ABSTRACT
The aim of this paper was to evaluate the potential protective effects of administration of rosemary extract against renal and testicular toxicity induced by aspartame in male rats. Three groups of rats were treated orally one time per two days for twelve weeks: first group was regarded as control, received distilled water, the second group was given aspartame at dose level of 1000 mg/kg b.wt, and the third was given aspartame with the same previous dose and rosemary at dose level of 125mg/kg b.wt. Our results showed that pretreatment with rosemary extract produced a highly significant decrease in the levels of serum urea, creatinine and potassium and a highly significant increase in sodium levels in serum. Also rosemary significantly attenuated the increase in lipid peroxidation, and enhanced the levels of reduced glutathione and antioxidant enzymes activities in kidney and testis compared to aspartame control, and an almost normal histological architecture of the kidney, was observed in the treated groups compared to aspartame control. Therefore, it can be concluded that the aqueous extract of rosemary possessed antilipid peroxidative and free radical scavenging activities.

KEYWORDS: Renal and Testicular Toxicity; Rosemary Extract; Oxidative Stress.

INTRODUCTION
Aspartame (L-aspartyl L-phenylalanine methylester) is a dipeptide artificial sweetener that is widely used as a non-nutritive sweetener in foods and drinks (Abhilash et al., 2011). Upon ingestion, aspartame is metabolised in the gastrointestinal tract into three components: aspartic acid, phenylalanine, and methanol (EFSA, 2006). It was reported that the administration of aspartame induced an oxidative stress in the liver and kidney of male albino rats (Iman and Mourad, 2011).

It was reported that plant phenolics are important exogenous multifunctional antioxidants (Dornan et al., 2003). Among the plants reported to present antioxidative activity,
Rosmarinus officinalis L. (common name, rosemary; family Labiatae), is known to be a rich source of active metabolites and is used in folk medicines (Abutbul et al., 2004). The most important constituents of rosemary are rosmarinic acid, carnosic acid, carnosol, rosmanol, flavonoids and other phenolic compounds (Zegura et al., 2011). Therefore, the present investigation was undertaken to test the effects of aspartame overdose on kidney and testis tissues in rats and to evaluate the possible protective effects of the aqueous rosemary extract against aspartame-induced damage.

**Material and Methods**

**Experimental animals:**
Adult male albino rats weighing between 120-140 g 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 under good ventilation and received water and standard balanced diet. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals stated by the Canadian Council on Animal Care (CCAC, 1993).

**Chemicals:**
Aspartame (diet-sweet® produced by Amriya pharmaceutical industries company in Amriya, Alexandria, Egypt) was purchased from a local pharmacy in tablet form. All other chemicals used for the investigation were of analytical grade.

**Rosemary leaves extract preparation:**
The dried rosemary (Rosmarinus officinalis L.) leaves were purchased from a local supermarket in Cairo (Cairo, Egypt). Leaves were cleaned, shade dried, powdered and extracted. The extract was prepared by refluxing leaves with bi-distilled H₂O for 36 hours (12 hours × 3). The cooled liquid extract was then transformed to powder by evaporating water. The powder was redissolved in bi-distilled water just before oral administration.
Doses and treatment:

The aspartame dose used in this study was 1000 mg/kg b. wt. This dose was previously reported to induce liver injury and leukocyte infiltration was observed in liver tissues of rats. This dose was dissolved in distilled H2O and given orally by gastric tube one time per two days for three months. The chosen dose of rosemary extract was 125 mg/kg b.wt. This dose was given by gastric tube (Abdella and Ahmed, 2009).

Experimental Design:

Animals were divided into three groups comprising six animals each designed as the following:

**Group (1):** was regarded as control group, received distilled water.

**Group (2):** administered aspartame at dose level of 1000 mg/kg b.wt.

**Group (3):** (administered rosemary & aspartame) was given rosemary at dose level of 125mg/kg b.w and aspartame 1000 mg/kg b.wt.

