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Simultaneous Administration of Aqueous Extract of *Rosmarinus officinal* with Nicotine Resulted in Prevention of Induced Hepatorenal Toxicity in Guinea Pigs

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Abstract: Rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals. The present work aimed to evaluate the effectiveness of aqueous extract of rosemary as a natural source of antioxidants to minimize the harmful effects of nicotine induced hepatorenal toxicity in Guinea pigs. In this study, twenty four adult male Guinea pigs were used for this study and divided into four groups. The first group was control group, the 2nd was the rosemary group orally received rosemary (220 mg/kg body weight /day), the 3rd was the experimental and received intraperitoneal injection of nicotine (6 mg/kg body weight /day), the 4th one co-administered intraperitoneal injection of nicotine (6 mg/kg body weight /day) and rosemary (220 mg/kg body weight /day) orally by gavage for 30 days. Blood samples were obtained for assessment of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyltransferase activities, total proteins, albumin, and globulin concentrations, albumin concentration/globulin concentration (A/G) ratio, urea, uric acid, creatinine, sodium ion, and potassium ion concentrations. In nicotine treated animals, the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyl transferase activities, urea, uric acid, creatinine, and potassium ion concentrations were significantly ($p<0.05$), increased as compared to the control group. On the other hand, serum total proteins, albumin, and sodium ion concentrations of nicotine treated Guinea pigs, were significantly ($p<0.05$), decreased compared with control animals. But, globulin concentrations and A/G ratio were non significantly changed. Co-administration of rosemary significantly improved all biochemical parameters. It can be concluded that, simultaneous administration of aqueous extract of *rosemary* with nicotine resulted in prevention of induced hepatorenal toxicity in Guinea pigs. It is recommended that the heavy smokers should be advised to take rosemary as antioxidant to prevent the hepatorenal toxicity. Further studies are necessary to elucidate exact mechanism of hepatorenal protection and potential usefulness of aqueous extract of rosemary as a protective agent against nicotine induced hepatorenal toxicity in clinical trials.

Keywords: Hepatoprotective, Renoprotective, Rosemary (*Rosmarinus officinalis*), Male Guinea Pig, Nicotine

1. Introduction

Cigarette smoking and of the use of other tobacco products became an important cause of increased mortality and morbidity in developed countries [1]. Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling nicotine [2]. It is a highly toxic organic compound containing nitrogen and alkaloid [3]. People who smoke and also who are exposed to cigarette smoke indirectly by breathing the air in the same environment are exposed to nicotine induced oxidative stress [4, 5], Oxidative stress would result in increased free radical injury in the tissue leading to extensive tissue damage with subsequent derangement of cell physiology [1]. As a consequence, these radicals interact with cell components such as lipids, proteins, DNA, RNA, carbohydrates and enzymes [4, 5]. So that smoking has an effect on the various metabolic and biological processes in the body [1]. During smoking, nicotine is rapidly absorbed into the circulatory system where more than 80% is metabolized in the liver. Liver is an important organ that has many tasks, and is responsible for processing drugs and other toxins to remove them from the body. Nicotine from heavy smoking increases

the risk of developing hepatocellular carcinoma, chronic liver diseases. In addition, nicotine increases the production of pro-inflammatory cytokins that would be involved in liver cell injury [6]. Also, nicotine is hepatotoxic [7 - 9], and nephrotoxic [10].

The body is engaged in a constant battle against damaging chemicals called free radicals or pro-oxidants to counter the harmful effects of free radicals, the body manufactures antioxidants to chemically neutralize them. However, the natural antioxidant system may not always be equal to the task. Sources of free radicals, such as cigarette smoke may overwhelm this defense mechanism [11]. The antioxidants are important species that possess the ability to protect the body from damage caused by free radicals induced oxidative stress [12]. Medicinal plants and their active principles have received great attention as potential antiperoxidative agent [13].

