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Research article

Mitigating Physiological and Histological Changes Induced by a High-Salt Diet in Male Albino Rats Using *Origanum majorana* and *Olea europaea* Leaf Extracts

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HIGHLIGHTS

- High NaCl feeding induces hypertension.
- Protective effects of marjoram and olive leaf extracts.
- A high-salt diet disrupted liver and kidney functions.
- High-salt diet caused sperm abnormalities and histopathological changes in vital organs.
- Restoring balance with natural extracts.

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ABSTRACT

This research explored the possible protective impact of marjoram and olive leaf extracts against hypertension triggered by a salt-rich diet in rats. Twenty male albino rats (*Rattus norvegicus*) were separated into four equal groups; the first group was the reference control, and the second group was provided 250 mg/Kg body weight of *Origanum majorana* leaves extract and 250 mg/Kg body weight of *Olea europaea* leaves extract daily. The third group was given freely a high 8% NaCl diet. The fourth group was administered an 8% salt diet supplemented with 250 mg/Kg body weight of *O. majorana* leaves extract and 250 mg/Kg body weight of *O. europaea* leaves extract. Samples were taken after 4 weeks of treatments. Feeding rats, a high sodium chloride diet consisting of 8% NaCl induces hypertension in rats. The high salt diet enhanced marked elevation in body weights and reduced most relative organ weights. The high salt feeding elevated sodium levels in hepatic and kidney tissues, in addition to a rise in the liver's MDA level. Feeding on a high salt diet reduced superoxide dismutase and catalase activities. It promoted considerable disruption in liver and kidney functions. It also triggered a decrease in sperm count and an increase in abnormal sperm morphology. The high salt feeding induced histological changes in the liver, kidney, brain, and testes of rats. Combined supplementation with both *O. majorana* and *O. europaea* extracts alleviated salt-induced abnormalities in rats.

1. Introduction

High sodium chloride (NaCl) consumption is a major dietary factor contributing to multiple organs' widespread physiological and histopathological alterations [1-3]. The primary mechanism involves the disruption of cellular and systemic homeostasis [4], leading to oxidative stress, inflammation, and lipid peroxidation (LPO) [5]. Chronic high salt consumption is closely linked to the development of hypertension [6] by disruptions of fluid balance and vascular function [7] and promotes systemic organ damage [8].

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Excessive sodium intake increases extracellular fluid volume by promoting water retention, which elevates blood pressure [9]. Additionally, high NaCl intake impairs the renin-angiotensin-aldosterone system (RAAS), resulting in vasoconstriction and elevated vascular resistance [10]. Sodium also influences vascular endothelial cells, causing oxidative stress, decreased nitric oxide (NO) availability, and impaired endothelial function contributing to cardiovascular complications [11].

High-salt diets exacerbate oxidative damage by promoting the generation of reactive oxygen species (ROS) and depleting the body's natural antioxidant reserves [12]. This oxidative imbalance drives LPO, damaging cellular membranes and contributing to systemic inflammation [13]. High NaCl consumption induces pathological changes in multiple organs. The liver experiences metabolic disruptions, oxidative damage, and inflammatory responses [14,15]. In the kidneys, high sodium intake accelerates fibrosis, impairs filtration, and promotes hypertension-related damage [16,17]. In the brain, sodium chloride contributes to neuroinflammation and oxidative damage, affecting cognitive functions [18,19]. The testes are also vulnerable, with oxidative stress impairing spermatogenesis and hormonal balance, potentially affecting reproductive health [20,21].

Given the systemic nature of these effects, there is increasing interest in natural, plant-derived interventions for mitigating the damage caused by high salt intake. Extracts from *Origanum majorana* (marjoram) and *Olea europaea* (olive) have shown potential in addressing oxidative stress and histological damage [22,23]. Marjoram is rich in flavonoids and polyphenols recognized for its strong radical scavenger and anti-inflammatory effects. At the same time, olive leaves are abundant in oleuropein and hydroxytyrosol, which improve vascular health and reduce oxidative stress [24,25]. Combined supplementation with olive and marjoram leaf extracts offers a promising strategy, as their bioactive compounds may synergistically mitigate oxidative stress, reduce LPO, and improve organ function.

