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Potential Protective Effects of Aqueous Extract of Majoram Leaves, Against Aspartame Induced Renal Toxicity in Female Rats

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Abstract: The present study aim to evaluate the possible protective influence of the aquous extract of majoram leaves (OM) against renal toxicity induced by aspartame (ASP) in female rats. Adult female rats (180± 20g) were divided into 9 groups (n=8) and daily treated orally for 6 months. Rats of G_1 (control) received distilled water, G_2 and G_3 (50 and 150 mg ASP/rat/day), while G_4 and G_5 (100 and 300 mg OM/rat/day) and G_{2*} - G_{5*} (ASP + OM). The kidney of each rat were histological examined and biochemical evaluation (kidney functions, oxidative stress and tumor markers) were performed. Our results revealed that, oral administration of ASP produced a significantly increase in the levels of serum urea, creatinine, galectin-1, lactate dehydrogenase (LDH), total antioxidant capacity (TAC) and nitric oxide (NO) compared to control and OM groups. The renal tubules were disrupted and lost their normal architecture in females treated with ASP. CO administration of OM with ASP significantly attenuated the increase of the biochemical parameter and an almost normal histological architecture of the kidney, was observed in the treated groups (G_{2*} - G_{5*}) compared to ASP groups. It can be concluded that the aqueous extract of majoram leaves (OM) have the potential to protect against renal toxicity induced by ASP.

Key words: O. Majoram · Aspartame · Urea · Creatinine · Galectin-1 · Lactate Dehydrogenase · Total Antioxidant Capacity, Nitric Oxide and Histopathology

INTRODUCTION

Aspartame (N-L-alpha-aspartyl-L-phenilalanine-1methylester) is one of the most widely used artificial sweeteners (180-200 times sweeter than sucrose) [1, 2]. The safety of aspartame (ASP) has been repeatedly questioned and associations between its intake and some aliments such as headache, nausea, depression, tiring, irritability, tachycardia, insomnia and dizziness have been hypothesized. It was demonstrated that ASP should be induced hepatotoxic and nephrotoxic effects in rats [3, 4]. The increase in incidence of cancer in aspartame treated rats could be related to its metabolite methanol [5]. On metabolism in humans and experimental animals, ASP is rapidly and completely metabolized to aspartic acid (40%), phenylalanine (50%) and methanol (10%). Methanol, a toxic metabolite is primarily metabolized to formaldehyde and then to formate; these processes are accompanied by the formation of superoxide anion and hydrogen peroxide [6, 7]. It was even supposed that

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aspartame could be a risk factor for brain cancer and could be worsen some pathologies as multiple sclerosis, epilepsy [8]. Other symptoms may include allergic reactions in up to 20% of cases (Urticaria, respiratory symptoms and edema of the lips, tongue, throat or salivary glands) or convulsive crises [9]. It was mentioned that the metabolites of aspartame can be toxic, principally to the liver and retina [10]. It was demonstrated that an increase of cell volume and decreased the density of cell in the fetal kidneys of rats whose mothers treated with aspartame [11] as well as serum urea, creatinine were significantly increased in rats that had received aspartame [12].

As tumor markers, galectins, a family of mammalian carbohydrate binding proteins, are involved in many processes important for tumor survival and dissemination, including proliferation, apoptosis, transcriptional regulation, intracellular signaling, cell adhesion and cell migration [13]. Experimental and clinical data demonstrate that Galectin-1 has a potential prognostic significance and is implicated in renal cell carcinoma (RCC) [14]. In addition, Lactate dehydrogenase (LDH) is a metabolic enzyme that catalyzes the inter conversion of pyruvate and lactate during glycolysis and gluconeogenesis, depending on nutrient availability [15]. LDH is widely expressed in tissues (heart and muscle, for example) as one of five different isoenzymes and it is detectable in serum. LDH levels are increased in response to tissue injury, necrosis, hypoxia, hemolysis and myocardial infarction [16-18]. Elevated baseline LDH levels are associated with clinically advanced disease and it should be considered when evaluating treatment options. On the other hand, microscopic examination of the kidney sections of the treated rats revealed cloudy swelling of the tubules with slight congestion within the interstitium [19].

