considered significantly and highly significantly different, respectively.

**Histological examinations:**

Autopsy samples were taken from the testes of rats in different groups and fixed in 10% formol saline. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slice microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain and then examined through the electric light microscope (Banchroft *et al*.; 1996).
RESULTS

Histological examination results showed that there was no histopathological alteration observed and the normal histological structure of the mature active seminiferous tubules with complete spermatogenic series were recorded in Fig.1. Congestion was observed in the intertubular blood vessels in case of ethanolic control group (Fig.2). While administration for pumpkin and parsley oils showed that there was no histopathological alteration observed as recorded in Fig.3, 4.

The alcoholic rats exhibited a significant decrease in testosterone level (T); while levels of LH and FSH were increased significantly in comparison to normal rats (Table 1). The treatment of these animals with oils of pumpkin and parsley induced a potential increase of the lowered serum T and diminished the elevated LH and FSH levels as compared with the alcoholic animals.

Table 2 showed a significant decrease in of antioxidant levels (reduced glutathione and catalase) in testis of alcohol group compared to the control one. In contrast, administration of pumpkin and parsley oils with alcohol showed significant changes in antioxidant capacities in testis when compared with the control group. Lipid peroxidation levels increased significantly in alcohol group compared to control one, in contrast, administration of pumpkin and parsley oils with alcohol showed significant decrease as compared to control one.

![Histological examination of the testis of normal control group.](image)

**Fig.1** Histological examination of the testis of normal control group.
Fig. 2 Histological examination of the testis of ethanolic control group.

Fig. 3 Histological examination of the testis of alcoholic treated with pumpkin oil group.

Fig. 4 Histological examination of the testis of alcoholic treated with parsley oil group.
Table 1: Effect of oral administration of pumpkin and parsley oils on the LH, FSH & testosterone levels in male alcoholic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>LH (mIU/ml)</th>
<th>% change</th>
<th>FSH (mIU/ml)</th>
<th>% change</th>
<th>Testosterone (ng/ml)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>0.19±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>0.23±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>8.30±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alcoholic control</td>
<td>0.26±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.84</td>
<td>0.32±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.13</td>
<td>5.85±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.52</td>
</tr>
<tr>
<td></td>
<td>Alcoholic treated with pumpkin oils</td>
<td>0.22±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-15.38</td>
<td>0.27±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-15.63</td>
<td>6.53±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.62</td>
</tr>
<tr>
<td></td>
<td>Alcoholic treated with parsley oils</td>
<td>0.21±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-19.23</td>
<td>0.25±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-21.88</td>
<td>7.42±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.84</td>
</tr>
<tr>
<td></td>
<td>F-probability</td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD at the 5% level</td>
<td>4.12x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
<td>6.49x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
<td>0.625</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD at the 1% level</td>
<td>5.62x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
<td>8.85x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
<td>0.841</td>
<td></td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
Table 2: Effect of oral administration of pumpkin and parsley oils on the lipid peroxidation level and reduced glutathione, catalase and glutathione transferase activities in male alcoholic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>LPx * (nmol/gm/hr)</th>
<th>% change</th>
<th>GSH * (mg/g)</th>
<th>% change</th>
<th>CAT * (U/gm)</th>
<th>% change</th>
<th>GST * (U/gm)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41.13 ± 2.00 b</td>
<td>-</td>
<td>71.03 ± 2.34 a</td>
<td>-</td>
<td>0.538 ± 0.065 b</td>
<td>-</td>
<td>6.14 ± 0.22 a</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alcoholic control</td>
<td>56.49 ± 6.46 a</td>
<td>37.35</td>
<td>59.63 ± 2.59 b</td>
<td>-16.05</td>
<td>0.400 ± 0.088 c</td>
<td>-25.65</td>
<td>6.51 ± 0.15 a</td>
<td>6.03</td>
<td></td>
</tr>
<tr>
<td>Alcoholic treated with pumpkin oil</td>
<td>36.37 ± 4.16 b</td>
<td>-35.62</td>
<td>68.93 ± 1.38 a</td>
<td>15.60</td>
<td>0.447 ± 0.049 b</td>
<td>11.75</td>
<td>6.56 ± 0.22 a</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Alcoholic treated with parsley oil</td>
<td>46.40 ± 4.85 ab</td>
<td>-17.86</td>
<td>68.66 ± 3.53 a</td>
<td>15.14</td>
<td>0.481 ± 0.003 b</td>
<td>20.25</td>
<td>6.51 ± 0.54 a</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

