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HEPATOPROTECTIVE EFFECT OF ROSEMARY EXTRACT AGAINST ASPARTAME-INDUCED LIVER TOXICITY IN MALE RATS

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ABSTRACT
There is a growing interest in using natural antioxidants to treat various pathological tissue conditions considering the role of oxidative stress in their pathogenesis. Rosemary (*Rosmarinus officinalis L., Lamiaceae*) is a woody perennial herb and has been considered as one of the most effective in anti-oxidative stress and anti-inflammatory activity. Aspartame (ASP) is a dietary low-calorie artificial sweetener and is widespread used in more than 6,000 products. Its metabolites amino acids, phenyl amine, aspartic acid and methanol in the gastrointestinal tract can be toxic, and it is considered as an oxidative stress producer and a multi-potential carcinogenic agent. This study aimed to evaluate the possible protective role of aqueous extract of rosemary (125 mg/ kg b.wt/day) against aspartame (250 mg/ kg b.wt/day)-induced injury in liver of adult male albino rats. In this work rosemary was co-administered, pre-administered and post-administered with aspartame. Results showed that after two months rosemary restored levels of nitric oxide, malondialdehyde, catalase, super oxide dismutase, glutathione, tumor necrotic factor alpha and alpha fetoprotein in liver tissue. Moreover, rosemary improved serum levels of alanine amino-transferase, aspartate amino transferase, total protein and albumin, urea, creatinine and uric acid. Additionally, rosemary improved serum disturbances of electrolytes induced by aspartame for, sodium, potassium, calcium and phosphorus. This study concluded that, rosemary extract alleviates the harmful effects of aspartame in rat's liver and has a grateful role in restoration of sodium potassium and calcium phosphorus hemostasis.

KEYWORDS: Rosemary, Antioxidants, Oxidative Stress, Liver, Aspartame, Rats.

INTRODUCTION
The health problems derived from oxidative stress and lipid oxidation have attracted the attention of researchers and consumers.[1,2] Numerous diseases, such as cancer, aging and ischemia are linked to dietary and biological lipid oxidation products.[3] Liver is the major and chief site for food metabolism, detoxifies and removes all endogenous and exogenous substances from body. The liver injuries and damage as result of oxidative stress impair liver functions and lead to many complications.[4] Aspartame (ASP) is a dietary low-calorie artificial sweetener and is widespread used over the last 30 years.[5] ASP is found in more than 6000 products, including yoghurt, soft drinks, candy, chewing gum, tabletop sweeteners fruit juices, jellies, gelatins and drugs such as vitamins and sugar-free cough drops.[6] However, ASP represents a higher source of oxidative stress since it is rapidly metabolized into methanol, phenylalanine and aspartic acid. Methanol is further metabolized by oxidation to formaldehyde and then to formate. These processes are accompanied by formation of superoxide anion and hydrogen peroxide which cause harmful effects of acute intoxication in many organs especially on liver functions.[7] Nowadays consumers are concerned about the negative effect of synthetic chemicals in food and therefore there is a growing interest in using natural extracts as alternatives for synthetic additives because of (a) their cooperation with other preservation methods (b) they are safe, and (c) their specific properties as antioxidant, antidiabetic, antimutagenic, antibacterial and antitoxigenic.[8] *Rosmarinus officinalis, L.* (rosemary) is an aromatic plant from the Lamiaceae family, grows in many parts of the world and rich in several antioxidants.[9] The most important antioxidant compounds of rosemary are carnosic acid, carnosol and rosmarinic acid.[10] In the United States and Europe, rosemary is a unique spice commercially available for use as an antioxidant and rosemary extracts have been used in the treatment of diseases as well as in food preservation, because it prevents oxidation and microbial contamination and nowadays in the European Union, rosemary extracts are added to food and beverages at levels of up to 400 mg/kg (as the sum of carnosic acid and carnosol).[8] On sight of the above-mentioned data, this study aimed to evaluate the possible protective role of aqueous extract of

rosemary against aspartame-induced injury in liver tissue of adult albino rats. This goal could be achieved through evaluating of ameliorating oxidative damage, pro-inflammatory cytokines and liver antioxidant markers; glutathione content, catalase and superoxide dismutase enzymes. In addition, liver functions and electrolytes levels were estimated.

MATERIALS AND METHODS

Experimental animals

Sixty male albino rats weighing 120-140 g were obtained from animal house of the National Research Center (Cairo). Rats were housed in plastic cages at a temperature of (24±2℃) under natural lighting conditions (12 h light/dark cycles). All rats were provided with water and food (standard laboratory diet). Animals were subjected to pre-experimental acclimatization for two weeks before starting the experiment. All animals cared for according to the guidelines for animal experiments which were approved by Ethical Committee of Ain Shams University, Cairo, Egypt.

Chemicals

Aspartame (C14H18N2O5)

Pure aspartame (ASP) powder, reagents were purchased from Sigma Chemical Company, Louis, Mo, USA.