When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease. Then, the maintenance of adequate antioxidant levels, but not over dosage, is essential to prevent or even manage a great number of disease conditions [20]. As antioxidant status markers, serum total antioxidant capacity (TAC) is the major advantage test to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound [20-23]. Also, Nitric oxide (NO) is a crucial molecule for biological systems. One of the biological functions of NO is a cytotoxic molecule influencing the ability of cells to kill bacteria, viruses and protozoa, as well as tumor cells, by activating macrophages. The damaging effects of NO on cellular proteins, DNA and lipids; and can lead to cell death, tissue injury and organ failure [24-26].

Marjoram (*Organiummajorana* L.) is an aromatic herbal medicine known to possess various therapeutic properties. It contains phenolic terpenoids, flavonoids, tannins and phenolic glycosides [27]. Marjoram is one of the most familiar kitchen herbs. It is cultivated for use of its aromatic leaves for flavoring and other culinary purposes. Many of the folklore medicinal claims about marjoram were confirmed in different experimental models [28]. It is a well-liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skin care, flatulence and stomach disorders [29, 30]. Marjoram has high antioxidant capacity due to its high polyphenol content [31-34]. The medicinal effects of marjoram are gastrointestinal tract stimulant, tonic, carminative, diaphoretic, hypoglycemic, diuretic as well as antibacterial [35].

Therefore, the present investigation was undertaken to test the effects of aspartame on kidney function and kidney tissues in rats and to evaluate the possible protective effects of the aqueous extract of marjoram leaves on the renal toxicity induce by aspartame.

MATERIALS AND METHODS

Experimental Animals: Adult female albino rats weighing 180±20g were used in the present study. The animals were obtained from the Animal House at King Fahad Medical Research Centre., Jeddah, S A. They were kept under observation for two weeks before the onset of the experiment to exclude any infection. The animals were kept at room temperature and exposed to natural daily light-dark cycles. Rats were fed ad libitum and clean water was continuously available. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals stated by the Canadian Council on Animal Care [36].

Experimental Materials: Aspartame (ASP) was purchased from pharmacy in the form of tablets (20 mg/table). The human ADI (Acceptable daily intake) of aspartame was 40 mg/kg [37].The equivalent dose for rat (180±20g) was converted to be 50 mg/rat [38, 39].ASP was dissolved in distilled water before administration by dose to each rat (50 and 150 mg) orally as in previous study on

hepatotoxicity [40]. Marjoram plant was purchased from the local market in Medina, SA. Authentication of the plant was carried out by Biology Department, Faculty of Science and Arts, Khulais, Jeddah University. The dried leaves were extracted by maceration in distilled water (200g/1000 ml) for day at 37-40°C [41]. The dried extract was dissolved in deionized distilled water to a final stock concentration (100 and 300 mg/rat).

Experimental Design

Groups: The rats were randomly and equally divided into nine groups, (n=8) and daily administrated orally for 6 months. G_1 (Control) was administrated distilled water. G_2 & G_3 (ASP) were administrated 50 and 150 mg ASP/rat, respectively), while G_4 & G_5 (OM) were administrated 100 and 300 mg OM/rat, respectively). Co-treated groups (ASP + OM), G_{2*} & G_{3*} were administrated (50 mg ASP +100 or 300 mg OM, respectively) and G_{4*} & G_{5*} were administrated (150 mg ASP and 100 or 300 mg OM, respectively).

Sample Collections: At the end of experiment period (6 months), all the animals were exposed to mild anesthesia. The blood was collected from Orbital sinus left for 15 min at room temperature and centrifuged at 3000 rpm for 15 min. Separated sera were then kept at -20°C until biochemical analysis.

Biochemical Measurements

Kidney Functions Measurements: Serum urea concentration was determined according to the Urease- modified Berthelot reaction [42] using the reagent kits purchased from Diamond Diagnostics, Egypt. Serum creatinine was determined by colorimetric procedure using kits from Biodiagnostic [43].