Despite their benefits, research examining the combined use of olive and marjoram extracts in addressing high salt-induced hypertension and systemic organ damage are scarce. Combining these extracts offers a promising protective strategy, as their complementary bioactive compounds may exert synergistic effects in countering the physiological and histological damage induced by high salt feeding. Therefore, the current study investigated the combined efficacy of *O. majorana* and *O. europaea* leaf extracts in mitigating NaCl-induced alterations in rat organs.

2. Materials and methods

2.1. Chemicals

Pure sodium chloride was purchased from El Naser Pharmaceutical Chemicals Co., Egypt.

2.2. Animals

Twenty male albino rats (*Rattus norvegicus*) four weeks old, weighing (70 ± 5g), were obtained from the Animal care facility, Faculty of Science, Suez Canal University, Egypt. The rats were housed in plastic housing and maintained on a 12-hour light-dark cycle with regulated temperature (25±2ºC) and relative humidity (45±5%). They were provided with a standard balanced diet and water *ad libitum,* and allowed a 7-day acclimatization period to the laboratory conditions. The experimental procedures was evaluated and authorized by the Research Ethics Committee of the Faculty of Science at Arish University, under approval number "ARU001". Our techniques followed the guidelines outlined in the 8th edition of the guide for the care and use of laboratory animals, ensuring strict adherence to ethical standards.

2.3. Preparation of marjoram and olive leaf extracts

The leaves of marjoram (*O. majorana*) and Olive (*O. europaea*) were collected from the Arish area, North Sinai, Egypt. The plants were examined and classified at the Faculty of Science, Arish University, by a specialist professor in plant taxonomy. The plant leaves were dried by leaving them at room temperature for one week. The desiccated leaves were crushed into powder. Ten grams of desiccated powder of each plant were dissolved in 100 mL of 70% ethyl alcohol to prepare a 10% (w/v) stock solution [26,27]. The mixture is stirred, and placed in a cold and dark place for 48-72 hours to extract the effective substances. Then, the extract was filtered. A rotary evaporator was used

to evaporate ethanol and get the dry extract, which was then stored in a tight and dark container, away from humidity and excess heat to preserve the extract's effectiveness.

2.4. Experimental design

After acclimatization, the rats were haphazardly assigned into four equal groups. The first group acted as the normal control, the second group received 250 mg/kg body weight of *O. majorana* leaves extract [28] and 250 mg/kg body weight of *O. europaea* leaves extract [29] daily. The third group was fed an unrestricted 8% NaCl diet [30]. The fourth group was given an 8% salt diet, supplemented with 250 mg/kg body weight of *O. majorana* leaves extract and 250 mg/kg body weight of *O. europaea* leaves extract.

2.5. Duration time

Upon completion of the experiment (28 days), all rats were euthanized utilizing ketamine (50 mg/kg body weight, intramuscular injection) and rapidly dissected. Tissues were removed, weighted, blotted on filter paper, and kept at –30ºC till additional biochemical examinations. The body weights of rats were taken weekly.

2.6. Non-invasive blood pressure measurement

The systolic and diastolic blood pressures of the rats were evaluated non-invasively with a tail-cuff plethysmography system (CODA®Monitor, Kent Scientific, Torrington, CT, USA), incorporating a specialized retraining chamber designed for animal comfort and stability.

1. **Acclimatization:** Rats were acclimated to the restrainer and device for five consecutive days (10-15 minutes per day) to minimize stress-related variability. The chamber allowed the tail to extend freely for easy access, reducing handling stress.

2. **Chamber design:** The chamber was constructed to securely restrain the animal's body while leaving the tail accessible. The chamber size was customized to prevent unnecessary movement, ensuring consistent positioning during measurements.

3. **Setup:** Measurements were conducted in a temperature-controlled environment (30-35 ºC) to ensure tail vasodilation and optimal blood flow.

4. **Measurement protocol:** The rat was placed into the chamber, and its tail extended through the opening. A pneumatic cuff with a sensor was placed around the proximal tail. The cuff was inflated to occlude blood flow and then deflated gradually, with oscillometric signals recorded to determine systolic and diastolic pressures.

5. **Data collection and validation:** Each rat underwent three consecutive measurements, and the average was used for analysis. Outliers caused by movement artifacts were excluded, and readings were repeated if necessary.

2.7. Tissue preparation for microscopic examination

Following the dissection of the animals, the liver, kidney, testes, and brain were extracted, dried on filter paper, and weighed. Representative samples were then placed in a 10% formalin solution and processed into paraffin for histological sectioning and staining.

