EFFECTS OF ROSEMARY EXTRACT SUPPLEMENTATION ON ETHANOL INDUCED LIVER INJURY IN ADULT MALE ALBINO RAT

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ABSTRACT

Background: Rosemary is a native Mediterranean small green herb. Its leaves contain many active constituents providing defense against oxidative stress from oxidizing agents and free radicals. The liver has a central role in the metabolism of many drugs. Chronic alcohol intake is associated with increased oxidative stress and decreased antioxidant enzymes. Objective: Evaluation of the effects of Rosemary extract supplementation on ethanol induced liver injury in adult male albino rats. Material and methods: Thirty adult male albino rats of local strain weighing 140 - 155 g were chosen to be the model of the present study. They were divided into three equal groups: Group I (Control group) received no treatment, Group II (Ethanol group) were subjected to induction of hepatotoxicity by daily administration of 45% liquid ethanol for six weeks, and Group III (Rosemary-treated group) were subjected to induction of hepatotoxicity and Rosemary supplementation for six weeks. Blood samples were withdrawn, serum was separated for determination of ALT, AST, ALP, SOD and MDA serum levels. Rats were killed and pelvis-abdominal cavities were opened. Livers were excised for studying histopathological changes. Results: Ethanol administration was associated with significant changes in the liver functions indicated by elevated serum liver enzymes and distorted liver morphology associated with disturbed oxidant-antioxidant status as indicated by elevated serum MDA and decreased SOD levels. These changes were improved by Rosemary administration but however, these improvements did not reach the basal level. Conclusion: Ethanol administration markedly disturbed the liver functions. Rosemary administration has a hepatoprotective effects against these changes. Keywords: Rosemary, alcoholic liver disease, oxidants-antioxidants.

INTRODUCTION

The liver has a central role in metabolism. Therefore, it is highly susceptible to chemical-induced toxicity. Chemical-induced liver injury is a major health problem and accounts for the majority of acute liver failure (Corsini and Bortolini, 2013). The pathophysiological mechanisms of chemical-induced hepatotoxicity are mostly associated with the production of reactive oxygen species (ROS), which induce oxidative stress and damage of the cellular macromolecules (Gu and Manautou, 2012). Oxidative stress has been recognized as a key factor in the pathophysiological changes observed in a wide range of liver diseases (Zhu et al., 2012). Natural antioxidant products have gained worldwide popularity due to their efficacy and safety. They are increasingly used to treat various pathological liver conditions (Zhang et al., 2013).

Rosmarinus officinalis L. is a native Mediterranean small green herb belonging to the family of Labiatae and commonly called Rosemary. Its leaves contain many
constituents such as phenolic acids, flavonoids, vitamin C, vitamin B, choline and rosmarinic acid that have shown antioxidant property, providing a defense against oxidative stress from oxidizing agents and free radicals (Anadon et al., 2008).

In traditional medicine, Rosemary has been used as a stimulant and mild analgesic, and it has been considered as one of the most effective herbs for treating headaches, poor circulation, inflammatory diseases and physical and mental fatigue. Rosemary has also been used empirically as a choleretic and hepatoprotective agent in folk medicine (Yu et al., 2013). Most pharmacological effects of Rosemary are the consequence of high antioxidant activity of its main chemical constituents especially carnosol and carnosic acid (Ngo et al., 2011).

Alcohol consumption is known to be associated with liver damage. The close relation between ethanol and liver damage is mainly due to the fact that about 80% of ingested alcohol is metabolized in the liver (Meier and Seitz, 2008). Excessive alcohol consumption not only enhances reactive oxygen species (ROS) generation, but also depletes antioxidants, thus creating a state of oxidative stress that leads to severe liver injury (Cederbaum et al., 2009).

The present work was designed to evaluate the effects of Rosemary extract supplementation on ethanol-induced liver injury in adult male albino rats.