Oxidative Stress Measurements: Total antioxidant capacity (TAC) was measured by using a Randox assay, Crumlin, County Antrim, United Kingdom [44]. The spectrophotometric determination of serum nitric oxide (NO) by commercial kit supplied from Roche Diagnostics Co [45].

Tumor Markers Measurements: Serum level of galectin-1 was determined with a galectin-1 sandwich enzyme-linked immunosorbent assay purchased from R&D system [46] lactate dehydrogenase (LDH) was measured in serum by colorimetric method using the reagent kits purchased from Roche diagnostics, Mannheim, Germany [47].

Histopathology: The control and treated animals were scarified after being fasted. Kidney from different groups were quickly removed and fixed by immersion in formaldehyde solution (10%). Haematoxylin and eosin stain were used to study the general histological structure of the kidney. The masson's trichrome stain [48] and Periodic Acid Schiff reaction (PAS) technique [49] were used to define the connective tissue elements (Collagen) and mucopolysaccharides and polysaccharides in the kidney.

Statistical Analysis: The collected data were statistically analyzed through SPSS package version 18. Quantitative data was represented as mean ± standard Error (SE). Quantitative comparisons were done through Analysis of Variance (ANOVA). The difference was considered significant at P-value ≤ 0.05 levels.

RESULTS

The results of the current study (Figs. 1 & 2) revealed that oral ASP (G_2 & G_3) administration caused marked renal dysfunction as evidenced by the significant increase in serum urea and creatinine levels ($P< 0.001$ & $P< 0.01$). Animals received OM (G_4& G_5) showed no significant differences in the level of urea and creatinine compared with the control group (G_1).A significant decrease ($P< 0.001$) was recorded in groups (G_{2*} - G_{5*}) compared with ASP groups (Figs. 1&2).

Concerning oxidative stress, our data in Figs. (3 & 4) demonstrated a significant increase ($P< 0.001$) in the level of total antioxidant capacity (TAC) and nitric oxide (NO) in ASP groups (G_2& G_3) compared to the control group (G_1). Serum TAC and NO levels were significantly higher in G_3 than G_2. The present result (Figs. 3&4), the level of TAC and NO were almost near to the control values. The amelioration occurred in high concentration of OM (G_3*&G_5*) is more than that in low concentration (G_2* &G_4*).

Lactate dehydrogenase (LDH) and galectin-1 assay used as Tumor markers. The mean serum levels of galectin-1and LDH in ASP, OM and ASP+ OM groups were shown in Figs. (5&6). There is a significant difference ($P< 0.001$) in serum level of galectin-1 and LDH in G_2 and G_3 which treated with ASP as compared to G_1 (control).