Rosemary (*Rosmarinus officinalis*) is a herb commonly used as spice and flavoring agents in food processing and is useful in treatment of many diseases [14, 15]. It is composed of dried leaves and flowers constitutes a particularly interesting source of biologically active phytochemicals as it contains a variety of phenolic compounds including carnosol, carnosic acid, rosmanol, 7-methyl-epirosemanol, isorosmanol, rosmadial and caffeic acid [14, 16]. Rosemary extracts have a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals [17]. The antioxidant activity of rosemary extract can be attributed mainly to two components, carnosic acid and carnosol [18]. It is useful in prevention of hepatotoxicity [19, 20], and nephrotoxicity [21]. The evidence reporting the amelioration by aqueous extract of rosemary in nicotine induced hepatorenal toxicity in Guinea pigs are hardly found. So, the present work aimed to evaluate ameliorating effect by aqueous extract of rosemary in nicotine induced hepatorenal toxicity in Guinea pigs.

2. Materials and Methods

2.1. Chemicals

Nicotine hydrogen tartrate salt [1-methyl-2- (3-pyridyl) pyrrolidine-bitartrate salt] was purchased from Sigma-Aldrich (St. Louis, MO, USA). The drug was dissolved in physiological saline (0.9% sodium chloride) and injected subcutaneously daily with 6 mg, nicotine / kg body weigh for 30 days. Nicotine 6 mg/kg body weight was prepared by mixing 60 mg of nicotine in 10 ml normal saline. A total of 1 ml /Kg body weight of the nicotine. The selection of the nicotine dose (6 mg/kg body weight) in the present study was based on approximate the plasma levels reported in heavy smokers [22] and previous published studies [23, 24].

Fresh rosemary (*Rosmarinus Officinalis* L) was collected from Surman city, West Libya between the month of May and June 2015. The plant was dried under shade at 25°C and the dried leaves of plant were grounded with a blender. Aqueous rosemary extract was prepared according to the method of Amin and Hamza [25]. Briefly, twenty gram of dried plants was slowly boiled in 100 ml of distilled water and heated for 30 minutes. The extracts were then filtered and directly administered orally by gavage to the animals at a volume of 5ml/kg body weight (220 mg/kg body weight). The selection of the rosemary dose (220 mg/ kg body weight) was based on previous published studies [15, 21, 26].

2.2. Animals

Twenty four adult male Guinea pigs (*Cavia porcellus*) weighting 450-600 gm were used for this study. The animals were obtained from animal house unit in the faculty of veterinary medicine, Tripoli University, Libya. The animals were housed in a room under standard conditions of ventilation, temperature (25 ± 2°C), humidity (60-70%) and light/dark condition (12/12). The animals were provided with tape water *ad libitum* and fed with the standard commercial chow. The animal procedures were performed in accordance with Guide Lines for Ethical Conduct in the Care and Use of Animals.

2.3. Experimental Design

After one week of acclimation, the animals were randomized and divided into four groups (6 Guinea pigs for each) as follow:

Group I (Control group): The animals received intraperitoneal injection of saline (0.5 ml/day) for 30 days.

Group II (Rosemary group): The animals received rosemary (220 mg/kg body weight /day) orally by gavage for 30 days.

Group III (Nicotine treated group): The animals received intraperitoneal injection of nicotine only (6 mg/kg body weight /day) for 30 days.

Group IV (Nicotine/rosemary co-administered): The animals received intraperitoneal injection of nicotine (6 mg/kg body weight /day) and rosemary (220 mg/kg body weight /day) orally by gavage for 30 days.

At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed under light ether anesthesia, then rapidly dissected and subjected to the following examinations:

2.4. Biochemical Analysis

Blood samples were drawn by cardiac puncture. The sample was collected in clean dry tube and centrifuged at 3000 rpm for 15 minutes then, serum was separated and kept in a deep freezer at -20◦C until biochemical measurements were carried out. The activities of Alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in serum according to the methods described by Reitman and Frankel [27]. Serum alkaline phosphatase (ALP) activity was determined according to Kind *et al.* [28]. Serum γ-GT activity was determined according to the method of Szas [29].