All the treatments were performed orally and one time per two days for three months. By the end of the experimental periods all groups were sacrificed under diethyl ether anaesthesia. Blood samples were collected from each rat, allowed to coagulate at room temperature then centrifuged at 3000 round per minute (r.p.m.) for 20 minutes. The clear, non-haemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at -20°C till used. The kidney and testes tissue samples were immediately removed from each animal, washed in ice cooled 0.15 M NaCl and blotted on a filter paper. Then the tissue 0.5 gm were homogenized in ten volumes of ice cold saline solution (pH; 7) using teflon homogenizer (Glas-Col, Terre Haute, USA) until a uniform suspension was obtained. The homogenate was kept in deep freezer at -20°C for biochemical assays.

Biochemical determinations:

Creatinine in serum was measured according to Young (2001) using kits obtained from diamond diagnostics, Egypt. Urea concentration was determined in serum according to the Urease-modified Berthelot reaction (Patton and Crouch, 1977) using the reagent kits purchased from Diamond Diagnostics, Egypt. Sodium concentration in serum was determined by colorimetric method according to Trinder (1951) & Maruna (1958) using kits obtained from Teco Diagnostics, USA. Potassium concentration in serum was determined by
turbidimetric tetraphenylborate method according to Hoeflmayr (1979) using kits obtained from Spectrum Diagnostics Egyptian co. for Biotechnology. luteinizing hormone (LH) concentration in serum was determined according to Braunstein et al. (1976) and serum follicle stimulating hormone (FSH) was determined according to the method of Odell et al. (1968) using reagent kits purchased from Monobind, INC. (USA). Testosterone concentration in serum was determined according to the method of Andreyko et al. (1986) using reagent kits purchased from Biosource Company, (Belgium). Glutathione level in kidney and testis homogenate was determined according to the colorimetric method of Beutler et al. (1863) using kits obtained from Bio Diagnostic Company, Egypt. Glutathione peroxidase (GPx. EC 1.11.1.9) activity in homogenates was determined according to the chemical method of Paglia and Valentine (1967). Glutathione-S-transferase (GST.EC 2.5.1.18) activity was assayed according to method suggested by Mannervik and Guthenberg (1981). The enzyme superoxide dismutase (SOD,EC 1.15.1.1) activity was assayed according to method suggested by Marklund and Marklund (1974). Malondialdehyde produced during lipid peroxidation was measured by the method of Preuss et al. (1998).

**Histopathology:**
Kidney and testis tissues were taken from the eviscerated animals in different groups and fixed in 10% formol saline for twenty four hour. 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 slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain then examined through the electric light microscope (Banchroft et al., 1996).

**Statistical analysis:**
The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT) (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.
Results

Aspartame administration resulted in a highly significant increase in the levels of serum urea, creatinine and potassium (P < 0.01), while a highly significant decrease in sodium concentration (P < 0.01) was observed when compared with normal rats. The treatment of aspartame-administered rats with rosemary extract produced a highly significant decrease in serum urea, creatinine and potassium levels (P < 0.01) and a highly significant increase in the sodium concentration (P < 0.01) in comparison with aspartame treated rats (table 1).

Concerning serum hormones related to testis function indicated in table 2, the aspartame-administered rats exhibited a highly significant decrease of serum FSH, LH and testosterone levels (P < 0.01) as compared to control group. On the other hand the treatment of aspartame-administered rats with rosemary extract produced a significant increase (P < 0.05) in serum FSH level, but a non significant change (P>0.05) in LH and testosterone levels when compared to corresponding groups.

Table 3 illustrates the effect on renal oxidative stress markers and various antioxidant enzymes activities. In aspartame treated rats glutathione contents (GSH) and glutathione-S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were highly significantly decreased (P < 0.01) in kidney, while lipid peroxidation level had a highly significant elevation (P < 0.01) as compared to control rats. On the other hand the glutathione level and GST, GPx and SOD activities were highly significantly increased (P < 0.01) as a result of administration of rosemary extract compared to aspartame treated rats. The rosemary extract produced a highly significant decrease (P < 0.01) of the elevated lipid peroxidation level in comparison with aspartame treated rats.