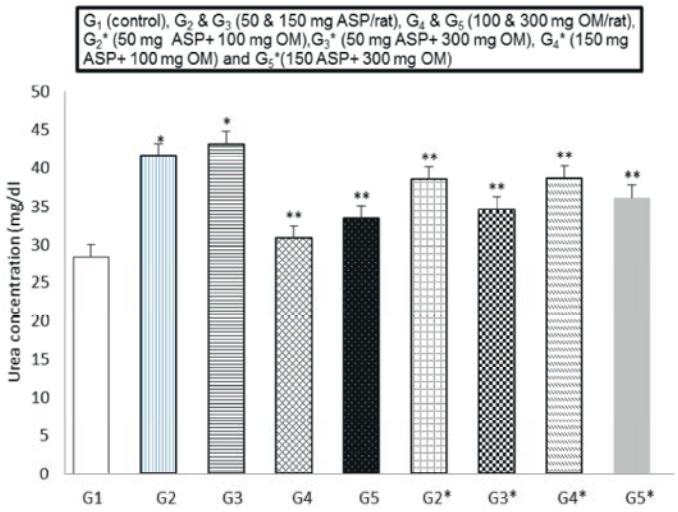

Fig. 1: Effect of ASP, OM and ASP+OM on renal urea

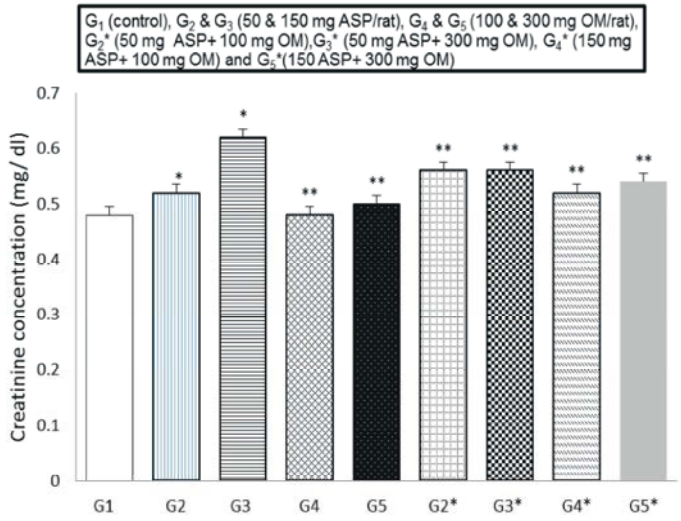

Fig. 2: Effect of ASP, OM and ASP+OM on renal creatinine

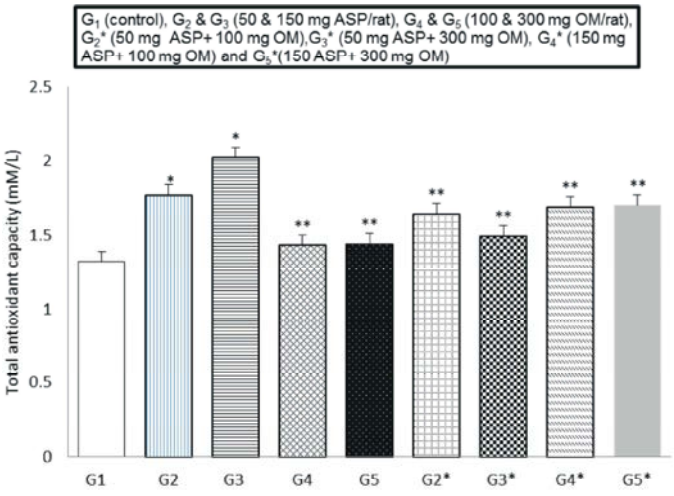

Fig. 3: Effect of ASP, OM and ASP+OM on total antioxidative capacity (TAC)

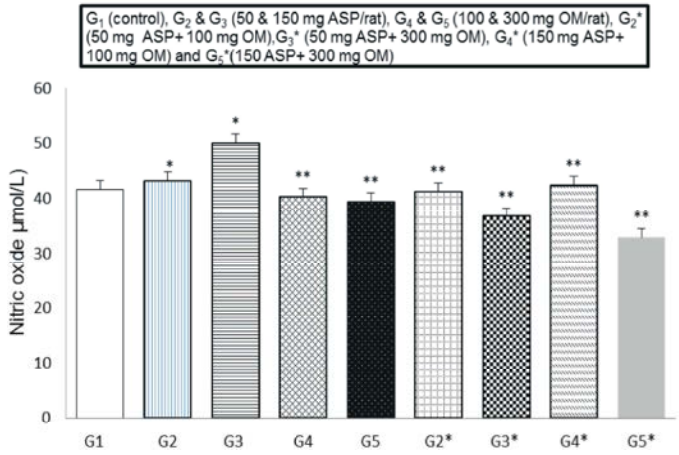

Fig. 4: Effect of ASP, OM and ASP+OM on nitric oxide level (NO)