Rosemary leaves extract preparation

The dried rosemary 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 H_2O for 36 hrs (12 hours × 3). The Cooled liquid extract was then transformed to powder by evaporating water. The powder was re-dissolved in bi-distilled water just before oral administration.[11]

Experimental design

After acclimatization period, animals were randomly distributed into 6 groups of 10 rats each as the following: The 1st group (Con.) left without treatment and kept as a control group. The 2nd group (Rose) was treated with a daily oral dose of rosemary extract (125 mg/ kg b.wt) for two months.[12] The 3rd group (Asp) was administrated a daily oral dose of aspartame (250 mg/ kg b.wt) dissolved in distilled water for two months.[13] The 4th group (Rose + Asp) was concomitantly treated with rosemary accompanied with aspartame as the same previous corresponding doses, period and route of administration. The 5th group (Rose then Asp) was administered rosemary for two hours then aspartame for two months. The 6th group (Asp then Rose) was administered aspartame for two months then rosemary for two months. At the end of the experiment, all rats were sacrificed under ether anthesis. Blood was collected in clean dry tubes, kept for 15 minutes and then centrifuged at 3000 rpm for 20 minutes. For the biochemical analysis; the sera were collected in Ependorph' tubes. Also, tissue samples from liver were dissected.

Biochemical analysis of liver tissue

Malondialdehyde level was evaluated by colorimetric method using lipid peroxide (Malondialdehyde) assay kit, Biodiagnostic, No. 25 29, Egypt according to the method described by Ohkawa et al.[14] Nitric Oxide (NO) level was determined by colorimetric method using Nitric Oxide assay kit purchased from Biodiagnostic Co.: No 25 33, Egypt, according to the methods of Montgomery and Dymock.[15] Catalase activity was determined by colorimetric method using catalase assay kit purchased from Bio-diagnostic Co.: No 25 17, Egypt, according to the method described by Aebi.[16] Glutathione content was determined according to the method of Beutler[17], using kits from Biodiagnostic, Egypt, No. 2524. Superoxide dismutase (SOD) activity was determined by colorimetric method using superoxide dismutase assay kit purchased from biodiagnostic Co. No 25 21, Egypt, according to the method described by Nishikimi.[18] Quantitative estimation of tumor necrosis factor-α (TNF- α) was carried out using Rat TNF-α ELISA kit, No. K0331196, Koma Biotech Inc., Korea, according to the method described by Intiso et al.[19] AFP was determined according to the manufacturer's instructions and guidelines (Biosource Europe S.A., Nivelles, Belgium) using rat AFP ELISA kit, No. MBS700622.

Biochemical analysis of serum

ALT and AST were determined using SPECTRUM alanine aminotransferase kits, E.C.2.6.1.2, Bio diagnostic, Egypt, according to the method described by Reitman and Frankel.[20] Total Protein was determined using SPECTRUM total protein kit, block 20008-piece 19 A, Diagnostics, Egypt, according to Gomal et al.[21] Albumin concentration was determined in serum according to the method of Doumas[22], using the reagent kits purchased from Diamond Diagnostics, Egypt. Urea concentration was estimated according to Young.[23], using reagent kits Purchased from Diamond Diagnostic, Egypt. Creatinine was determined according to Tietz[24], using reagent kits from Diamond Diagnostic, Egypt. Uric acid concentration was estimated according to Trivedi et al.[25], using reagent kits Purchased from Diamond Diagnostic, Egypt. Sodium and potassium were determined according to Bishop et al.[26] The measurement of inorganic phosphorus in serum is determined according to Goldenberg and Fernandez.[27] Calcium was determined according to method of Gindler and King.[28]

RESULTS AND DISCUSSION

The current study focused on the effect of aqueous extract of rosemary on the toxicity induced by artificial sweetener, aspartame on liver of rats. Rosemary (*Rosmarinus officinalis L.*) is a household plant which grown in several parts of the world, it is used in folk medicine, as an antispasmodic in renal colic and

dysmenorrhea, in dismissing respiratory disorders, and to stimulate growth of hair.[29] It was reported that rosemary has the potential to satisfy free radicals and improve the antioxidant status in rat tissues.[11] In the current study rosemary was co-administered, pre-administered and post-administered with aspartame. Notably, the present study revealed that there are no significant changes between rats received rosemary alone and control, this is in accordance with Al-Gholam et al.[30]

It is known that oxidative stress results from an imbalance between the cellular production of reactive oxygen species (ROS) and the antioxidant mechanisms remove them.[31] In the current study, aspartame administration induced a significant increase ($p<0.001$) in lipid peroxidation as MDA and NO levels in liver. While a significant decrease ($p<0.001$) in liver catalase, superoxide dismutase (SOD) activities and glutathione (GSH) contents were recorded when compared to control rats (Table 1). In consistence with the present findings, Mourad[32] observed a significant elevation in lipid peroxidation in liver and kidney, as well as a decrease in liver GSH level and a decrease in SOD activity in liver and kidney. The current study suggested that the administration of ASP for two months is considered as a remarkable inducer of oxidative stress and inhibitor of antioxidant defense system in rat liver. This is in accordance with Iyaswamy et al.[33] who concluded that ASP acts as a chemical stressor by altering liver function homeostasis and increasing protein oxidative damage and this promotes cell apoptosis and hepatotoxicity. These results attributed to the fact that ASP generates ROS and stimulates liver lipid peroxidation (LPO) which related to imbalance between the generation of free radicals and antioxidants. Excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can produce tissue damage by reacting with various cell components resulting in DNA strand breaks, LPO and protein oxidation. This chronic oxidative process can also lead to a progressive weakening of the antioxidant defense system.[34] Furthermore, nitric oxide (NO) and its oxidative metabolite, peroxynitrite ($ONOO^-$) are referred to as reactive nitrogen species (RNS). NO toxicity is related to its capacity to combine with superoxide anions (O^{-2}) to form peroxynitrite ($ONOO^-$), an oxidizing free radical that causes DNA fragmentation and lipid oxidation. In the mitochondria, $ONOO^-$ acts on the respiratory chain (I-IV) complex and manganese superoxide dismutase (Mn SOD) to produce superoxide anions and hydrogen peroxide (H_2O_2) respectively.[35] Also, these alternations after ASP administration is mainly related to its metabolite methanol which is metabolized by oxidation to formaldehyde and then to format. These processes are accompanied by the formation of free radicals.[36] So, MDA increased because the prime targets for free radical reactions are the unsaturated bonds in membrane lipids in the liver tissues. Consequent peroxidation results in a loss in membrane fluidity and receptor alignment.[37]