The data showing the effect on oxidative stress markers and various antioxidant enzymes activities in the testis are represented in table IV. The administration of aspartame to albino rats produced a significant decrease (P < 0.05) in GSH contents, and a highly significant decrease (P < 0.01) in GPx and SOD activities in testis, while a significant increase (P < 0.05) in lipid peroxidation was observed in testis as compared to control rats. The treatment of aspartame-administered rats with rosemary extract induced a significant increase (P < 0.05) in GSH contents, a highly significant increase (P < 0.01) in activities of GPx and SOD in testis, and a highly significant decrease (P < 0.01) in lipid peroxidation, while GST activity showed a non significant (P>0.05) change in the experimental groups as compared to the control group.
Histological examination:

The histopathological findings in kidney sections from the three experimental groups are highlighted in fig.1. The kidney sections of control rats exhibit normal histological structure of the glomeruli and tubules at the cortex and there was no histopathological alteration (Fig. 1A). Aspartame administration resulted in congestion in the sclerotic cortical blood vessels with swelling in the lining epithelial cells of the tubules (Fig. 1B). On the other hand the treatment of aspartame-administered rats with rosemary extract showed that there was no histopathological alteration detected (Fig. 1C).

Fig.2 represents the histopathological changes in testicular sections of rats. Examination of testes of the control group revealed that there was no histopathological alteration and the normal histological structure of the mature active seminiferous tubules with complete spermatogenic series (Fig. 2 A). Administration of aspartame alone resulted in hyalinization in the sperms in the lumen of some tubules (Fig. 2 B). The treatment of aspartame-administered rats with rosemary extract revealed that the sperm of some tubules still exhibited hyalinization (Fig. 2 B).

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Urea (mg / dl)</th>
<th>% change</th>
<th>Creatinine (mg/dl)</th>
<th>% change</th>
<th>Na⁺ (mEq /L)</th>
<th>% change</th>
<th>K⁺ (mmol /L)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.6 ± 0.98 b</td>
<td>-</td>
<td>0.565 ± 0.009 b</td>
<td>-</td>
<td>152.77 ± 0.88 a</td>
<td>-</td>
<td>6.52 ± 0.097 b</td>
<td>-</td>
</tr>
<tr>
<td>Aspartame</td>
<td>47.3 ± 1.25 a</td>
<td>36.7</td>
<td>0.686 ± 0.017 a</td>
<td>21.41</td>
<td>139.94 ± 1.28 b</td>
<td>-8.39</td>
<td>7.68 ± 0.07 a</td>
<td>17.79</td>
</tr>
<tr>
<td>Aspartame + Rosemary</td>
<td>36.58 ± 1.02 b</td>
<td>-22.66</td>
<td>0.56 ± 0.0288 b</td>
<td>-18.36</td>
<td>152.96 ± 2.78 a</td>
<td>9.3</td>
<td>6.16 ± 0.18 b</td>
<td>-19.79</td>
</tr>
</tbody>
</table>

F-Probability P<0.001 P<0.001 P<0.001 P<0.001
LSD at the level 5% 3.29 0.0611 2.67 0.383
LSD at the level 1% 4.56 0.084 3.69 0.5301

- Data are expressed as Mean ± standard error.
- Means, shared only superscript symbol (s) are not significantly different.
- Number of animals in each group is six.
Table 2: Effect on serum levels of FSH, LH, and testosterone

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>FSH (µIU/L)</th>
<th>% change</th>
<th>LH (µIU/L)</th>
<th>% change</th>
<th>Testosterone (ng/ml)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.91 ± 0.217 a</td>
<td>-</td>
<td>6.13 ± 0.06 a</td>
<td>-</td>
<td>4.28 ± 0.16 a</td>
<td>-</td>
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<tr>
<td>Aspartame</td>
<td>3.94 ± 0.043 c</td>
<td>-33.33</td>
<td>4.11 ± 0.347 b</td>
<td>-32.95</td>
<td>3.08 ± 0.09 b</td>
<td>-28.03</td>
</tr>
<tr>
<td>Aspartame + Rosemary</td>
<td>4.41 ± 0.059 b</td>
<td>11.92</td>
<td>4.005 ± 0.41 b</td>
<td>-2.55</td>
<td>3.25 ± 0.11 b</td>
<td>5.52</td>
</tr>
</tbody>
</table>