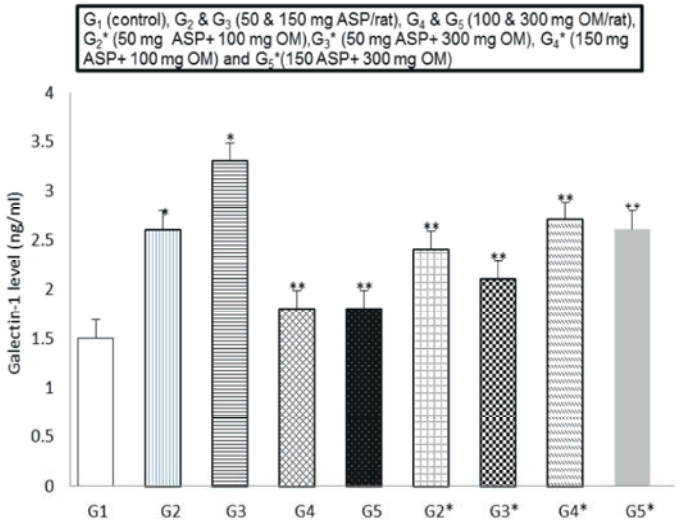

Fig. 5: Effect of ASP, OM and ASP+OM on galectin-1 level

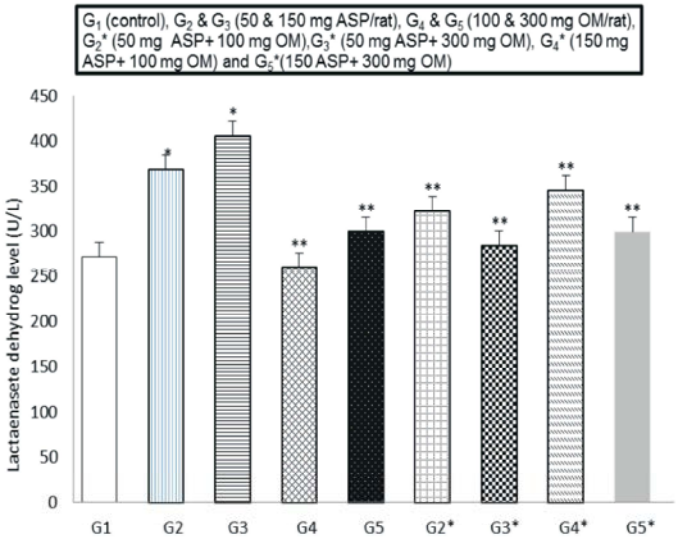

Fig. 6: Effect of ASP, OM and ASP+OM on lactate dehydrogenase levels

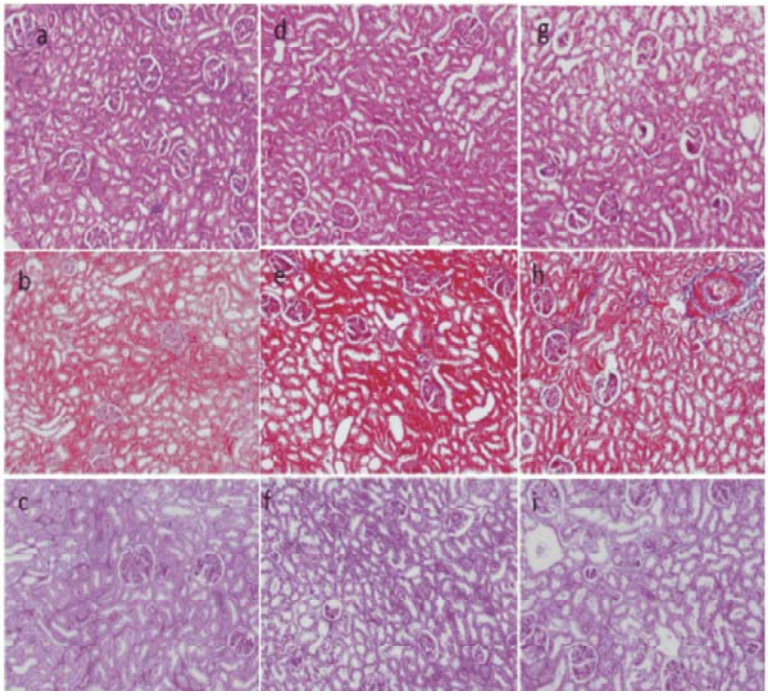

Fig. 7: (a-c) The transverse sections of the kidney of female rats of G_1 (Control) and G_4& G_5 (100 and 300 mg OM\rat\day).(d-f) The transverse sections of female rats of the kidney of G_2 (50 mg ASP/kg bw) and (g-h) The transverse sections of the kidney of G_3 (150 mgASP/kg bw) for 6 month.