Serum total proteins concentration was determined

according to Biuret method explained by Weichselbaum [30]. Serum albumin concentration was determined according the method of Doumas *et al*. [31]. Serum globulin concentration was determined according to the formula: Globulin = total protein–albumin.

The ratio of serum albumin concentration /globulin concentration (A/G) was determined as albumin / globulin level. Serum urea measurement was based upon the cleavage of urea with urease [32]. Serum uric acid was determined [33]. Serum creatinine was measured without protein precipitation [34]. Sodium concentration in serum was determined by colorimetric method according to Trinder [35], and Maruna [36]. Potassium concentration in serum was determined by turbidimetric tetraphenylborate method according to Hoeflmayr [37]. Using Chiron diagnostics kits.

2.5. Statistical Analysis

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using one way ANOVA. The results were considered statistically significant when $p < 0.05$.

3. Results

Biochemical parameters in serum of the different groups are shown in Table 1. Guinea pigs that received intraperitoneal injection of nicotine only (6 mg/kg body weight /day) for 30 days had significantly ($p<0.05$), increased the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyl - transferase activities, urea, uric acid, creatinine, and potassium ion concentrations as compared to the control Guinea pigs. Co-administration of nicotine with rosemary were significantly ($p<0.05$) prevented the changes recorded in serum liver function serum enzymes activities, and serum kidney function parameters as compared with control group. On the other hand, serum total proteins, albumin, and sodium ion concentrations of nicotine treated Guinea pigs were significantly ($p<0.05$) decreased as compared to the control Guinea pigs, but, globulin concentrations and A/G ratio were non significantly changed as compared to the control Guinea pigs. Co-administration of nicotine with rosemary were significantly ($p<0.05$) prevented the changes recorded in serum total proteins, albumin, and sodium ion concentrations as compared with control group.

Table 1. *Effect administration of Guinea pigs to aqueous extract of rosemary and/ or nicotine on serum biochemical parameters.*

Parameters	Groups			
	Control	Rosemary	Nicotine	Nicotine + Rosemary
	Mean + SD	Mean + SD	Mean + SD	Mean + SD
Alanine aminotransferase (U/L)	57.50±2.43	59.00±2.38	75.33±3.25[a]	66.14±2.11[b]
Aspartate aminotransferase (U/L)	83.83±2.91	85.17±2.85	110.83±6.67[a]	98.7±3.97[b]
Alkaline phosphatase (U/L)	41.5±1.50	44.17±2.97	55.17±1.34[a]	48.8±2.41[b]
γ- glutamyl transferase activities (U/L)	19.67±1.11	20.83±1.41	28.55±0.96[a]	23.10±1.20[b]
Total proteins concentration (g/dl)	6.75±0.10	6.95±0.21	5.90±0.19[a]	6.54±0.14[b]
Albumin concentration (g/dl)	3.70±0.13	3.53±0.11	3.13±0.06[a]	3.56±0.09[b]
Globulin concentration (g/dl)	3.05±0.21	3.42±0.28	2.77±0.20	2.99±0.06
A/G ratio	1.22±0.13	1.04±0.11	1.13±0.08	1.19±0.05
Urea concentration (mg/dl)	21.50±1.71	22.33±1.97	30.17±3.24[a]	26.00±1.53[b]
Creatinine concentration (mg/dl)	0.50±0.04	0.51±0.02	1.02±0.12[a]	0.78±0.06[b]
Uric acid concentration (mg/dl)	1.35±0.06	1.33±0.06	1.66±0.09[a]	1.48±0.07[b]
Sodium ion concentration (mmol/L)	136.5±2.06	137.2±1.34	132.0±1.91[a]	135.6±1.25[b]
Potassium ion concentration (mmol/L)	4.65±0.19	4.48±0.13	5.68±0.35[a]	5.01±0.27[b]

a: Significant differences as compared with control group ($P < 0.05$). b: Significant differences as compared with nicotine treated group ($P < 0.05$).
All data are mean of 6 individuals.