2.8. Histological methods

Histological sections, 5 microns thick, were formulated and stained with hematoxylin and eosin. Microscopic examination of the samples was performed blindly using an Olympus BX43 microscope [31].

2.9. Biochemical analysis

Sodium was measured using spectrum kits, Cairo, Egypt, according to Henry et al. [32]. Oxidative stress parameters were assessed using BioDiagnostic kits (Cairo, Egypt). Catalase (CAT) activity was assessed using the Aebi method

[33]. The concentration of malondialdehyde (MDA) was evaluated using the method of Ohkawa et al. [34]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were quantified using the modified version of the method of Schumann and Klauke [35]. Uric acid levels was quantified utilizing Spinreact kits (Spain) and the technique of Fossati et al. [36]. Creatinine was determined with Diamond Diagnostics kits (Holliston, USA) using the Heinegard and Tiderstrom technique [37].

2.10. Statistical analysis

The results are presented as means ± standard deviation (SD) for five rats per group. Data from the control and treated groups were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. A probability-value of <0.05 was considered statistically significant. Data analysis was conducted leveraging the statistical Package for Social Sciences (SPSS) software, version 22.0 for windows.

3. Results

3.1 Body weights

The results showed that salt-rich diet feeding induced considerable elevation ($P<0.05$) in body weights after two and four weeks of treatment, by 38.0% and 33.25%, respectively (Fig. 1). On the other hand, combined supplementation with both olive and marjoram extracts restores normal levels.

3.2 Organ weights

The results showed that high salt feeding induced significant ($P<0.05$) decrease in relative liver and testicular weights by 17.94% and 44.5% after 4 weeks of treatment, respectively. However relative kidney and brain weights remain unaffected after 4 weeks of treatment (Fig. 1). On the other hand, combined supplementation with both olive and marjoram extracts not only reversed these effects, but also elevated relative kidney, brain, and testis significantly ($p<0.5$) by 42.6, 65.76 and 46.07%, respectively, compared with normal controls after 4 weeks of treatment.

3.3 Blood pressure

The 8% salt feeding induced a considerable elevation in systolic blood pressure of rats after 2 and 4 weeks of treatment with magnitudes of 19.25 and 22.35% compared with normal controls, respectively (Fig. 2). It also enhanced significant ($P<0.05$) elevation in diastolic blood pressure by 24.71 and 28.94% compared with normal controls following 2 and 4 weeks of treatment, respectively. In contrast, combined supplementation with marjoram and olive extracts restores blood pressure to approach control.

3.4 Biochemistry analysis

After 4 weeks of high sodium chloride intake, there was a notable rise in MDA levels in hepatic tissue by 131% and sodium levels in liver and kidney tissues by 21.95% and 17.87%, respectively in comparison to the normal control group (Fig. 3). SOD and CAT activities were reduced significantly ($p<0.05$), by 21.87 and 27.38% compared to normal rats, upon 4 weeks of high NaCl administration, respectively. It enhanced considerable disruption in hepatic (AST & ALT) and renal (urea & creatinine) functions (Table 1). High salt feeding caused a substantial decrease in sperm count, and considerable elevation in abnormal sperm morphology; however, plasma testosterone and sperm motility are approximately not affected (Table 2). Nevertheless, supplementation with both marjoram and olive leaf extracts ameliorated these effects.

3.5 histopathology findings

The tissue architecture of the kidney, liver, rain, and testis in the control group displayed normal cellular organization, with well-defined structures and appropriate cellular integrity. Similarly, treatment with *O. majorana* and *O. europaea* leaves extracts resulted in tissue architectures of these organs that were more or less similar to the control group (Fig. 4-7), showing preserved cellular morphology and minimal disruption. The effects of high NaCl feeding on rats' histopathology were detected. A marked impairment of the renal tubules through glomeruli congestion, signs of tubular cell lining vacuolation, and intra-tubular hemorrhage could be seen after 4 weeks of salt

ingestion (Fig. 4). Hepatocytes showed the formation of vacuoles in the cytoplasm and abnormal structure organization after chronic high salt ingestion (Fig. 5). On the other hand, brain cells displayed signs of cell atrophy and surrounded vacuolation after administering 8% salt diet for 28 days (Fig. 6). It was observed that the germinal testicular epithelium was adversely affected, as evidenced by the wide separation of the cells and a decrease in spermatid count within the lumen in rats chronically given high salt diet (Fig. 7). Conversely, the dosage of *O. majorana* and *O. europaea* leaf extracts significantly improved tissue damage. These findings suggest that both plant extracts offer protective effects against potential damage caused by high NaCl exposure.