The increase in galectin-1 and LDH in G_3 was more than G_2. On the other hand, the co- treated groups (G_2* – G_5*) were treated with ASP+OM, produced a significant decrease as compared to treated groups with ASP.

Histopathology

The Negative (G_1) and OM Control (G_3& G_4): Examination of serial transverse sections of the kidney of control (G_1) and treated group with OM (G_4& G_5) showed no detectable differences in the histological structure of their kidney. The cortex of the kidney was composed of many uriniferous tubules each one was formed of nephron and collecting tubules. The nephron was composed of a renal Malpighian corpuscles, proximal tubules and distal tubules. The Malpighian corpuscles were formed of glomerulus and Bowman's capsule (Fig. 7a). The Bowman's capsule was formed of double layer, an inner visceral layer formed of podocytes and an outer partial layer formed of squamous epithelium, in between the two layers there was a capsular space (Fig. 7a). Masson's trichrome stain showed the normal distribution of the collagen fibers in the tissue of kidney (Fig. 7b). Periodic Acid Schiff reaction showed the normal distribution of PAS positive material in the renal cells (Fig. 7c).

The Chronic Effect of Aspartame (ASP): Examination of serial transverse sections of the kidney of treated group (G_2) (50 mg ASP/rat/day) showed that the structural changes were mainly manifested in the renal tubules rather than the Malpighian corpuscles. The renal tubules were disrupted and lost their normal architecture. The wall of some renal tubule were fragmented and not continuous. Some tubular epithelium appeared degenerated with variable sized vacuoles. Other tubular epithelium were distorted and separated from the basement membrane. Focal areas of tubular necrosis in the form of ill-defined cell membranes with pyknotic nuclei were observed (Fig. 7d). Masson's trichrome stain showed moderate increase in the collagen fibers deposition in the kidney (Fig. 7e) and Periodic Acid Schiff reaction showed moderate reduction of the distribution of the PAS positive material content in the kidney (Fig. 7f) compared with the control groups. Nevertheless, the rat treated with 150 mg of ASP (G_3), the kidney showed extensive lesion of its cells. The structural changes were manifested in both renal tubules and Malpighian corpuscles, most renal tubules showed swelling of their cells to the extend that their lumena were obliterated. The Malpighian corpuscles showed disfigured glomerulus. There were focal degeneration and

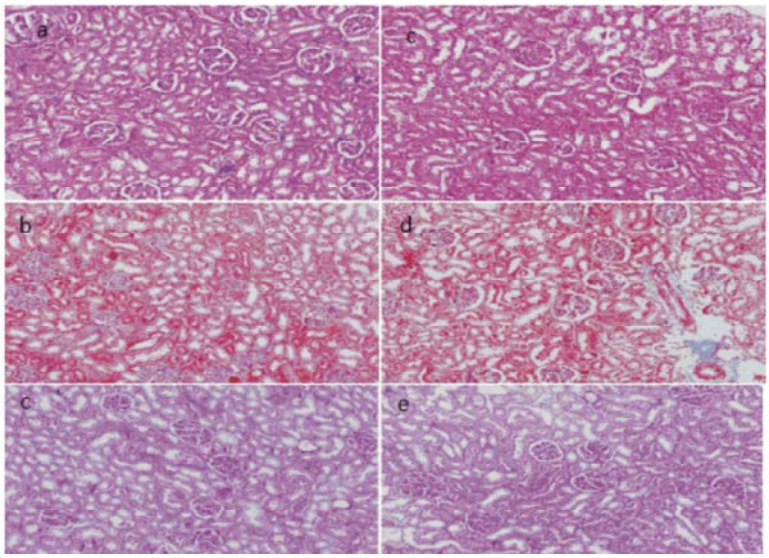

Fig. 8: (a-c) The transverse sections of the kidney of female rats of G_{2*} (50 mg ASP+ 100 mg OM/ rat/day) and (d-f) The transverse sections of the kidney of G_{4*} (150 mg ASP + 100 mg OM/rat/ day) for 6 month

fragmentation of both partial and visceral layers of the Bowman's capsules. Masson's trichrome stain showed marked increase in the collagen fibers deposition in the kidney (Fig. 7h) and Periodic Acid Schiff reaction showed marked reduction of the distribution of the PAS positive material content in the kidney (Fig. 7i) compared with G_1.