F-Probability

- P<0.001
- P<0.001
- P<0.001

LSD at the level
- 5% 0.399 0.944 0.373
- 1% 0.5528 1.305 0.517

- Data are expressed as Mean ± standard error.
- Means, shared only superscript symbol (s) are not significantly different.
- Number of animals in each group is six.
Table 3: Effect on kidney oxidative stress and antioxidant defense system parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>GSH* (mg/g)</th>
<th>% change</th>
<th>GST* (U/g)</th>
<th>% change</th>
<th>GPx* (mU/g)</th>
<th>% change</th>
<th>SOD* (U/g)</th>
<th>% change</th>
<th>LPO* (n mol/100 mg)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5.6±0.15 a</td>
<td>-</td>
<td>349.4±31 ^a</td>
<td>-</td>
<td>784.5±21.8 ^a</td>
<td>-</td>
<td>0.94±0.01 ^a</td>
<td>-</td>
<td>12.6±0.36 ^b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Asp</td>
<td>4.5±0.2 cd</td>
<td>-19.75</td>
<td>219.6±15.4^b</td>
<td>-37.14</td>
<td>613.1±25 ^b</td>
<td>-21.84</td>
<td>0.74±0.03 ^b</td>
<td>-20.43</td>
<td>21.2±1.34 ^a</td>
<td>68.76</td>
</tr>
<tr>
<td></td>
<td>Asp + RE</td>
<td>5.2±0.2 ^ab</td>
<td>15.52</td>
<td>341.3±20.7 ^a</td>
<td>55.41</td>
<td>730.2±12.6 ^a</td>
<td>19.09</td>
<td>0.89±0.03 ^a</td>
<td>20.27</td>
<td>13.8±0.9 ^b</td>
<td>-34.99</td>
</tr>
<tr>
<td>F-Probability</td>
<td></td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD at the level 5%</td>
<td></td>
<td>0.55</td>
<td>70.2</td>
<td>61.8</td>
<td>0.073</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LSD at the level 1%</td>
<td></td>
<td>0.76</td>
<td>97.1</td>
<td>85.5</td>
<td>0.1</td>
<td>4.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± standard error.
- Means, shared only superscript symbol (s) are not significantly different.
- Number of animals in each group is six.
- * GSH (Reduced glutathione), GST (Glutathione-S-transferase), GPx (Glutathione peroxidase), SOD (Superoxide dismutase), LPO (lipid peroxidation), Aspartame (Asp) and Rosemary extract (RE).

Table 4: Effect on testis oxidative stress and antioxidant defense system parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>GSH* (mg/g)</th>
<th>% change</th>
<th>GST* (U/g)</th>
<th>% change</th>
<th>GPx* (mU/g)</th>
<th>% change</th>
<th>SOD (U/g)</th>
<th>% change</th>
<th>LPO* (n mol/100 mg)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>3.52±0.14 ^a</td>
<td>-</td>
<td>197.6±10.7 ^a</td>
<td>-</td>
<td>830.1±20.5 ^a</td>
<td>-</td>
<td>0.89±0.02 ^a</td>
<td>-</td>
<td>18.7±1.004 ^b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Asp</td>
<td>2.97±0.1 ^b</td>
<td>-15.62</td>
<td>159.5±12.4 ^a</td>
<td>-19.28</td>
<td>643.96±39.6 ^b</td>
<td>-22.42</td>
<td>0.55±0.05 ^b</td>
<td>-39.32</td>
<td>26.77±2.37 ^a</td>
<td>42.69</td>
</tr>
<tr>
<td></td>
<td>Asp + RE</td>
<td>3.49±0.14 ^a</td>
<td>17.5</td>
<td>183.01±8.8 ^a</td>
<td>14.75</td>
<td>766.4±19 ^a</td>
<td>19.01</td>
<td>0.82±0.02 ^a</td>
<td>51.85</td>
<td>17.4±2.42 ^b</td>
<td>-35.01</td>
</tr>
<tr>
<td>F-Probability</td>
<td></td>
<td>P&lt;0.1</td>
<td>P&lt;0.1</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LSD at the level 5%</td>
<td></td>
<td>0.398</td>
<td>-</td>
<td>84.5</td>
<td>0.109</td>
<td>6.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD at the level 1%</td>
<td></td>
<td>0.55</td>
<td>-</td>
<td>116.8</td>
<td>0.151</td>
<td>8.51</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± standard error.
- Means, shared only superscript symbol (s) are not significantly different.
- Number of animals in each group is six.
- * GSH (Reduced glutathione), GST (Glutathione-S-transferase), GPx (Glutathione peroxidase), SOD (Superoxide dismutase), LPO (lipid peroxidation), Aspartame (Asp) and Rosemary extract (RE).
Fig. 1 A - Kidney of control group, showing normal histological structure in the glomeruli (g) and tubules (t) at the cortex. (H&E x 64)

Fig. 1 B - Kidney of aspartame group, showing congestion in sclerotic (s) blood vessels with swelling in the lining epithelial cells of the tubules (t). (H&E x 64).