4. Discussion

Oxidative stress results from an imbalance between the cellular production of reactive oxygen species and the antioxidant mechanisms that remove them [36]. The relationship between the amount of products of oxidative metabolism and natural scavengers of free radicals determines the outcome of tissue damage. Overproduction of reactive oxygen metabolites and a reduction in antioxidant mechanisms have been reported due to acute or chronic smoke exposure [39]. However; nicotine which is a major toxic component of cigarette smoke has been shown to produce diffuse damage to endothelium and plays a major role in the development of numerous human disease or disorders [40].

The present study demonstrated that nicotine treatment caused significant increases in the serum ALT, AST, ALP and Ɣ-GT activities and decreases in the serum total proteins, albumin and indicating impaired liver function. Similar results were also reported by Jang *et al.*, [41] and Sharif *et al*., [42]. Fahim *et al*., [43] reported rise in both hepatic ALT and AST levels following i.p nicotine injection (1mg/Kg) for 3 weeks in mice. Another study observed over expression of ALP level and other genes involved in osteoblast maturation and differentiation in osteoblasts in response to subtoxic nicotine administration in humans [44]. Also, Mahmoud and Amer [45] found that significant elevations in the activities of ALT, AST, and alkaline phosphatase in liver homogenate of nicotine treated rats compared with control group. These results may be attributed to the state of hypoxia of the parenchyma for contracting fibrous tissue and the increased permeability of

hepatic cell membrane due to nicotine treatment which release ALT enzyme into the circulation. The increased level of ALT is marked as liver parenchymal cell destruction induced by nicotine treatment. The elevation in serum ALT activity observed in the present study may reflect hepatotoxic potency of subchronic exposure of nicotine on liver. This effect could be an essential process for the liver to restore the balance of different free amino acids that might have been disturbed throughout recovering mechanisms. It has been established that the liver is the sole source for the synthesis of albumin, fibrinogen and most of Alpha and β globulins, while the immunoglobulin are formed in the lymphoid tissues by the plasma cell [46]. Accordingly, the liver affected by nicotine may suffer from dysfunctions and this may modify the synthesis and metabolism of proteins. This might explain the significant decrease observed in the total serum proteins in Guinea pigs treated with nicotine. The results are also in accordance with the work of Sershen *et al.,* [47] who found that, injection of nicotine produced inhibition of protein synthesis, due to exposure to cigarette smoke.

The elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances [48]. Nicotine and its metabolites are eliminated from kidney, these organs are adversely influenced by nicotine. Membrane lipids are vital for the maintenance and integrity of cell function, the breakdown of membrane phospholipids and lipid peroxidation due to the generation of free radicals are expected to change membrane structure, fluidity, transport and antigenic properties, all of which play an important role in the pathogenesis of organ disorders [49]. Indeed, increasing evidence suggests that chronic cigarette smoking adversely influences the prognosis of nephropathies [49, 50].

In the present study, the serum urea, creatinine and uric acids were significantly increased in Guinea pigs treated with nicotine compared with control animals suggesting an impairment of kidney function. The observed alterations in renal function parameters are in line with the reports by these findings are agreement with the results of other studies (10 & 51-54]. This is agree with the findings of Ahmed *et al.,* [55] who found that the levels of creatinine and urea were significantly higher in smoker group when compared with the control group. There is evidence that an increase in renal retention of uric acid can occur in cases of acute or chronic renal disease/ failure [56]. Several mechanisms may be operative in inducing renal vasoconstriction and vascular damage. Nicotine increases plasma levels of vasoconstrictors including catecholamines, arginine, vasopressin and endothelin-1 [57]. Cigarette smoke damages endothelial cells, and nicotine induces smooth muscle cell proliferation [58]. Other study attributed the renovascular resistance to activation of the sympathetic nervous system [59]. These effects could be attributed to changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [60].

