

Modulating Efficiency of γ -Irradiated Rosemary in Improving the Hepatic Antioxidant Status of Ethanol Administrated 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, rosmaridiphenol 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 administrated 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 irradiation 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: $3^{\circ}\text{C}/\text{min}$ 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 ($140 \pm 20\text{g}$). The animals were housed under conditions of controlled temperature ($30 \pm 2^{\circ}\text{C}$) 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 through 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 using the method reported by 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 Prencipe [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: $\text{LDL-C (mg/dl)} = \text{TC} - (\text{TG}/5 + \text{HDL-C})$, $\text{vLDL (mg/dl)} = \text{TG}/5$. 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 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).

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 -Pinene (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

Compounds	RT(min)	Constituents of essential oil	
		Raw	10 kGy
α -Pinene	5.204	8.46	2.40
B- Pinene	5.338	0.64	-
1,8-Cineol	6.603	14.49	33.68
γ -Terpinene	7.345	4.50	1.50
B-Terpineol	8.403	0.30	-
α -Terpinolen	8.476	2.84	22.63
Camphor	8.768	20.85	-
Borneol	9.766	0.08	7.88
α -Terpineol	10.605	0.94	-
Verbenone	11.031	2.90	1.40
Benzaldehyde	11.687	1.35	-
Propanol	11.858	-	0.51
Myrtanol	12.052	0.64	-
Cinnannaldehyde	12.332	0.91	-
Endobornyl	12.478	0.46	-
Phenol	12.575	-	0.56
Promecarb	12.916	0.08	1.49
α -Copaene	14.558	-	5.75
Caryophyllene	14.777	18.37	6.32
α -Humulene	15.349	0.71	0.06
Naphthalene	15.641	1.60	1.26
Delta-Cadinene	17.027	9.59	0.97
Caryophyllene oxide	17.721	2.70	1.03
Tau-Cadinol	17.794	0.45	0.56
Cyclopentaneacetic acid	17.988	-	0.57
β -Elemene	18.085	0.53	-
Caryophylla	18.158	-	1.02
Benzocycloheptane	19.995	0.80	-
Anymol	20.081	-	1.81
α -Androst	20.652	0.53	0.33
Unknown	20.923	5.28	8.27

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

Parameters	I	II	III	IV
AST (U/ml)	25.87 ± 1.74 ^a	49.43 ± 2.93 ^c	31.38 ± 2.29 ^b	30.89 ± 2.29 ^b
ALT(U/ml)	15.78 ± 1.18 ^a	44.18 ± 2.11 ^c	25.37 ± 1.95 ^b	24.15 ± 1.62 ^b
ALP (U/100 ml)	9.12 ± 0.37 ^a	16.95 ± 0.71 ^c	11.22 ± 0.57 ^b	11.07 ± 0.53 ^b
GGT(U/ml)	3.38 ± 0.27 ^a	6.09 ± 0.44 ^c	4.51 ± 0.47 ^b	4.42 ± 0.39 ^b
Bilirubin(mg/dl)	0.61 ± 0.02 ^a	1.13 ± 0.03 ^c	0.71 ± 0.02 ^b	0.70 ± 0.03 ^b

Values are expressed as means ± S.E. (n=7).

Values in the same row with different superscripts are differing significantly at P<0.05.

Table 2: Effect of rosemary supplementation on hepatic markers in the serum of ethanol administered rats.

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, spasmogenic 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 -Terpinolen 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 -Terpineol, α -Terpineol, benzaldehyde and camphene) in irradiated rosemary essential oil samples, in addition to the enhancement of antibacterial activity and of scavenging activity. Moreover, Pérez et al. [14] reported that sanitizing dry rosemary with gamma radiation gives rise to extracts with improved antioxidant properties which could be of use in the food and pharmaceutical industries.

The mechanism by which radiation induces changes in the volatile oil composition could presumably be due to the sensitivity of the components of the volatile oil, the changes in molecules configuration due to radiation, the changes resulted from the oxidation and hydroxylation of the aromatic rings of terpens and the possible degradation of some essential oil constituents during gamma irradiation as well as the radiolytic effect and possible production of free radicals [40].

1,8-cineole is one of important essential oil that has high antioxidant activity; thus the elevation of its value by γ -irradiation resulted in increasing the biological efficiency of γ -irradiated rosemary. Ciftci et al. [40] concluded that cineole showed antioxidant activity and eliminated oxidative stress induced by persistent environmental

Parameters	I	II	III	IV
TC (mg/dl)	157.41 ± 3.29 ^a	212.54 ± 4.18 ^c	185.46 ± 3.36 ^b	183.89 ± 3.49 ^b
TG (mg/dl)	114.66 ± 3.51 ^a	184.19 ± 2.68 ^c	153.46 ± 2.67 ^b	150.32 ± 2.56 ^b
HDL-C (mg/dl)	46.63 ± 1.72 ^a	36.85 ± 1.82 ^c	41.08 ± 1.51 ^b	41.73 ± 1.51 ^b
LDL-C (mg/dl)	87.85 ± 2.57 ^a	138.85 ± 2.02 ^c	113.69 ± 2.15 ^b	112.10 ± 2.37 ^b
vLDL-C (mg/dl)	22.93 ± 1.25 ^a	36.84 ± 1.73 ^c	30.69 ± 1.47 ^b	30.06 ± 1.66 ^b

Values are expressed as means ± S.E. (n=7).