SOD is an important in the cell defense through dismutation of superoxide radicals to H_2O_2 and O_2. Also, catalase detoxifies H_2O_2 into H_2O and O_2. Moreover, SOD protects catalase against superoxide anion and catalase protects SOD against inactivation by H_2O_2. Thus, SOD and catalase act as the enzymatic anti-oxidative defense mechanism against reactive oxygen species.[38] Moreover, GSH acts as the non-enzymatic anti-oxidative defense because it reacts with nitric oxide and protects the cellular system against the toxic effects of lipid peroxidation.[39] The decrease in GSH activity observed in the present study seems to have been caused by methanol, because methanol metabolism depends upon GSH[40], 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.[32]

In this study, the oxidative and anti-oxidative markers were ameliorated significantly comparing to ASP group after the treatment of rosemary extract either companied with aspartame for two months (Rose +Asp group) or 2 hours pre-treatment of aspartame for two months (Rose then Asp group) or two months after aspartame treatment (Asp then Rose group) (Table 1). The protective effect of rosemary can be described that rosemary has a high scavenging capability of reactive oxygen and nitrogen species, mostly free radicals, as thought to be one of the main mechanisms of the antioxidant achievement displayed by phenolic compounds, proteins.[41] In addition, 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 radical damage and to reduce hepatotoxicity.[42] Moreover, Nieto et al.[8] reviewed that the antioxidant action of rosemary is taken via activation of redox-signaling pathways through Nuclear factor E2-related factor 2(Nrf2)-dependent transcriptional regulation. Nrf2 is a transcription factor that coordinates the basal and stress-inducible activation of a vast array of cytoprotective genes. Additionally, the antioxidant activities of carnosic acid and carnosol are due to their ability to maintain or increase glutathione peroxidase and SOD as well as reduce MDA through inhibition of lipid peroxidation and the effects of both components on membrane lipid peroxidation is higher than the effects of artificial antioxidants.[43]

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine secreted by macrophages, neutrophils, T-cells and natural killer (NK) cells. It plays a major role in growth regulation, inflammation, tumor genesis, tumor metastasis and autoimmune diseases. Moreover, TNF-α is contributed to the manifestation of the pathological and systemic inflammatory response and ultimately to the development of organ failure.[44,45] A Fetoprotein (AFP) is an oncofetal gene and has been used as a screening test for early primary hepatocellular cancer and

serve as predictive markers for the development of hepatocellular carcinoma.[46] The present findings showed that ASP caused a significant elevation ($p<0.001$) in the levels of both TNF-α and AFP comparing to controls. On the other hand, these levels were improved in all groups treated with rosemary extract comparing to aspartame group (Table 1). These elevations in TNF-α and AFP may be related to liver cell injury which caused by the accumulation of formaldehyde adducts and oxidative stress followed by ASP intake and such effects of aspartame cause functional alterations of proteins and DNA mutations, autoimmunity, cell death or malignant transformation.[47] Additionally, damage of liver cells by ASP might be second result of the activation of Kupffer cells which secrete TNF-α, interleukins, reactive oxygen and nitrogen species, these mediators could act directly on hepatocytes to cause cell death.[48] Moreover Arafa and Aly[48] pointed that more AFP expression can be induced in rat hepatocytes following toxic injury and liver damage where continuous hepatocellular damage occurs, and hepatocellular regeneration is supposed to take place. Consequently, the scavenger capacity of rosemary for free radicals is considered the main cause of the improvement of TNF-α and AFP. Recent articles reviewed that rosemary targets several deregulated pathways related with cancer, inflammation and apoptotic related proteins.[41,49] Furthermore, the antioxidant molecules from rosemary not only act as free radical scavengers but also play a role by regulating the activity and/or expression of certain enzymatic systems associated in appropriate physiological processes like apoptosis, tumor promotion and intracellular signal transduction.[49] More in details, carnosic acid and carnosol have powerful anti-inflammatory and anti-cancer activities.[50]

Table (1): Effect of Rosemary (Rose) or / and Aspartame (Asp) on hepatic oxidative stress, anti-oxidants, tumor necrotic factor alpha and alpha-fetoprotein contents in male rats.

