Modulating Efficiency of γ-Irradiated Rosemary in Improving the Hepatic Antioxidant Status of Ethanol Administered Rats

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Abstract

Alcoholic liver disease represents a spectrum of clinical illness and morphological changes such as hepatic inflammation and necrosis (alcoholic hepatitis). Among natural antioxidants, rosemary contains several antioxidant oil and phenolic components that exhibit hepatoprotective effect. This study aimed to investigate the antioxidant effect of dietary supplementation with γ-irradiated rosemary in ethanol induced liver injury in rats. Rosemary essential oil was analyzed by gas chromatography/mass spectrometry (GC/MS). The results of biological study revealed that dietary supplementation of either raw or γ-irradiated rosemary following ethanol administration exerts remarkable modulating effect by reducing the level of total bilirubin, the activity of transaminases, gamma glutamyl transferase and serum alkaline phosphatase, decreasing the concentration of some lipid contents, malondialdehyde and xanthine oxidase activity. Also, supplementation of dietary rosemary resulted in elevation of high density lipoprotein level, reduced glutathione content and enhances the activity of xanthine oxidase dehydrogenase, superoxide dismutase and catalase. Thus, gamma-irradiated rosemary could be incorporated to the diet as a nutritional supplement, to augment the liver’s defences against oxidative stress.

Keywords: Liver diseases; Rosemary; Essential oil; Gamma-irradiation; Antioxidants

Introduction

Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to many different diseases [1]. Alcohol administration is one of the most common causes of chronic liver disease in the world and it was found that alcohol affects the liver, through not only nutritional disturbances but also its direct toxicity, because its predominant metabolism in the liver is associated with oxidation-reduction changes and oxidative stress [2]. The body’s natural defenses against free radicals (e.g. antioxidants) are inhibited by alcohol consumption resulting in the increasing of liver damage [3,4].

There has been a great deal of interest in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases [4]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness [5].

One of these herbs is Rosemary or Rosmarinus officinalis L. (Labiatae) which is an evergreen perennial shrub grown in many parts of the world. It has been used as medicinal plants in folk medicine, but Rosmarinus itself was used for asthma, bronchitis, cold, flu, digestive, anaemia, hypertension, insomnia, labyrinthitis, sluggishness memory, tachycardia, vitiligo, for high cholesterol and diabetes disease [6-9]. Rosemary contains caffeic acid, carnosol, rosmarinic acid and rosmarinic acid, all of which are potent antioxidants as well as anti-inflammatory agents. Due to its antioxidants, rosemary can help prevent cataracts and the natural acids present in rosemary help in protecting the body’s cells and DNA from free radical damage. It is also a good source of antioxidant vitamin E (alpha tocopherol) and other important antioxidants [10]. Moreover, the volatile oils in rosemary also help reduce inflammation that contributes to liver and heart disease [11].

Especially during picking, processing and packing, rosemary is susceptible to contamination by pathogenic microorganisms [12]. Gamma radiation is a highly effective means of inhibiting the growth of undesirable microbes and avoiding the occurrence of food-transmitted diseases. This is substantiated by the fact that an increasing number of countries have adopted irradiation as a way to ensure the hygienic quality of dehydrated foods [13]. The international safe dose clearance is up to 10 kGy, though some countries, including Argentina, have increased this level to 30 kGy without any harmful effects being observed [14]. Also, the effect of irradiation on some of the compounds responsible for antioxidant activity in rosemary has been reported by Koseki et al. [15], Calucci et al. [16] and El-Beltagi et al. [17].

The objective of the study is therefore to evaluate the efficiency of dietary supplementation with raw and γ-irradiated rosemary in improving the hepatic antioxidant status of ethanol administered rats.

Material and Methods

Materials

Rosemary (Rosmarinus officinalis L.) powder and standard commercial rodent diet were purchased from local herbal market (Cairo, Egypt), while ethanol was purchased from Sigma Company.

Gamma radiation process

The samples of dry rosemary powder were transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a 60Co source at a dose rate of 4.70 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

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GC-MS analysis of rosemary essential oil

**Extraction of essential oil:** The essential oils of rosemary were obtained by water distillation in a glass apparatus for 3 hours. The separated volatile oils were dried over anhydrous sodium sulphate before hold glass bottles at -20°C according to Guenther [18].