**MATERIAL AND METHODS**

The experimental protocol and animal handling were approved and performed according to the guidelines of animal use of the Ethical committee of Faculty of Medicine - Al-Azhar University. Thirty adult male albino rats of local strain weighing 140 - 155 g were chosen to be the model of the present study. They were left for two weeks in the laboratory room before any experimental interference for acclimatization with free access to water and rat chow pellets. Rats were kept in suitable cages (40 x 30 x 30 per 5 rats) at room temperature with the natural light/dark cycle. Rats were divided into three equal groups:

**Group I (Control group)** received no treatment and served as control group.

**Group II (Ethanol group)** were subjected to induction hepatotoxicity by daily administration of 0.5 ml of 45% liquid ethanol by intragastric tube for six weeks (Adaramoye et al., 2009).

**Group III (Rosemary group)** were subjected to combined induction of hepatotoxicity as group II and daily administration of 0.5 ml of Rosemary leaves extract by intragastric tube for six weeks (Abdul-Rahim and Taha, 2011).

**Rosemary extract:** Rosemary was purchased from local herbal market. Fifty grams of it was soaked in 150 ml boiled water for three hours, filtered with carbon silica cloth and the filtrate was stored in a refrigerator at 4 °C. Each rat was daily administrated 0.5 ml of Rosemary leaves extract by intragastric tube for six weeks (Abdul-Rahim and Taha, 2011).

At the end of the experimental period, blood samples were withdrawn. Serum was separated for determination of alanine aminotransferase and aspartate aminotransferase (Silverman et al., 1995), alkaline phosphatase (Overgaard et al.,
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1996), malondialdehyde (Yoshioka et al., 1979) and super oxide dismutase (Minami and Yoshikawa, 1979). Samples were stored at –20 °C until assayed. Following that, rats were killed and their abdominal cavities were opened. Livers were excised, kept in 10% formalin solution. Paraffin blocks were made and different sections at multiple levels were obtained. Slides were then stained by Hematoxylin and Eosin stains, and examined using a light microscope for evaluation of histopathological changes.

Statistical analysis: Data input and analysis were done using SPSS computer program. All results were expressed as mean ± standard error. Mean values of the different groups were compared using a one-way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. P value < 0.05 was accepted to denote a significant difference.

RESULTS

Results of the present work showed that ethanol administration led to significant increase in serum ALT level from 41.16 ± 1.2 u/ml to 62.83 ± 1.92 u/ml (+ 52.65 %), significant increase in serum AST level from 46.34 ± 2.52 u/ml to 71.91 ± 2.83 u/ml (+ 55.18 %), significant increase in serum ALP level from 78.24 ± 1.39 u/l to 92.89 ± 2.18 u/l (+ 18.72 %), significant increase in serum MDA level from 25.45 ± 1.01 ?mol/l to 53.76 ± 1.56 ?mol/l (+ 111.24 %), and significant decrease in serum SOD level from 9.02 ± 0.83 mg/dl to 3.98 ± 0.62 mg/dl (-55.88 % - Table 1).

Administration of Rosemary extract led to significant decrease in serum ALT level from 62.83 ± 1.92 u/ml to 47.32 ± 1.28 u/ml (-24.69 %), significant decrease in serum AST level from 71.91 ± 2.83 u/ml to 51.48 ± 1.89 u/ml (-28.41 %), significant decrease in serum ALP level from 92.89 ± 2.18 u/l to 83.42 ± 1.64 u/l (-10.39 %), significant decrease in serum MDA level from 53.76 ± 1.56 ?mol/l to 41.86 ± 1.41 ?mol/l (-22.14 %), and significant increase in serum SOD level from 3.98 ± 0.62 mg/dl to 6.27 ± 0.43 mg/dl (+57.53 % - Table 1).

When compared to group I, results of the present work showed that administration of Rosemary extract led to significant increase in serum ALT level from 41.16 ± 1.2 u/ml to 47.32 ± 1.28 u/ml (+14.97 %), insignificant increase in serum AST level from 46.34 ± 2.52 u/ml to 51.48 ± 1.89 u/ml (+11.09 %), significant increase in serum ALP level from 78.24 ± 1.39 u/l to 83.42 ± 1.64 u/l (+6.62 %), significant increase in serum MDA level from 25.45 ± 1.01 ?mol/l to 41.86 ± 1.41 ?mol/l (+64.48 %), and significant decrease in serum SOD level from 9.02 ± 0.83 mg/dl to 6.27 ± 0.43 mg/dl (-30.49 % - Table 1).