The Protective Effect of Aqueous Extract of Majoram Leaves (OM): The female rats treated with 50 mg ASP +100 mg OM (G_2*) showed that the tissue of kidney restored some of its normal architecture. The structural changes were manifested in renal tubules, some renal tubules showed swelling of their cells to the extent that their lumena were obliterated (Fig. 8a). Masson's trichrome stain showed restoration of most of the distribution of the collagen fibers (Fig. 8 b) and Periodic Acid Schiff reaction showed slight reduction of the distribution of the PAS positive material content in the kidney (Fig. 8c) as compared with the treated groups (G_2& G_3) but it appeared moderately increased when compared with the control group (G_1). The G_{4*} showed that the few renal tubules and Malpighian corpuscles were restored their structure (Fig. 8d). Masson's trichrome stain showed slight increase in the collagen fibers deposition in the kidney (Fig. 8e), Periodic Acid Schiff reaction showed moderate reduction of the distribution of the PAS positive material content in the kidney (Fig. 8f) as compared with G_1. However, in both group (G_{3*} & G_{5*}) the structures of the kidney restored their normal structures and became normally as that of control groups.

DISSCUSION

Aspartame (ASP) is the most used artificial sweetener in the world. ASP may exert toxic effects when administered at very high doses, although species susceptibility varies considerably [50]. The ASP induced deterioration of kidney function was evidenced by the elevated serum creatinine and urea levels. The elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic [51, 52]. Serum creatinine concentration is the most widely used marker in estimating glomerular filtration rate (GFR) [53]. Renal lesions associated with ASP treatment (G_2, 50 mg & G_3, 150 mg), mainly the structural changes in renal tubules and Malpighian corpuscles. These were in agreement with the previously reported study of the ASP renal toxicity [12]. Our results confirmed that nephrotoxicity is a major dose-limiting side effect of aspartame. This suggestion was supported by the study of Waggas *et al.* [40] and Soffritti *et al.* [54]. Co-administration of OM attenuated the ASP induced renal toxicity and oxidative stress by decreasing urea, creatinine, NO and TAC in serum.

The development of ASP renal toxicity in the present study was associated with an increase in serum TAC and NO levels, indicating that oxidative stress plays a crucial role in the pathogenesis of ASP renal toxicity. These alterations may be due to the production of high number of free radical by methanol metabolite of aspartame. Our results confirm previous findings

associating significantly increasing serum TAC levels with progression of chronic renal failure and this elevated of TAC levels were strongly correlated to both serum creatinine and uric acid levels [55]. It was observed that TAC was higher in patients with chronic renal injury, as a result of serum urea accumulation [56]. Aspartame consumption can induce oxidative stress and renal toxicity [57]. In the present study, co-treatment with OM prevented the increase of serum TAC and NO associated with ASP administration. Previous experiment shows that volatile oil, alcoholic and aqueous extracts of OM had a protective effect on lead-induced hepato-renal intoxication and chromosomal aberrations [58].

Our results shows that ASP-induced a significant increase in serum galactin-1 level in both doses used (50 & 150 mg). Galactin-1 had significant increased expression in metastatic and primary renal cell carcinoma (RCC) tumors [59, 60]. It represents a potential prognostic marker for RCC patients [61]. Galactin-1 can interact with different proteins and has been shown to contribute to cancer pathogenesis through pathways involved in immune suppression [11]. Our results revealed that co-treatment with OM prevented the increase of serum galactin-1 level associated with ASP administration.

Our results show an increase in lactate dehydrogenase (LDH) level in ASP administrated groups (G_2&G_3) comparing to control. These results confirm the contribution of ASP in Renal Cell Carcinoma (RCC). Serum LDH is a prognostic and a predictive biomarker for the survival benefit in RCC [17].