Group	MDA (nmol /g tissue)	NO (µmol /g tissue)	Catalase (µmol/g tissue	GSH (µmol/g tissue)	SOD µmol/g tissue	TNF-α (ng/g tissue)	AFP (ng/g tissue)
Con.	24.95±0.73	2.30±0.13	4.17±0.13	29.00±3.19	2.58±0.14	7.03± 0.27	5.94±0.29
Rose	28.80±0.26	2.53±0.12	3.81±0.19	28.40±1.28	2.33±0.08	7.68± 0.14	7.39±0.14
	N.S	N.S	N.S	N.S	N.S	N.S	N.S
	15.43%	10.00%	-8.63%	-2.06%	-9.68%	9.24%	24.41%
Asp	69.66±3.94	4.44±0.16	0.77±0.03	10.83±0.65	0.58±0.10	26.16±1.85	41.66±2.60
	a***	a***	a***	a***	a***	a***	a***
	179.19%	93.04%	-81.53%	-62.65%	-77.51%	272.11%	601.34%
Rose + Asp	28.18±0.59	1.86±0.05	4.75±0.27	35.45±0.42	3.82±0.20	4.10± 0.32	3.58±1.03
	b***	b***	b***	b***	b**	b***	b***
	12.94%	-19.13%	13.90%	22.24%	48.06	-41.67%	-39.73%
Rose then Asp	26.43±0.53	2.01±0.04	2.95±0.17	27.10±0.29	2.23±0.10	9.28±0.39	6.13±0.32
	b***	b***	b***	b***	b***	b***	b***
	5.93%	-12.60%	-29.25%	-6.55%	-13.56	32.00%	3.19%
Asp then Rose	33.00±0.36	2.58±0.13	2.83±0.26	26.91±0.42	2.20±0.06	10.06±0.12	7.10±0.26
	b***	b***	b***	b***	b***	b***	b***
	32.26%	12.17%	-32.13%	-7.20%	-14.72%	43.10%	19.52%

*The results are presented as Mean ± SE for 6 rats /group *** = P <0.001. **= p<0.01. N.S= non-significant. a: Significant change from control group. b: Significant change from Asp group. Con= normal control group, Rose = Rosemary group, Asp = aspartame group, Rose +Asp =Rosemary and aspartame group, Rose then Asp =Rosemary two hours then aspartame group, Asp then Rose = Aspartame then rosemary group. (%): percent of change from control value.*

The present results showed that at the level of serum analysis, the treatment of aspartame caused significant elevation ($p<0.001$) in the activities of liver enzymes (ALT and AST), in addition to significant decline ($p<0.001$) in total protein and albumin comparing to controls (Table 2). Rosemary extract treatment led to limited ALT and AST activities and increase in total protein and albumin comparing to aspartame group possibly because of the remarkable antioxidant property of Rosemary that drastically attenuated liver tissue damage. The increase in serum ALT, AST activities gives another confirmation for liver injury caused by oxidative stress which induced by aspartame. Accordance with Iyaswamy et al.[33], ALT and AST located in the cytosol but escape out from the cell into extracellular fluids and blood flowing due to changes in the permeability of hepatocyte membranes due to increased lipid peroxidation induced by oxidative stress. More to the point decreased total protein and albumin also may be related to decrease of liver enzymes. According to[36] the decreased liver enzymes may affect the metabolism and regulation of amino acids and this may lead to starvation of the cells and might affect the synthesis of nuclear proteins, nucleic acids and phospholipids. Additionally, hepatocytes establish a main source of serum protein, the alternations in serum

protein and albumin indicating disability of protein synthesis from liver cells and it is also a sign of increased catabolism than anabolism due to toxicity of ASP. As well, the results of the present study are in conformity with those reported by Iyaswamy et al.[33] who demonstrates that long-term consumption of aspartame may induce changes on the redox status of liver functions; however, aspartame and its metabolite methanol could induce liver damage via the mechanism of apoptosis and bring out hepatotoxicity. The marked regulation of liver enzymes, total protein and albumin after rosemary treatment may prove that rosemary conserves the structural integrity of liver against ASP-induced injury through the capacity to ROS scavenger as well as through stimulation of endogenous antioxidant defense system.[51] Another consequence of the rosemary effect capable of inactivate free radicals and blocking them from approaching biomolecules in the biological system as proteins, amino acids, DNA, polyunsaturated fatty acids, lipoproteins and sugar.[52]

Serum analysis also showed that aspartame caused elevation in urea, uric acid ($p<0.001$) and creatinine ($p<0.01$) (Table 2). This is another indication for liver dysfunctions as high urea levels mean that activities of the urea cycle enzymes increased which increase the capacity of the liver to synthesize urea and this may relate to the increased catabolism than anabolism of proteins. This suggestion was supported by previous studies.[53, 54] Also, the elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances.[55] In accordance with these results, it was investigated that methanol administration significantly increased serum urea and creatinine levels.[56] Additionally, as mentioned in Maiuolo et al.[57] The liver is one of the main organs for endogenous production of uric acid, and it is eliminated by kidney. The balance of uric acid formation and excretion is driven by several enzymatic pathways which regulated by pathophysiological factors as metabolic products and free radical species. Furthermore, hyperuricemia is independently associated with the severity of liver damage and elevation of liver enzymes, AST and ALT which recorded in the present study.[58] In addition, the elevations of urea and uric acid also reflect the severity of kidney dysfunction. This suggestion was supported by the previous studies of Adaramoye and Akanni[59] who reported that this is occurred in association with a justly sudden fall in glomerular filtration rate because of methanol. Methanol the metabolite of aspartame that enters the proximal tubular cells, binds to anionic phospholipids and inducing abnormal function and metabolism of intracellular membranes and other organelles, and developed injury in the tubular epithelial cells in kidney.[60]

As shown in (Table 2), after treatment with rosemary restoration of urea and uric acid was obtained while serum creatinine showed no significant change ($p>0.05$). This data is accordance with Ardalan et al.[61] According to Essawy et al.[51], rosemary extract alleviates the toxicity induced by aspartame on the kidney through antioxidant potency and stimulation of endogenous antioxidant defense system.