* Histopathological study (figures 1 - 6): Liver of the control group showed normal pattern of liver architecture with maintained hepatic lobule containing central venule and normally arranged hepatocytes separated by blood sinusoids (figure 1). Ethanol administration led to distorted trabecular structure of the hepatic lobules. The cytoplasm enlarged and contained empty vacuole-like spaces. Some sinusoids were overfilled with erythrocytes. Local polymorphonuclear infiltration, mild steatosis and duct proliferation (figures 2 - 4). Liver tissue of
rats treated by Rosemary showed marked improvement of hepatocytes morphology with evidence of regenerative activity, reduced cytoplasmic vacuolations, sinusoidal dilation and polymorphonuclear infiltration (figures 5 and 6).

Table (1): Changes in the measured parameters in tested groups (Mean ± S.E).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean ± S.E.</th>
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<tbody>
<tr>
<td></td>
<td>Group I (n=10)</td>
<td>Group II (n=10)</td>
</tr>
<tr>
<td>ALT (u/ml)</td>
<td>41.16 ± 1.2</td>
<td>62.83 ± 1.92</td>
</tr>
<tr>
<td></td>
<td>+ 52.65 %* a</td>
<td>- 24.69 %* b</td>
</tr>
<tr>
<td>AST (u/ml)</td>
<td>46.34 ± 2.52</td>
<td>71.91 ± 2.83</td>
</tr>
<tr>
<td></td>
<td>+ 55.18 %* a</td>
<td>- 28.41 %* b</td>
</tr>
<tr>
<td>ALP (u/l)</td>
<td>78.24 ± 1.39</td>
<td>92.89 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>+ 18.72 %* a</td>
<td>- 10.39 %* b</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>25.45 ± 1.01</td>
<td>53.76 ± 1.56</td>
</tr>
<tr>
<td></td>
<td>+ 111.24 %* a</td>
<td>- 22.14 %* b</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>9.02 ± 0.83</td>
<td>3.98 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>- 55.88 %* a</td>
<td>+ 57.53 %* b</td>
</tr>
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- Group II was compared to group I (a).
- Group III was compared to group II (b).
- Group III was compared to group I (c).
- n: No. of rats in each group.

* Significant.

Figure (1): Normal liver structures in control group (H & E x 125).

Figure (2): PNL infiltration in ethanol group (H & E x 125).
DISCUSSION

The present work was designed to evaluate the effects of Rosemary extract supplementation on ethanol-induced liver injury in adult male albino rats.

Administration of 45% ethanol led to hepatotoxicity indicated by elevated serum levels of ALT, AST and ALP in addition to disturbed oxidant-antioxidant status evidenced by elevated serum MDA and reduced SOD levels. These results agreed with Adaramoye et al. (2009) who reported that chronic ethanol administration led to hepatotoxicity as evidenced by the increased levels of serum ALT, AST and ALP, and enhanced formation of MDA up to 70% associated with reduced SOD level in animals treated by 45% ethanol. Jang et al. (2014) reported that chronic ethanol administration resulted in a clear hepatotoxicity as evidenced by the increased plasma AST and ALT and significantly decrease in the activities of the hepatic antioxidant enzymes SOD and CAT. It has been reported that the high levels of AST and ALT in the plasma compartment are central indications of the degree of liver damage caused by ethanol administration (Panda et al., 2012).