**Separation and identification of chemical components of the essential oil:** Separation and identification of essential oil components were performed by using Gas chromatography instrument, Model Hewlett-Packard- MS (5970) series II at the Agriculture Research Centre, Giza, Egypt. Condition analysis are as follows: Column-30 m hp Methyl silicon 0.1 mm; Temperature: Initial 60°C; Rate: 3°C/min up to 240°C; Carrier gas: Helium 1.0 ml/min; Injection port; Temperature: 250°C; Detector temperature: 270°C; Integration: By using HP software Data; Injection volume: 0.3 ml. The isolated peaks were identified by matching with data from the library of mass spectra and compared to those of authentic compounds and published data [19]. Quantitative determination was carried out based on peak area integration.

**Animals**

The experiments were conducted on male albino rats (140 ± 20g). The animals were housed under conditions of controlled temperature (30 ± 2°C) with natural light. Food and water were provided ad-libitum.

**Study design**

The animal were randomly divided into 4 groups, each consisted of 7 rats.

**Group I:** rats were fed on balanced diet for 8 weeks, served as control.

**Group II:** rats were fed on balanced diet for 8 weeks and received daily oral dose of 20% (v/v) ethanol 5ml/100g body weight daily for four weeks [4].

**Group III:** rats received daily oral dose of 20% (v/v) ethanol (5 ml/100 g B.wt./day) for 4 weeks followed by dietary raw rosemary (1%W/W) for 4 weeks.

**Group IV:** rats received daily oral dose of 20% (v/v) ethanol (5 ml/100 g B.wt./day) for 4 weeks followed by dietary irradiated rosemary (1%W/W) for 4 weeks.

At the end of the experiment, animals from each group were sacrificed 24 hrs post the last dose of treatment. Blood samples were collected though heart puncture after light anaesthesia and allowed to coagulate and centrifuged to obtain serum for biochemical analysis. Also, liver tissue was removed for biochemical investigation.

**Biochemical analysis**

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel [20], serum gamma glutamyl transferase (GGT) was assessed according to Rosalk [21] as well as serum alkaline phosphatase activity (ALP) was assessed according to Kind and King [22]. Total bilirubin was analyzed according to Rosalk [21] as well as serum alkaline phosphatase activity (ALP) was assessed according to Malloy and Evelyn [23]. In addition, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain et al. [24], Fossati and Prenrice [25] and Demacker et al. [26], respectively, while low-density lipoprotein cholesterol and very low-density lipoprotein-cholesterol were evaluated according to Friedwald et al. [27] and Norbert [28] formulas, respectively by the following equations: LDL-C (mg/dl)=TC-(TG/5+HDL-C), vLDL (mg/dl)=TG/5. The lipid peroxidation was determined colorimetrically as malondialdehyde (MDA) [29]. Hepatic xanthine oxidase (XO) and xanthine dehydrogenase (XDH) were determined according to Kaminski and Jewezska [30]. Hepatic glutathione content (GSH) and the activity of superoxides dismutase (SOD) and catalase (CAT) were measured by the method of Gross et al. [31], Minami and Youshikawa [32] and Nie [33], respectively.

**Statistical analysis**

Statistical analyses were performed using computer program. Statistical Packages for Social Science (SPSS) [34] and values were compared to each other using one-way analysis of variance (ANOVA).

**Results**

Rosemary essential oils were analyzed by GC-MS chromatograms and the results revealed that the main components of the raw samples were camphor (20.85%), caryophyllene (18.37%), 1, 8-cineole (14.49%), Δ-Cadinene (9.59%) and α-Finene (8.46%). While, the main components of irradiated rosemary essential oil (10 Kgy) were 1, 8-cineole (33.68%), α-Terpinolen (22.63%) and Borneol (7.88%) (Table 1).

The activity of AST, ALT, ALP and GGT as well as the concentration of serum bilirubin for different animal groups were given in table 2. Oral administration of ethanol induced significant elevation in the activity of these liver enzymes and the level of total bilirubin as compared to the values of control at P<0.05. Whereas, treatment of EtOH-rats with raw or irradiated rosemary showed a significant reduction in these enzymes activity and total bilirubin level as compared to ethanol administrated rats.

The mean values of serum TC, TG, LDL-C and vLDL-C were significantly increased, while the mean value of HDL-C was significantly decreased.