The present study shows that, treatment of Guinea pigs with nicotine were caused a significant decrease in serum sodium ions and increase in serum potassium ions concentrations compared with control group. This is in agreement with Hozayen *et al.,* [61] who found that, 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. The Na+, K+- AT Pase 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 [62].

Rosemary is used in folk medicine, as an antispasmodic in renal colic and dysmenorrhea, in relieving respiratory disorders, and to stimulate growth of hair [63]. The aqueous extract of rosemary used as a drug with strong antioxidant properties for eliminating the generated free radicals, reinforce the antioxidant system and prevent oxidative stress [64].

The present study, revealed that co-administration of nicotine and aqueous extract of rosemary significantly decreased the elevations in the serum ALT, AST, ALP, and Ɣ-GT activities and increased the levels of serum total proteins and albumin a compared with nicotine treated group. This results are run in parallel with the results of Abd El Kader *et al.,* [65] who found that, a significant improving effect of pre-treatment with rosemary on the altered activities of serum ALT, AST, GGT and ALP induced by Pb-acetate intoxication. The observed decrease in these serum marker enzymes shows that rosemary preserves the structural integrity of liver against lead-induced damage. Mannaa *et al.,* [66] reported that, the hepatotoxic effects of $AlCl_3$, as indicated by significant augmentations of serum ALT and AST levels can be modified by rosemary supplementation in combination with $AlCl_3$. These protective effects of rosemary may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of polyphenolic compounds (66, 67). Phytochemical studies have shown that rosemary contains essential oils, terpenoids, flavonoids and alkaloids. Some of its constituents such as rosmarinic acid have been reported as powerful antioxidant protecting against free radicals damage and to reduce hepatotoxicity [68]. 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, or xenobiotic-metabolizing enzymes in liver [69].

In the present study, co-administration of rosemary to animals treated with nicotine were significantly decreased the serum urea, creatinine and uric acid compared with nicotine treated group. This is in agreement with Azab *et al.,* [21] who found that co-treatment of gentamicin and rosemary aqueous extract significantly decreased the serum urea, creatinine and uric acid compared with gentamicin treated group. 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 [61]. Sahu *et al.,* [70] 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. Pre-administration of rosemary alleviates the harmful effects induced by lead acetate by improvement the kidney functions [63]. Mannaa et al., [66] reported that, renal dysfunctions of $AlCl_3$, as indicated by significant augmentations of serum urea and creatinine levels, can be modified by rosemary supplementation in combination with $AlCl_3$. The rosemary aqueous extract alleviates the toxicity induced by lead on the kidney through stimulation of endogenous antioxidant defense system [65]. Also, the rosemary aqueous extract alleviates the nephrotoxicity induced by CCL_4 in albino rats [15]. 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 [71]. The protective effect of rosemary can be explained that rosemary extract has a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, as thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals [17]. Also, Rosemary extracts are able to donate electrons to reactive radicals, converting them to more stable and on reactive species, therefore preventing them from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems [15, 26, 61 & 72].

This study shows that, co-administration of nicotine and aqueous extract of rosemary to Guinea pigs were caused a significant increase of sodium ions and decrease potassium ions concentrations compared with nicotine treated group. This is in agreement with Hozayen *et al.*, [61] who reported that, 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 [71].

The biological activities of rosemary aqueous extracts are mainly attributed to their high concentration of phenolic constituents namely carnosic and rosmarinic acids that are recognized as natural antioxidants [73, 74]. Many studies reported that the preventive effects of rosemary and its extracts are attributed to its antioxidant activity [75].

5. Conclusion

The present study concluded that, simultaneous administration of aqueous extract of *rosemary* with nicotine resulted in prevention of induced hepatorenal toxicity in Guinea pigs. It is recommended that the heavy smokers should be advised to take rosemary as antioxidant to prevent the hepatorenal toxicity. Further studies are necessary to elucidate exact mechanism of hepatorenal protection and potential usefulness of aqueous extract of rosemary as a protective agent against nicotine induced hepatorenal toxicity in clinical trials.

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