Table (2): Effect of Rosemary (Rose.) or / and Aspartame (Asp.) on some serum analysis of male rats.

Group	ALT (IU/L)	AST (IU/L)	Total protein (g/dl)	Albumin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
Con.	48.16±2.9	148.00± 4.72	7.36±0.11	4.01±0.19	35.50±1.66	0.25±0.01	5.78±0.17
Rose	43.16± 0.87	140.50±3. 50	7.56±0.05	3.98±0.04	39.33±0.33	0.21±0.01	5.33±0.09
	N.S	N.S	N.S	N.S	N.S	N.S	N.S
	-10.38%	-5.06%	2.71%	-0.74%	10.78%	-16.00%	-7.78%
Asp	113.66±6.14	299.16± 5.97	5.35± 0.16	2.61±0.11	45.66±1.35	0.35±0.01	8.46±0.60
	a***	a***	a***	a***	a***	a**	a***
	136.00%	102.13%	-27.30%	-34.91%	28.61%	40.00%	46.36%
Rose +Asp	54.16± 1.07	151.50± 3.28	7.55±0.11	3.66±0.04	37.50±1.33	0.40±0.04	6.13±0.08
	b***	b***	b***	b***	b**	N.S	b***
	12.45%	2.36%	2.58%	-8.72%	5.63%	60.00%	6.05%
Rose then Asp	59.21± 1.39	168.20± 2.09	7.31± 0.10	3.45±0.06	41.83±1.85	0.40±0.02	6.25±0.08
	b***	b***	b***	b***	N.S	N.S	b***
	22.94%	13.64%	-0.67%	-13.96%	17.83%	60.00%	8.13%
Asp then Rose	59.00± 0.96	160.16± 3.20	7.08±0.11	3.43±0.18	29.16±2.35	0.33±0.02	6.10±0.10
	b***	b***	b***	b***	b***	N.S	b***
	22.50%	8.10%	-3.80%	-14.46%	-17.85%	32.00%	5.53%

*The results are presented as Mean ± SE for 6 rats /group. *** = P <0.001, **= P<0.01, N.S= non-significant a: Significant change from control group. b: Significant change from Asp group. Con= normal control group, Rose = Rosemary group, Asp = aspartame group. Rose +Asp =Rosemary and aspartame group, Rose then Asp =Rosemary two hours then aspartame group, Asp then Rose = Aspartame then rosemary group. (%): percent of change from control value.*

All over the body, electrolytes as sodium and potassium play the critical roles in regulation of nerve and muscle functions and preserve acid-base and water balance. Also, calcium and phosphorus play a vital role in transmission of nerve impulse, muscular contraction, hormone secretion, blood coagulation and intercellular adhesion, skeletal development and cell signaling.[62,63] The kidney plays the vital role in the homeostasis of these electrolytes through glomerular filtration and tubular reabsorption and/or secretion. The disturbances in kidney functions lead to unbalance and disorders over all the body in these electrolytes.[64] In the current study aspartame caused significant disturbances ($p<0.001$) in serum sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and phosphorus (Ph) levels (Table 3). In more details, the results obtained a decrease in serum (Na^+) with an increase in K^+ levels. Similarly, a decrease in Ca^{2+} with an increase in Ph levels was recorded. This means that aspartame caused disorders of mineral metabolism and electrolytes hemostasis. According to[40], lower Na^+ indicates inability of kidney to conserve sodium and increase in potassium may be due to reduced excretion of K^+ worse by leakage of intracellular potassium into blood stream. And this is because lesions in renal tubular epithelium induced by methanol metabolite of aspartame. Also, according to Hozayen et al.[65], these disturbances in Na^+ and K^+ may be due to inhibition of membrane bound enzyme (Na^+, K^+ ATPase) activity due to the indirect action of ASP metabolites as methanol on the membrane bilayer, through lipid peroxidation by free radicals. Likewise, decreased calcium level by aspartame may be due to inhibition of Ca^{2+} ATPase. This enzyme is responsible for active Ca^{2+} transport and is sensitive to hydro peroxides and free radicals from methanol metabolite of aspartame and this led to oxidative modification of thiol groups in this enzyme.[66] Moreover, methanol enhances deposition of Ca^{2+} and causes an increase in the urinary excretion of it. According to Saleh[60], these results may establish that there was association between aspartame and kidney stones as Ca^{2+} accumulation is the most common reason for the formation of kidney stones. Similarly, increased phosphorus ($p<0.001$) by aspartame in blood means that kidneys do not excrete enough phosphate.[67] And this is may be due to renal cells injury induced by aspartame.[68] Also, increased phosphorus in aspartame-based group may be due to increasing the absorption of phosphate by aspartame or its amino acids.[61] The treatment of aspartame administered rats with rosemary extract induced a significant ($p<0.001$) improvement in electrolytes in comparison with aspartame treated group (Table 3). In accordance with Hozayen et al.[65] and as discussed before, this may be due to the antioxidant properties of rosemary which improve injured renal cells. As well as the role of rosemary in regulating the activity and/or expression of certain enzymatic systems associated in physiological processes. Furthermore, mechanistic pathways of cyto-protection exerted by rosemary include (i) potent free radical scavenging activity and the ability to increase the cellular content of glutathione (ii) Ability to regulate membrane permeability, (iii) enhancement of detoxifying activity, and (iv) anti-apoptotic, anti-necrotic and anti-carcinogenic activities.