The present study highlights the effect of the aqueous extract of marjoram on oral administration of aspartame. OM is a useful in decreasing LDH level. Previous studies revealed that the essential oil of Origanum decreased lactate dehydrogenase (LDH) [62]. The observed normalization trend of following aqueous extract of majoram treatment could possibly due to scavenging effect of its components [63].

In a carcinogenicity study on aspartame (ASP), [64,65] were provided of a relationship between ASP treatment and transitional cell hyperplasia [64]. Observed a dose-related, statistically significant increase in the incidence of dysplastic hyperplasia and carcinoma of the renal pelvis in females compared with the controls.

In the current study, examination of serial transverse sections of the kidney of rats treated by 50 mg ASP (G_2) and 150 mg ASP (G_3) showed that the structural changes were mainly manifested in the renal tubules and Malpighian corpuscles. The walls of renal tubule were fragmented and tubular epithelium appeared degenerated with variable sized vacuoles. The Malpighian corpuscles showed disfigured glomerulus. However, the rat in G_3, the changes became worse where the kidney showed extensive lesion of its cells. This results is supported by [65] who mentioned that the adult male rats administrated aspartame (50mg/kg) produced histological changes in large number of renal tubules where their lining cells appeared degenerated with pyknosis or absence of the nuclei. The increased cell volume and decreased numerical cell density in the fetal kidneys of rats whose mothers treated with aspartame reported [11]. The artificial sweeteners (Saccharin, cyclamate, aspartameand acesulfame-k) use was found to be positively associated with UTT risk [66].

In the present work, the degenerative changes which were observed in the kidney could be related to the hazardous effects of the metabolites of aspartame on the cell [7]. In the current study, the some renal tubules showed swelling of their cells to the extent that their lumena were obliterated. These swelling of the cells might be due to fatty degeneration and aedematous changes respectively [67].

In the present work, the degenerative changes, which were observed in the kidney, could be related to the hazardous effects of the metabolites of aspartame on the cell proteins [7, 68]. In the current work showed increased collagen fiber deposition and reduction of the PAS positive material in the kidney tissues. The increase in collagen fiber deposition and reduction of the PAS were moderate in rats of G_2 and marked in G_3. This finding was in line with [60,69]. In present study showed that rates (G_4& G_5) treated with 100 & 300 mg OM/rat, their kidney showed the normal structures, deposition of collagen fibers and the distribution of ASP positive material. Indicating that this plant does not have toxic effect. This is in agreement with previous studies [70, 71].

The current study demonstrate that OM has protective effects against the nephrotoxicity induced by aspartame in female rats, which are in agreement with [72, 73]. The observed improvements may be revealed to the presence of many antioxidant components found in OM. The feeding of rats with marjoram was able to reduce and sometimes completely remove the toxic effect of Carbon tetrachloride (CCl4) [74, 75]. The *Origanummajorana* L. (OM) showed apparent protective

and curative effect on Cd-induced hepatotoxicity and nephrotoxicity, suggested [71]. On the other hand, our study showed that rates treated with ASP + OM (G_{3*} and G_{5*}), their kidney restored their normal structures, deposition of collagen fibers and the distribution of PAS positive material. This indicate the effect of OM is dose dependent which proved [76]. We attribute the ability of marjoram to help the kidney to restore their structures to the antioxidant activity of marjoram. The antioxidant and antitumor activities of OM have been reported in various studies [70, 77-79] proved the ability of OM to prevent liver dysfunction and alterations of antioxidative parameters induced by cadmium may be due to the fact that OM possess scavenging free radicals properties as it has been reported to have antioxidant properties. The aqueous extracts of OM exhibited therapeutic and chelating effects against Cd induced nephrotoxicity. However, the therapeutic effect was more potent than its chelating effect [80]. Therefore, it could be recommended that populations exposed to Cd can use the aqueous extract of OM.

CONCLUTION

To conclude, the present study demonstrated that aspartame elicited deleterious renal toxic effects. OM prevented aspartame-induced renal toxicity. Therefore, this study recommends that intake of marjoram leaves as a drink may have protective effects against aspartame that may cause renal toxicity and oxidative stress. However, further clinical studies are required to assess the safety and benefits of marjoram.

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