**Table 1:** Effect of γ-irradiation on constituents (% of rosemary essential oil.

| Compounds          | RT(min) | Constituents of essential oil |
|--------------------|---------|------------------------------|
|                    |         | Raw                         |
|                    |         | 10 Kgy                      |
| α-Pinene           | 5.204   | 8.46                        |
| β-Pinene           | 5.338   | 0.64                        |
| 1,8-Cineole        | 8.603   | 14.49                       |
| γ-Terpine          | 7.345   | 4.50                        |
| B-Terpine          | 8.403   | 0.30                        |
| α-Terpineolen      | 8.768   | 2.84                        |
| Camphor            | 9.766   | 0.08                        |
| Borneol            | 10.605  | 0.94                        |
| Verbenone           | 11.031  | 2.90                        |
| Benzaldehyde       | 11.687  | 1.35                        |
| Propanol           | 11.858  | -                           |
| Myrtanol           | 12.052  | 0.64                        |
| Cinnamaldehyde     | 12.332  | 0.91                         |
| Endobornyl         | 12.478  | 0.46                         |
| Phenol             | 12.575  | -                           |
| Promecarb          | 12.916  | 0.08                        |
| α-Copaene          | 14.558  | 5.75                         |
| Caryophyline       | 14.777  | 18.37                        |
| α-Humulene         | 15.349  | 0.71                        |
| Naphthalene        | 15.641  | 1.60                        |
| Dels-Cadinene      | 17.027  | 9.99                        |
| Caryophyline oxide | 17.721  | 2.70                        |
| Tau-Cadinol        | 17.794  | 0.45                        |
| Cyclopentaneeacetic acid | 17.988 | 0.57                       |
| β-Elemene          | 18.085  | 0.53                        |
| Caryophylla        | 18.188  | -                           |
| Benzoic cyclohexanate | 19.995 | 0.80                         |
| Anymol             | 20.081  | -                           |
| α-Androst           | 20.652  | 0.53                        |
| Unknown            | 20.923  | 5.28                        |
decreased in ethanol group compared to control group. EtOH-rats dietary supplemented with raw and γ-irradiated rosemary showed an obvious decrease in the mean values of serum TC, TG, LDL-C and vLDL-C associated with a noticeable increase in HDL-C concentration compared to ethanol group (Table 3).

Ethanol intake was associated with marked decrease in level of hepatic GSH content and the activity of XDH, SOD and CAT accompanied by significant increase in the values of MDA and XO activity. Treatment of rats with rosemary (raw and irradiated) following ethanol administration resulted in improvement in the activity of the antioxidant enzymes and elevation of GSH content as well as reduction of MDA and XO levels (Table 4).

**Discussion**

Excessive intake of alcohol causes severe damage to the liver, which may become cirrhotic. Rosemary constituents have a therapeutic potential in the treatment or prevention of bronchial asthma, spasmodicogenic disorders, diabetes mellitus, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischemic heart diseases, cataract, cancer and poor sperm motility [5,35-38].

From the results of this study, it could be observed that some essential oils were disappeared and some new components were appeared in irradiated rosemary as well as the values of other essential oils such as 1,8-cineole and α-Terpinolene were increased under the effect of γ-irradiation (10 kGy). These results were in agreement with Lee et al. [39] who found that radiation dose up to 10 kGy resulted in appearance of new components (bicyclo, phenol and α-Copaene) and disappearance of some components (β-Terpinene, α-Terpinol, benzaldehyde and camphene) in irradiated rosemary essential oil samples, in addition to the enhancement of antibacterial activity and of scavenging activity. Moreover, Perez et al. [14] reported that sanitizing dry rosemary with gamma radiation gives rise to extracts and of scavenging activity. Moreover, Pérez et al. [14] reported that the hepatoprotective properties of rosemary via the ductase system (XO and XDH), GSH, SOD and CAT of ethanol administered rats. Also, Hussein et al. [4] observed a significant increase in the activity of serum liver enzymes ALT, AST and γGT and the concentration of total bilirubin compared to ethanol group. These finding are in accordance with the results of Fahim et al. [44], who reported that administration of rosemary extract (150 mg/kg body weight) to rats for 3 weeks produced pronounced hepatoprotective effect. Also, Aruoma et al. [45] exhibited the hepatoprotective properties of rosemary via the retardation of oxidative degradation of lipids. It was also previously proved that rosemarinic and carnosic acids contain mixtures of natural antioxidants inhibited LDL oxidation and have the ability to prevent the deposition of triglycerides in the liver [46-48]. Moreover, Abd El-Ghany et al. [49] obtained that the inclusion of rosemary powder and rosemary extract to the liver injured rats ameliorated liver enzyme activities compared with CCl4-rats. However, ethanol administrated-rats received either raw or γ-irradiated rosemary for 4 weeks had a significant amelioration in the activity of ALT, AST, ALP and γGT and total bilirubin. Rajakrishnan and Menon [42] indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Das et al. [43] reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzyme γGT into blood. Also, Hussein et al. [4] observed a significant increase in the activity of serum liver enzymes ALT; AST and γGT in ethanol group compare to control group.