Table (3): Effect of Rosemary (Rose.) or / and Aspartame (Asp.) on serum electrolytes of male rats.

Group	Na (mEq/L)	K (mEq/L)	Ph (Mg/dl)	Ca (Mg/dl)
Con.	133.66±1.02	4.06±0.15	3.83±0.09	8.95±0.13
Rose	135.16±1.01	4.06±0.04	4.18±0.10	8.90±0.08
	N.S	N.S	N.S	N.S
	1.12%	0.00%	9.13%	-0.55%
Asp	124.83±1.40	5.91±0.12	5.08±0.24	7.70±0.20
	a***	a***	a***	a***
	-6.60%	45.56%	32.63%	-13.96%
Rose +Asp	136.83±0.90	3.86±0.04	3.60±0.07	9.45±0.18
	b***	b***	b***	b***
	2.37%	-4.92%	-6.00%	5.58%
Rose then Asp	133.83±0.74	4.00±0.04	4.10±0.08	8.80±0.08
	b***	b***	b***	b***
	0.12%	-1.47%	7.04%	-1.67%
Asp then Rose	135.33±1.02	4.08±0.06	4.10±0.06	8.88±0.10
	b***	b***	b***	b***
	1.24%	0.49%	7.04%	-0.78%

*The results are presented as Mean ± SE for 6 rats /group. *** = P <0.001 N.S= non-significant. a: Significant change from control group. b: Significant change from Asp group. Con= normal control group, Rose = Rosemary group, Asp= aspartame group, Rose +Asp =Rosemary and aspartame group, Rose then Asp =Rosemary two hours then aspartame group, Asp then Rose = Aspartame then rosemary group. (%): percent of change from control value.*

CONCLUSION

Upon the results obtained in the current study, it can be concluded that, rosemary extract alleviates the harmful effects of aspartame in rat's liver through free radical scavenging, anti-lipid peroxidative, anti-inflammatory and anti-tumor activities. Notably, rosemary has a grateful role in restoration of sodium potassium and calcium phosphorus hemostasis. In this regard the current study has brought a convincing indication preferring the usage of natural antioxidants like rosemary as a protective strategy against toxicity induced by aspartame.

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REFERENCES

1. Colitti, M., et al., *Oxidative stress and nutraceuticals in the modulation of the immune function: Current knowledge in animals of veterinary interest.* Antioxidants, 2019; 8(1): 28.
2. Jamshidi, S., et al., *Preventive Effect of Different Wild Pistachio Oils on Oxidative Stress Markers, Liver Enzymes, and Histopathological Findings in a Metabolic Syndrome Model.* Galen Medical Journal, 2019; 1238.
3. Sottero, B., et al., *Lipid oxidation derived aldehydes and oxysterols between health and disease.* European Journal of Lipid Science and Technology, 2019; 121(1): 1700047.
4. Ahmad, N., et al., *Correlation Between Oxidative Stress and Liver Toxicity*, 2018.
5. Haighton, L., et al., *Evaluation of aspartame cancer epidemiology studies based on quality appraisal criteria.* Regulatory Toxicology and Pharmacology, 2019.
6. Al-Qudsi, F.M. and M.M. Al-Hasan, *In utero exposure to commercial artificial sweeteners affects mice development and mammary gland structure.* Environmental Science and Pollution Research, 2019; 26(5): 5054-5064.
7. Choudhary, A.K. and Y.Y. Lee, *Neurophysiological symptoms and aspartame: What is the connection?* Nutritional neuroscience, 2018; 21(5): 306-316.
8. Nieto, G., G. Ros, and J. Castillo, *Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis, L.): A Review.* Medicines, 2018; 5(3): 98.
9. Pandey, S. and D. Sahoo, *Identification of gene expression logical invariants in Arabidopsis.* Plant Direct, 2019; 3(3): e00123.
10. Lucarini, R., et al., *Hepatoprotective effect of Rosmarinus officinalis and rosmarinic acid on acetaminophen-induced liver damage.* Emirates Journal of Food and Agriculture, 2014: 878-884.
11. Botsoglou, N., et al., *Potential of long-term dietary administration of rosemary in improving the antioxidant status of rat tissues following carbon tetrachloride intoxication.* Food and chemical toxicology, 2010; 48(3): 944-950.
12. Abdella, E.M. and R. Ahmed, *Suppression of doxorubicin apoptotic, histopathologic, mutagenic and oxidative stress effects in male mice bone marrow and testis tissues by aqueous rosemary leaves extract.* Iranian Journal of Cancer Prevention, 2012; 2(1): 35-49.
13. Christian, B., et al., *Chronic aspartame affects T-maze performance, brain cholinergic receptors and Na+, K+-ATPase in rats.* Pharmacology biochemistry and behavior, 2004; 78(1): 121-127.
14. Ohkawa, H., N. Ohishi, and K. Yagi, *Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.* Analytical biochemistry, 1979; 95(2): 351-358.
15. Montgomery, H. and J.F. Dymock, *Determination of nitrite in water*. 1961, ROYAL SOC CHEMISTRY THOMAS GRAHAM HOUSE, SCIENCE PARK, MILTON RD, CAMBRIDGE …. p. 414.
16. Aebi, H., *[13] Catalase in vitro*, in *Methods in enzymology*. 1984, Elsevier. p. 121-126.
17. Beutler, E., *Improved method for the determination of blood glutathione.* J. lab. clin. Med., 1963; 61: 882-888.
18. Nishikimi, M., N. Rao, and K. Yogi, *Colorimetric determination of super oxide dismutase.* Biochem. Bioph. Common, 1972; 46: 849-854.
19. Intiso, D., et al., *Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke patients.* Neurological Sciences, 2004; 24(6): 390-396.
20. Reitman, S. and S. Frankel, *Glutamic–pyruvate transaminase assay by colorimetric method.* American Journal of Clinical Pathology, 1957; 28(1): 56-63.
21. Gomal, A., C. Bardawill, and M. David, *Colorimetric method for total protein determination.* J Biol Chemi, 1949; 177: 751.
22. Doumas, B.T., W.A. Watson, and H.G. Biggs, *Albumin standards and the measurement of serum albumin with bromcresol green.* Clinica chimica acta, 1971; 31(1): 87-96.
23. Young, D., *Effects of drugs on Clinical Lab*. 1995, Tests, 4th ed AACC Press.
24. Tietz, N., *Textbook of clinical chemistry WB Saunders.* Philadelphia, 1986: 1279.
25. Trivedi, R.C., et al., *New enzymatic method for serum uric acid at 500 nm.* Clinical Chemistry, 1978; 24(11): 1908-1911.
26. Bishop, M., F. Duben-Engelkirk, and E. Fody, *Clinical Chemistry, principles, procedures , correlations 4th ed.* Lippinicott Williams & Wilkins California USA, 2000: 345-354.
27. Goldenberg, H. and A. Fernandez, *Simplified method for the estimation of inorganic phosphorus in body fluids.* Clinical chemistry, 1966; 12(12): 871-882.
28. Gindler, E.M. and J.D. King, *Rapid colorimetric determination of calcium in biologic fluids with*