Fig. 1 C - Kidney of (aspartame+ rosemary) group, showing intact normal histological structure of the glomeruli (g) and tubules(t). (H&E x 64).

Fig. 2 A - Testis of control group, showing normal histological structure of the mature active seminiferous tubules with complete spermatogenic series (s). (H&E x 40).
Fig. 2 B - Testis of aspartame group, showing hyalinization in the lumen of some seminiferous tubules (s). (H&E x 40).

Fig. 2 C - Testis of (aspartame+rosemary) group, showing hyalinization in the spermatozoa hyalinization in the lumen of some seminiferous tubules (h). (H&E x 64).

Discussion

The present study highlights the effect of the aqueous rosemary extract on overdose consumption of aspartame. Rosemary (Rosmarinus officinalis L.) is a common household plant grown in many parts of the world, it is used in folk medicine, as an antispasmodic in renal colic and dysmenorrhea, in relieving respiratory disorders, and to stimulate growth of hair (Nusier et al., 2007). It was reported that rosemary has the potential to quench free radicals and improve the antioxidant status in rat tissues (Botsoglou et al., 2010).

In our study there was a highly significant increase in the levels of urea and creatinine in the serum of aspartame-induced rats when compared to normal rats. The elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances (Hummadi, 2012). In accordance with these results, it was investigated that methanol administration significantly increased serum urea and creatinine levels (Parthasarathy et al., 2006). Methanol is one of the aspartame metabolites which is a toxicant and causes systematic toxicity (Tsakiris et al., 2006). The treatment of aspartame administered rats with rosemary extract induced a highly significant decrease in the levels of
urea and creatinine when compared with corresponding groups. These are in agreement with Sahu et al. (2011) who found that carnosic acid treatment (100 mg/kg/ day oral) before cisplatin administration, efficiently reduced acute nephrotoxicity by preventing the increase in blood urea nitrogen and serum creatinine level.

The Na⁺, K⁺-ATPase is a complex membrane protein that utilizes ATP to transport three Na⁺ ions out of cells and two K⁺ ions in against their concentration gradients (Lingrel, 2010). The administration of aspartame showed a highly significant decrease in serum sodium concentration and increasing in potassium concentration when compared to normal rats, this action may be due to inhibition of Na⁺, K⁺-ATPase activity. This agree with Simintzi et al. (2008) who found a reduction of the frontal cortex membrane Na⁺, K⁺-ATPase activity due to the indirect action of ASP metabolites, especially those of methanol on the membrane bilayer, through production of free radicals and lipid peroxidation. The treatment of aspartame administered rats with rosemary extract induced a significant increase in serum sodium and decrease in potassium levels in comparison with corresponding groups. This may be due to the antioxidant properties of extracts of rosemary leaves. It is generally assumed that these antioxidant molecules from rosemary may act as free radical scavengers but additionally might play a role by regulating the activity and/or expression of certain enzymatic systems implicated in relevant physiological processes like apoptosis, tumour promotion and intracellular signal transduction (Perez-Fons et al., 2006).

In the present study, the histological appearance of renal tissues confirmed the previous results. The histopathological profile of the rat treated with aspartame showed congestion in the sclerotic cortical blood vessels with swelling in the lining epithelial cells of the tubules. This agree with El Haliem et al. (2011) who found in the renal cortex, some glomeruli were shrunken and there was loss of brush border of tubular epithelium, most of the proximal convoluted tubules showed heterochromatic nuclei with a dilated nuclear envelope, mitochondria with destroyed cristae, and numerous lysosomes. Administration of rosemary extract with aspartame normalized these defects in the histological architecture of the kidney. These agree with Sahu et al. (2011) who found that pre and post administration of carnosic acid (100 mg/kg) with cisplatin dose on 5th day revealed predominant normal kidney morphology with only occasional degenerating tubules.