However, ethanol administrated-rats received either raw or γ-irradiated rosemary for 4 weeks had a significant amelioration in the activity of ALT, AST, ALP and γGT and the concentration of total bilirubin compared to ethanol group. These finding are in accordance with the results of Fahim et al. [44], who reported that administration of rosemary extract (150 mg/kg body weight) to rats for 3 weeks produced pronounced hepatoprotective effect. Also, Aruoma et al. [45] exhibited the hepatoprotective properties of rosemary via the retardation of oxidative degradation of lipids. It was also previously proved that rosemarinic and carnosic acids contain mixtures of natural antioxidants inhibited LDL oxidation and have the ability to prevent the deposition of triglycerides in the liver [46-48]. Moreover, Abd El-Ghany et al. [49] obtained that the inclusion of rosemary powder and rosemary extract to the liver injured rats ameliorated liver enzyme activities compared with CCl4-rats. However, ethanol administrated-rats received either raw or γ-irradiated rosemary for 4 weeks had a significant amelioration in the activity of ALT, AST, ALP and γGT and total bilirubin. Rajakrishnan and Menon [42] indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Das et al. [43] reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzyme γGT into blood. Also, Hussein et al. [4] observed a significant increase in the activity of serum liver enzymes ALT; AST and γGT in ethanol group compare to control group.

Several studies demonstrated that alcohol intake is associated with changes in serum lipid concentrations and whole-body lipid balance [4,50]. In the present study, there was a significant increase in the mean values of serum TC, TG, LDL-C and vLDL-C accompanied by a significant decrease in the mean value of serum HDL-C in ethanol group. These results were in agreement with Kumar et al. [41] who concluded that irradiation of rosemary oil can be used to obtain products with improved antioxidant activity and eliminates oxidative stress induced by persistent environmental pollutants (2,3,7,8-Tetrachlorodibenzodioxin) in rats in a time-dependent manner. Also, Santos et al. [41] reported that the hepatic necrosis induced by D-galactosamine/lipopolysaccharide (GALN/LPS) was greatly reduced by 1,8 cineole treatment.
indicative of ethanol induced oxidative stress in the liver leading to the decrease in CAT activity in the hepatic tissue of rats treated with 2 g/kg ETOH for a period of 4 weeks.

In this study, inclusion of rosemary powder (raw or γ-irradiated) to ETOH-rats provided anti-lipoperoxidant activity, as it reduced the formation of MDA and significantly decreased in XO activity associated with an obvious elevation in GSH content and the activity of XDH, SOD and CAT in liver. Bakirel et al. [65] found that long-term treatment of diabetes with the highest dose of the Rosmarinus officinalis extract had reversed the activities of the antioxidant enzymes, which might be due to decreased oxidative stress as evidenced by decreased lipid peroxidation. The authors reported that the Rosmarinus officinalis extract due to presence of several bioactive antioxidant principles and their synergistic properties may be caused an improving effect in antioxidant status. Moreover, Khalil et al. [66] observed a significant decrease in oxidative stress markers including serum TBARS and nitric oxide (NO). Serum enzymatic (glutathione transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidants (vitamin C and reduced glutathione) were found to be increased by the administration of Rosmarinus officinalis.

Conclusion

In conclusion, the data obtained in the present investigation confirmed the well known effect of ethanol in decreasing the antioxidant enzymes in liver tissues which may be due to the production of high amount of ROS. These effects were reversed by the treatment of rats with 1% of dietary γ-irradiated rosemary suggesting that rosemary has the potential to inhibit lipid peroxidation and improve the antioxidant status in rat liver. Hence, rosemary might be utilized as a nutritional supplement or a functional food component against liver injury. Moreover, the present data revealed that radiation dose (10 kGy) can improve the quality of rosemary essential oil by increasing the value of some essential oil such as 1,8-cineole.

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