methylthymol blue. American Journal of Clinical Pathology, 1972; 58(4): 376-382.
29. Nusier, M.K., H.N. Bataineh, and H.M. Daradkah, *Adverse effects of rosemary (Rosmarinus officinalis L.) on reproductive function in adult male rats.* Experimental biology and medicine, 2007; 232(6): 809-813.
30. Al-Gholam, M.A., et al., *Protective effect of rosemary on acrylamide motor neurotoxicity in spinal cord of rat offspring: postnatal follow-up study.* Anatomy & cell biology, 2016; 49(1): 34-49.
31. Kalita, J., et al., *Oxidative stress in status epilepticus: A clinical-radiological correlation.* Brain research, 2019; 1704: 85-93.
32. Mourad, M., *Effect of aspartame on some oxidative stress parameters in liver and kidney of rats.* African Journal of Pharmacy and Pharmacology, 2011; 5(6): 678-862.
33. Iyaswamy, A., et al., *Disruption of redox homeostasis in liver function and activation of apoptosis on consumption of aspartame in folate deficient rat model.* Journal of nutrition & intermediary metabolism, 2017; 8: 41-50.
34. Hamza, R.Z., et al., *Effect of l-carnitine on aspartame-induced oxidative stress, histopathological changes, and genotoxicity in liver of male rats.* Journal of basic and clinical physiology and pharmacology, 2019.
35. Gulec, M., A. Gurel, and F. Armutcu, *Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats.* Molecular and cellular biochemistry, 2006; 290(1-2): 61-67.
36. Kumar Choudhary, A., S. Selvaraj, and R. Sheela Devi, *Aspartame induce modification in membrane bound and antioxidant enzymes in liver and kidney of Wistar albino rats.* Current Nutrition & Food Science, 2014; 10(4): 275-287.
37. El-Sokkary, G.H., B.M. Khidr, and M. Saleh Sh, *Aspartame-induced oxidative stress on liver and kidney in normal and diabetic adult male rat.* IJAR, 2016; 6: 511-513.
38. Mapuskar, K.A., et al. *Utilizing Superoxide Dismutase Mimetics to Enhance Radiation Therapy Response While Protecting Normal Tissues*. in *Seminars in radiation oncology*. 2019. Elsevier.
39. Casuso, R.A. and J.R. Huertas, *Antioxidant Supplements in Obesity and Metabolic Syndrome: Angels or Demons*, in *Obesity*. 2018, Elsevier. p. 263-275.
40. Choudhary, A.K. and R.S. Devi, *Serum biochemical responses under oxidative stress of aspartame in* wistar *albino rats.* Asian Pacific Journal of Tropical Disease, 2014; 4: S403-S410.
41. Andrade, J.M., et al., *Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity.* Future science OA, 2018; 4(4): FSO283.
42. Azab, A.E. and M.O. Albasha, *Simultaneous administration of aqueous extract of Rosmarinus officinal with nicotine resulted in prevention of induced hepatorenal toxicity in Guinea pigs.* American Journal of Bioscience and Bioengineering, 2015; 3(5): 80-86.
43. Li, Z.-j., et al., *Comparison of the antioxidant effects of carnosic acid and synthetic antioxidants* on *tara seed oil.* Chemistry Central Journal, 2018; 12(1): 37.
44. Salvi, V., et al., *Cytokine Targeting by miRNAs in Autoimmune Diseases.* Frontiers in immunology, 2019: 10.
45. Wang, X., et al., *Proinflammatory macrophages impair skeletal muscle differentiation in obesity* through *secretion of tumor necrosis factor-α via sustained activation of p38 mitogen-activated protein kinase.* Journal of cellular physiology, 2019; 234(3): 2566-2580.
46. Yang, J.D. and T. Patel, *Early Detection of Hepatocellular Carcinoma: Expanding the Utility of Circulating Tumor Markers.* Hepatology, 2018.
47. Aboshanady, A.M., et al., *The effect of aspartame ingestion in pregnant female albino rats on placental and fetal weights, umbilical cord length, and histology of fetal pancreas.* Tanta Medical Journal, 2018; 46(2): 114.
48. Arafa, M.M. and S. Aly, *Ameliorating effect of folic acid to Aspartame toxicity in rats.* J. Vet. Sci, 2014; 1(1): 26-37.
49. de Oliveira, J.R., S.E.A. Camargo, and L.D. de Oliveira, *Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent.* Journal of biomedical science, 2019; 26(1): 5.
50. Gad, A.S. and A.F. Sayd, *Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review.* Food and Nutrition Sciences, 2015; 6(01): 179.
51. Essawy, A.E., et al., *Dual protective effect of ginger and rosemary extracts against CCl 4-induced hepatotoxicity in rats.* Environmental Science and Pollution Research, 2018; 25(20): 19510-19517.
52. Bashandy, M.A., *Role of rosemary (Rosmarinus officinalis) extract on carbimazole induced alteration in pancreas of adult male albino rat (histological, immuno-histochemicaland ultrastructural study*. Journal of American Science, 2018; 14(1).
53. Alsoufi, M.A., R.A. Aziz, and Z.G. Hussein, *Effect of some artificial sweeteners consumption in biochemical parameters of rats.* Curr. Res. Microbiol. Biotechnol, 2017; 5: 1095-1099.
54. Meyburg, J., et al., *Human heterologous liver cells transiently improve hyperammonemia and ureagenesis in individuals with severe urea cycle disorders.* Journal of inherited metabolic disease, 2018; 41(1): 81-90.
55. Hummadi, L.A., *Histopathological and ultrastructural changes in renal corpuscle of female rats topical application by p-phenylenediamine.* International Journal of Zoological Research, 2012; 8(3): 106-120.