Values in the same row with different superscripts are differing significantly at P<0.05.

Table 3: Effect of rosemary supplementation on serum lipid profile of ethanol administered rats.

Parameters	I	II	III	IV
MDA (n mol/ml)	173.51 ± 3.44 ^a	378.52 ± 3.59 ^c	223.06 ± 3.77 ^b	212.56 ± 3.37 ^b
XO (mU/mg protein)	2.23 ± 0.06 ^a	3.56 ± 0.07 ^c	2.51 ± 0.04 ^b	2.44 ± 0.04 ^b
XDH (mU/mg protein)	3.13 ± 0.18 ^a	1.72 ± 0.11 ^c	2.77 ± 0.18 ^b	2.86 ± 0.16 ^b
GSH (mg/g tissue)	25.14 ± 1.48 ^a	16.04 ± 1.31 ^c	22.92 ± 1.56 ^b	23.64 ± 1.42 ^b
SOD (U/mg protein)	45.07 ± 2.25 ^a	34.92 ± 2.55 ^c	41.28 ± 1.85 ^b	42.41 ± 1.73 ^b
CAT (U/g protein)	3.44 ± 0.05 ^a	1.83 ± 0.03 ^c	2.89 ± 0.04 ^b	2.93 ± 0.05 ^b

Values are expressed as means ± S.E. (n=7).

Values in the same row with different superscripts are differing significantly at P<0.05.

Table 4: Effect of rosemary supplementation on MDA, hepatic xanthine oxidoreductase system (XO and XDH), GSH, SOD and CAT of ethanol administered rats.

pollutant (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.

In this study, alcohol intake increased the mean values of liver enzymes (ALT, AST, ALP and γ GT) 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 γ GT into blood. Also, Hussein et al. [4] observed a significant increase in the activity of serum liver enzymes ALT, AST and γ GT 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 γ GT 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 rosmarinic and carnolic 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.

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.

[51] who reported that ethanol blocks fat oxidation and favours fat accumulation. The accumulation of fat in liver acts as a stimulus for the secretion of lipoproteins into the blood stream and the development of hyperlipidemia.

Administration of raw and γ -irradiated rosemary powder after ethanol intake caused significant declines in the lipid contents associated with an obvious increase in HDL-C concentration. Aljamal et al. [52] showed that the administration of rosemary decreased the level of cholesterol, triglycerides, LDL, and increased HDL in diabetic rats. Also, the authors indicated that the administration of rosemary shows better lipid profile in both normal and diabetic rats. Moreover, it seemed that rosemary possess a variety of important constituents such as caffeic acid and rosmarinic acid that have antioxidant effect and hypolipidemic potential. This may be an indication of progressive metabolic control of rosemary on mechanisms involved in elimination of the lipids from the body [5]. In addition to a well documented role in reverse cholesterol transport, HDL-cholesterol has been recognized to have several other important cardio protective properties including the ability to protect LDL from oxidative modification [53].

It was found that the inflammatory reactions and oxidative stress play a major role in alcohol hepatotoxicity [54]. In this investigation, there was a significant increase in MDA concentration accompanied by conversion of hepatic XDH to XO as a result of ethanol administration. Das and Vasudevan [55,56] observed increased lipid peroxidation with ethanol in their dose dependent studies. During ethanol metabolism, potentially dangerous byproducts are generated including reactive oxygen species (ROS) [2] which react with membrane lipids and cause lipid peroxidation leading to cell death [57]. Metabolism of ethanol to acetaldehyde leads to the conversion of a portion of tissue xanthine dehydrogenase (XDH) to xanthine oxidase (XO) due to an increase in cellular NADH as a result of ethanol oxidation with subsequent production of superoxide anion radicals [58].

Also, the present study revealed that the level of GSH content and the activity of SOD and CAT were significantly decreased in the liver of ethanol-administrated rats. In agreement, Masalkar and Abhang [59] found a decrease in antioxidant statue in alcoholic patients and showed that increased generation of free radicals and deficiencies of dietary antioxidants can be important etiological factor in alcoholic liver disease. Husain and Somani [60] reported a significant decrease in GSH content with EtOH treatment (1.6 g/kg) in hepatic tissue of rats. Also, Das and Vasudevan [57] in their alcohol dose dependent studies observed a significant decrease in GSH content in the hepatic tissue of rats. The decrease in GSH content after ethanol intoxication reflects the impaired conversion of glutathione of oxidized form to reduced form [61] thus alters the GSH/GSSG ratio. The increase in GSH/GSSG ratio in the liver of EtOH fed rats and inhibition of glutathione reductase are indicative of ethanol induced oxidative stress in the liver leading to the decreased antioxidant enzyme capacity [60].

The decrease in SOD activity in blood was reported by Husain and Somani [60] and in hepatic tissue [62] has also been reported during EtOH intoxication. The over-production of superoxide radicals due to EtOH intoxication implies low activity of SOD under ethanol induced oxidative stress in the hepatic tissue. The significant decrease in SOD activity due to ethanol administration indicates inefficient scavenging of reactive oxygen species (ROS) which might be implicated to oxidative inactivation of enzymes [63]. Moreover, Bindu et al. [64] reported a significant decrease in CAT activity with 4 g/kg EtOH treatment for a period of 50 days in rats. Das and Vasudevan [56] found a significant

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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