Results of the present work were also in agreement with Bhopale et al. (2015)
who reported that the plasma ALT, AST, ALP and LDH levels increased in ethanol-fed rats. Osna et al. (2016) reported that ethanol treatment significantly elevated serum AST and ALT levels. Thomes et al. (2017) also reported elevated levels of serum ALT, hepatic lipid peroxides and triglycerides in all ethanol-fed mice. It has also been reported that ethanol treatment decrease the defense enzymatic system including superoxide dismutase, catalase and glutathione peroxidase. In addition, malondialdehyde (MDA) and toxicity biomarker levels such as aspartate transaminase (AST) and alanine transaminase (ALT) and alkaline phosphatase (ALP) and gamma-glutamyl transaminase (GGT) activities elevate after chronic ethanol treatment (Kamoun et al., 2017).

The disturbed oxidant-antioxidant markers observed in the present work were also reported by Gong et al. (2016) who stated that ethanol consumption led to a significant increase in the malondialdehyde (MDA) and nitric oxide levels and a significant decrease in superoxide dismutase (SOD) and glutathione peroxidase activities.

Oxidative stress and lipid accumulation play important roles in alcohol-induced liver injury. The predominant factor causing ethanol-associated liver damage is the generation of oxidative stress. Ethanol administration provokes an oxidative imbalance through a number of pathways, including the generation of reactive oxygen species and alteration of the defense mechanisms (Singal et al., 2011). Also, ethanol enhances cell apoptosis by mitochondrial damage which could be one of the important mechanisms for alcoholic-induced liver injury (Jang et al., 2014).

Results of the present work showed that Rosemary extract administration led to reduction of the elevated serum levels of ALT, AST and ALP in addition to enhanced oxidant-antioxidant status evidenced by decreased serum MDA and increased serum SOD levels. These results were in agreement with Xiang et al. (2013) who reported that carnosic acid inhibited liver damage and disorder of lipid metabolism evidenced by decreased serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Raskovic et al. (2014) has also reported that AST and ALT activities were increased in liver injury mainly due to leakage of these enzymes from damaged hepatocytes into the bloodstream. The treatment of animals with Rosemary attenuated these parameters of hepatotoxicity. Also, the elevated MDA level suggests oxidative damage of cell membranes. They concluded that Rosemary supplementation partially normalizes the altered biochemical parameters, and significantly reverses the oxidative stress-related parameters in the experimental model of liver injury. Zhao et al. (2015) has reported that dietary Rosemary supplementation significantly reduces body weight gain, percent of fat, plasma ALT, AST, triglycerides, free fatty acids and plasma and liver malondialdehyde levels in mice fed with a high fat diet.

It has been reported that in the rat model of alcohol-induced liver injury, supplementation with the Rosemary extracted active constituent (carnosic acid) significantly decreased serum amino-
transferase, triglyceride and total cholesterol levels. Additionally, it inhibits oxidative stress, inflammation and cell death (Gao et al., 2016).

Results of the present work were in agreement with Wang et al. (2017) who reported that feeding with Rosemary extract significantly increases the enzyme activity of superoxide dismutase (SOD) and catalase (CAT), and significantly decreases the level of malondialdehyde.

Hepatoprotective effects of Rosemary could be attributed to its active constituents especially carnosic acid which was reported to have various pharmacological effects such as smooth muscle relaxant, anti-inflammatory and antioxidant providing free radical scavenging activity and exerts beneficial effects on preventing hepatotoxicity by limiting the extent of lipid peroxidation and hence cell membrane injuries (Raskovic et al., 2014).

Results of the present work showed that ethanol administration led to distorted structure of the hepatic lobules, vacuolated cytoplasm, polymorphonuclear infiltration, mild steatosis and duct proliferation. These results were in agreement with Jang et al. (2014) who reported that liver sections of rats treated with ethanol showed severe histopathological alterations appearing as centrilobular areas with necrosis and different degenerative changes such as ballooning, fatty and hydropic degeneration in association with inflammatory cell infiltration and dilation in the central vein. Bhopale et al. (2015) reported that liver histology of ethanol-fed rats shows inflammation, fatty change (cytoplasmic vacuolization) and midzonal pronounced fatty infiltration ranging from micro- to macrovacuolization. Also, Osna et al. (2016) have reported that, in the ethanol-treated rat, all rats exhibited a panlobular microvesicular pattern of steatosis and low levels of macrosteatosis, signs of inflammatory changes and hepatocyte cell injury but no fibrosis was seen.