56. Parthasarathy, N.J., et al., *Methanol-induced oxidative stress in rat lymphoid organs.* Journal of occupational health, 2006; 48(1): 20-27.
57. Maiuolo, J., et al., *Regulation of uric acid metabolism and excretion.* International journal of cardiology, 2016; 213: 8-14.
58. Shih, M.-H., et al., *Association between serum uric acid and nonalcoholic fatty liver disease in the US population.* Journal of the Formosan Medical Association, 2015; 114(4): 314-320.
59. Adaramoye, O.A. and O.O. Akanni, *Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats.* Journal of basic and clinical physiology and pharmacology, 2016; 27(1): 29-37.
60. Saleh, A.A.S., *Anti-neuroinflammatory and antioxidant effects of N-acetyl cysteine in long-term consumption of artificial sweetener aspartame in the rat cerebral cortex.* The Journal of Basic & Applied Zoology, 2015; 72: 73-80.
61. Ardalan, M.R., et al., *Nephrotoxic Effect of Aspartame as an Artificial Sweetener: a Brief Review.* Iranian journal of kidney diseases, 2017; 11(5): 339.
62. Blaine, J., M. Chonchol, and M. Levi, *Renal control of calcium, phosphate, and magnesium homeostasis.* Clinical Journal of the American Society of Nephrology, 2015; 10(7): 1257-1272.
63. Shaker, J.L. and L. Deftos, *Calcium and phosphate homeostasis*, in *Endotext [Internet]*. 2018, MDText. com, Inc.
64. Hou, Y.C., C.L. Lu, and K.C. Lu, *Mineral bone disorders in chronic kidney disease.* Nephrology, 2018; 23: 88-94.
65. Hozayen, W., H. Soliman, and E.M. Desouky, *Potential protective effects of rosemary extract, against aspartame toxicity in male rats.* J Inter Acad Res Multidisc, 2014; 2(6): 111-125.
66. Choudhary, A.K. and S.D. Rathinasamy, *Effect of long intake of aspartame on ionic imbalance in immune organs of immunized wistar albino rats.* Biomedicine & Aging Pathology, 2014; 4(3): 243-249.
67. Marks, J., The role of SLC34A2 in intestinal phosphate absorption and phosphate homeostasis. Pflügers Archiv-European Journal of Physiology, 2019; 471(1): 165-173.
68. Al-Eisa, R.A., et al., The Influence of L-carnitine on Aspartame Toxicity n Kidney of Male Rats. International Journal of pharmacology, 2018; 1(8): 1118-1127.