In aspartame administered rats, it was found that the levels of serum testosterone, FSH and LH were highly significantly decreased in comparision with control rats. The low level of LH and FSH gonadotrophic hormones, as well as the inhibition of the synthesis and the
secretion of testosterone is related to neuronal dystrophy induced by aspartame in the specific regions of hypothalamus which control the activity of pituitary, induced damages in all cellular ultrastructures of the adenohypophysis (Puica et al., 2009). In agreement with our results it was reported that exposure to formaldehyde decreased serum testosterone levels (Ozen et al., 2005). The treatment of aspartame administered rats with rosemary extract showed a significant increase in FSH but non significant increase in the level of serum testosterone and LH compared to corresponding groups. These results are consistent with histological structure degenerations of testis.

The histopathology analysis of the test is revealed signs of toxicity after administration of aspartame. Administration of aspartame alone resulted in hyalinization in the sperms in the lumen of some tubules. Puica et al. (2009) stated that administration of 2 mg/kg b.w. of ASP induced alteration in the structure of the pituitary LH-FSH cells and they have few secretion granules. Administration of rosemary extract cannot protect testes against aspartame-induced histopathological changes.

Oxidative stress results from an imbalance between the cellular production of reactive oxygen species (ROS) and the antioxidant mechanisms that remove them (Posadas et al., 2009). Aspartame administration induced a highly significant increase in lipid peroxidation, a highly significant decrease in glutathione (GSH) content and a highly significant decrease in glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities in kidney when compared to normal rats. Also aspartame produced a significant increase in LPO, a significant decrease in GSH and a highly significant decrease in GPx and SOD activities in testis when compared to normal rats. In consistence with our findings, it was observed a significant elevation in LPO level in liver and kidney, also reported a decrease in liver GSH level and a decrease in SOD activity in liver and kidney (Iman and Mourad, 2011). Parthasarathy et al. (2006) investigated that methanol administration significantly increased LPO level in the lymphoid organs; also, Zararsiz et al. (2007) recorded a significant increase in LPO level in the kidney of rats after treatment with formaldehyde.

The decrease in GSH activity observed in the present study seems to have been caused by methanol, because methanol metabolism depends upon GSH, or may be caused by its rapid reaction with the highly reactive compound, formaldehyde, which is generated during methanol metabolism forming nucleophilic adducts and/ or lipid peroxidation products (Iman and Mourad, 2011). The decrease in activity of antioxidant enzymes is probably related with
the action formaldehyde and free radicals, formaldehyde readily reacts with the amino acids of soluble proteins leading to hydroxymethyl derivatives and intra and intermolecular bridges in proteins, also Free radicals formed during the methanol oxidation can cause formation of protein peroxides, these changes may result in denaturation, aggregation and fragmentation of proteins, altering physicochemical properties and potentially losing of enzymatic activities (Abhilash et al., 2011).

The treatment of aspartame administered rats with rosemary extract induced a highly significant decrease in lipid peroxidation level in kidney and testis. Also, a highly significant increase in glutathione content was observed. The activity of antioxidant enzymes in kidney and testis increased significantly when compared with corresponding groups. Aqueous extract of rosemary has an anti-lipoperoxidant activity, as it reduced the formation of malonaldehyde significantly in a dose dependent manner (Soyal et al., 2007).

In agreement with our results, it was found that carnosic acid (CA) treatment reduced lipid peroxidation in rat tissue and increased the reduced level of GSH and activity of GST, GPx and SOD in kidney of the cisplatin-treated animals. In addition, it was reported that LPO, GSH, SOD, and GPx levels were restored by the administration of CA (Xiang et al., 2013).

In contrast to our results, Slamenova et al. (2011) observed that no significant changes were found in the activity of the most important antioxidant enzymes, superoxide dismutase and glutathione peroxidase in rat testicular cells against DNA-damaging oxidative agents.

In conclusion, the result of this study demonstrates that rosemary extract has potent protective action upon aspartame-induced toxicity in rats kidney and possessed antilipid peroxidative and free radical scavenging activities. However, further studies are necessary to assess the safety and benefits of rosemary in humans.

References


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