In the present work, Rosemary supplementation led to improved histopathological changes of the liver section of ethanol-induced liver injury.

These results were in agreement with Xiang et al. (2013) who reported that the histopathological examination demonstrated that Rosemary extracted active constituent (carnosic acid) could improve pathological abnormalities and reduce the immigration of inflammatory cells in liver tissues. Zhao et al. (2015) has reported decreased lipid accumulation in hepatocytes in mice administrated Rosemary in comparison with that of high fat-diet-fed mice. El-Naggar et al. (2016) has been reported that examination of the liver sections of the mice that were treated with Rosemary showed improvement in the liver architecture, and the histological appearance of the hepatocytes was nearly similar to that of the control mice.

It has also been reported that administration of Rosemary extracted active constituent (rosmarinic acid) stimulated hepatocyte proliferation indicating that rosmarinic acid is potentially useful to promote liver regeneration (Lou et al., 2016).

CONCLUSION

Rosemary leaves extract mediated hepatoprotective effects through
scavenging of harmful free radicals and stimulated hepatocyte proliferation.

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REFERENCES


تأثر استخدام خلاصة الروزمارى على إصابات الكبد المحدثة بالإيثانول في ذكور الجرذان البيضاء البالغة

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خلاصة البحث: الروزمارى هو شجيرة صغيرة منشأها حوض البحر المتوسط. تحتوي أوراقه على العديد من المواد الفعالة المجابة للإجهاد التأكسدي بسب المواد المؤكسدة والشوارد الحرة. يؤدّي الكبد دورا هاما في عملية الأيض لمعظم المواد الداخلة للجسم ولذلك يتعرض للإصابات بالسموم أكثر من غيره من الأعضاء. وقد تبين تأثير الإجهاد التأكسدي على أمراض الكبد ومالها. والكحول هو أحد المسابات لأمراض الكبد (الكبد الدهني والثليفات) والتي تحدث غالبا بسبب خلل التوازن بين المواد المؤكسدة ومضافات الأكسدة بالجسم.

الهدف من البحث: تقييم استخدام خلاصة الروزمارى على إصابات الكبد المحدثة بالإيثانول في ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: استخدم في هذا العمل ثلاثون ذكر من السلالة المحلية من الجرذان البيضاء البالغة. تم تقسيمهم إلى ثلاثة مجموعات متساوية: المجموعة الأولى (مجموعة ضابطة)، والمجموعة الثانية (مجموعة اختبار) تعرضت لإحداث السمية الكبدية عن طريق تناول الإيثانول بتركيز 40 % يوميا لمدة ستة أسابيع. المجموعة الثالثة (مجموعة اختبار) تعرضت لإحداث السمية الكبدية بالإيثانول مع تناول خلاصة الروزمارى يوميا لمدة ستة أسابيع. وفي نهاية العمل تم سحب عينات دم وفصل المصل لقياس مستويات إنزيمات الكبد (أمينوترايسفيريز، أسينات، أمينوترايسفيريز، الكالاين فوسفاتي)، بالإضافة إلى بعض عوامل ومضافات التأكسد. ثم قنطت الجرذان وأخذن عينات من الكبد لدراسة التغيرات الهيستولوجية الممكن حدوثها.

النتائج: أدى تناول الإيثانول إلى زيادة تركيزات إنزيمات الكبد مع اضطراب التوازن بين عوامل ومضافات التأكسد بالإضافة إلى بعض التغيرات الهيستولوجية في عينات الأنسجة وقد تحسن هذه الاضطرابات إثر تناول خلاصة الروزمارى.

الاستنتاج: الروزمارى كأحد مضادات الأكسدة الطبيعية ذو أهمية في حماية الكبد من الأمراض المزمنة والتي يكون سبباً الرئيسية اضطراب التوازن بين عوامل ومضافات التأكسد بالجسم.