| F-probability                           | P<0.001                      | P<0.01            | P<0.001              | P>0.05 |
| LSD at 5%                               | 13.725                       | 7.601             | 0.183                 | -      |
| LSD at 1%                               | 18.718                       | 10.366            | 0.250                 | -      |

* LPx (Lipid peroxidation), GSH (Reduced glutathione), CAT(Catalase) and GST(Glutathione-S-transferase).

Data are expressed as Mean ± standard error.

Number of animals in each group is six.

Means, shared only superscript symbol (s) are not significantly different.
DISCUSSION

Parsley oil and their combination with alcohol caused a significant increase in GSH content compared to alcohol alone. Since the content of reduced GSH is the important indicator of antioxidative status, we can conclude that positive synergism was observed.

Acute (Oner-Iyidogan et al., 2001) and chronic (Lieber, 2005) exposure to ethanol lead to increased free radical and lipid peroxide formation. Decreased GSH and CAT activity seems to indicate the ethanol-induced oxidative stress (Husain et al., 2001).

Testicular membranes are rich in polyphenolic fatty acids that are prone to oxidative decomposition, and it is likely that the resulting lipid peroxidation contributes to the membrane injury and gonadal dysfunction that occurs as a result of alcohol abuse and/or chronic use (Aydilek et al., 2004). In alcohol group, decreased tissue CAT and GSH-Px and elevated MDA obviously reveal the damage caused by free radicals (Hozayen et al., 2014).

Reactive oxygen species generated in the tissues are efficiently scavenged by enzymatic antioxidant system (such as SOD, CAT and GSH-Px) and nonenzymatic antioxidants (such as vitamin A, C, and E) (Husain et al., 2001) and carotenoids (Genc et al., 1998). The antioxidant capacity of common fruits and vegetables had been analyzed in various studies (Wu et al., 2004).

The present study showed increased FSH levels in alcoholics. It is the direct toxic effect of alcohol on the testis that leads to decreased seminiferous tubular function. The FSH elevation is due to the absence of testicular feedback regulation at the pituitary level.

The present results correlate with those of Gumus et al. (1998), who reported that serum FSH levels are higher in chronic alcoholics. Similar findings had been reported by Van Thiel et al. (1987), who found that FSH levels increased in alcohol-fed animals (Hozayen, 2013). Because alcohol enters into the testis directly, causing decreases in spermatogenesis and T synthesis, it causes the increased level of LH found in chronic alcoholics. The results of this study are supported by those of Heinz et al. (1995), Sengupta et al. (1991), and Bannister and Lowosky (1987), who found that LH levels were increased in chronic alcoholics. In the present study, the decreased T and increased LH levels in alcoholics suggest that the major effect of alcohol on plasma T in humans is exerted on the testis at a peripheral site rather than on the hypothalamic–pituitary axis at a central site.

Both acute and chronic alcohol intoxication result in dose dependent suppression of plasma T levels in normal men. Alcohol-induced suppression of male T is due to a direct effect on the biosynthetic processes in the testes. Increased LH levels after alcohol-induced
suppression of T in men and male monkeys is consistent with established mechanisms of negative feedback of LH secretory activity.

CONCLUSIONS:

It is evident from the results of the present study that chronic alcoholism suppresses both blood hormonal levels and reduces semen quality. In Leydig cells, alcohol decreases the male sex hormone T. Because of the suppression of blood levels of T in alcoholics, the pituitary feedback regulation is altered. The pituitary LH is increased in blood as a compensatory mechanism. The pituitary FSH level in blood is increased because of the loss of seminiferous tubular function in alcoholics. This study has proved beyond doubt that chronic alcohol consumption has a detrimental effect on male reproductive hormones.

REFERENCES


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