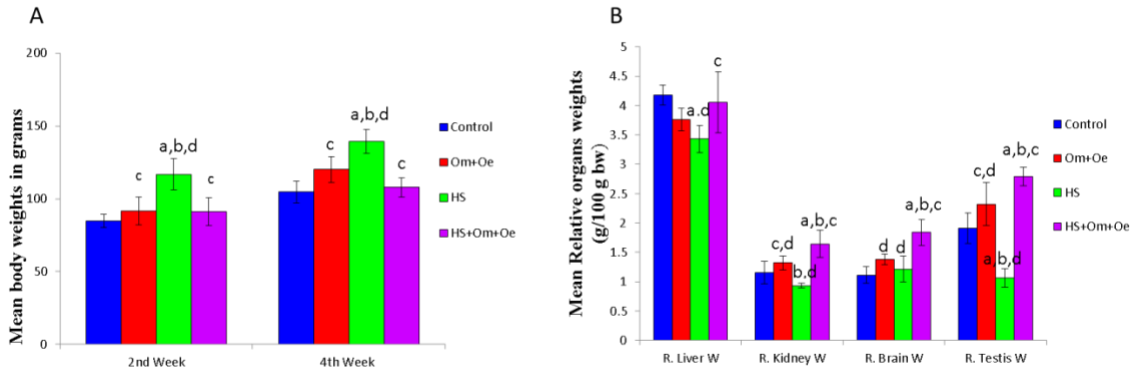

Fig. 1. The impact of high salt feeding on body weight (A) and relative organ weights (B) (g/100 g bw) of rats. Om: *O. majorana*, Oe: *O. europaea*; HS: high salt; bw: body weight. Data are presented as means ± SD for five rats in each group. a: Significantly different from control group at $p<0.05$ (Tukey's *post-hoc* test), b: significantly different from *O. majorana* and *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test), c: significantly different from high salt group at $p<0.05$ (Tukey's *post-hoc* test), d: significantly different from high salt + *O. majorana* + *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test).

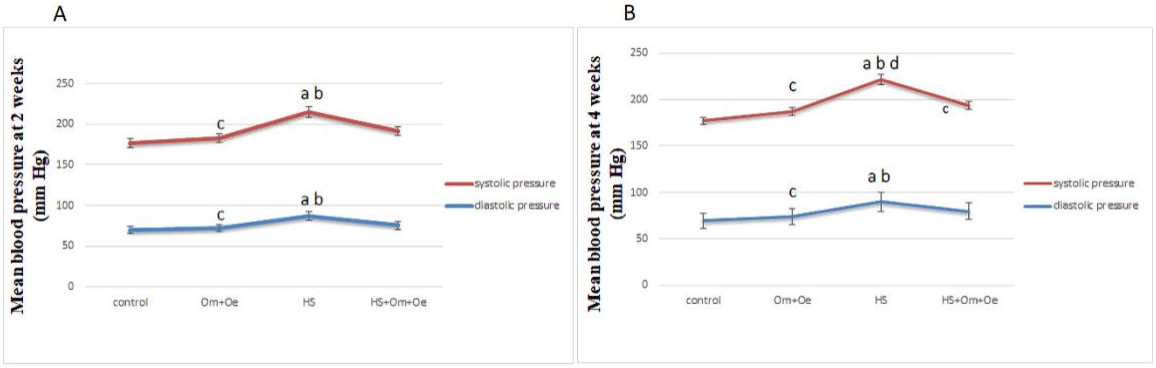

Fig. 2. Effect of high salt feeding on mean systolic and diastolic blood pressure in rats after 2 weeks of treatment (A) and 4 weeks of treatment (B). Om: *O. majorana*, Oe: *O. europaea*; HS: high salt; Data are presented as means ± SD for five rats in each group. a: Significantly different from the control group at $p<0.05$ (Tukey's *post-hoc* test), b: significantly different from *O. majorana* and *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test), c: significantly different from high salt group at $p<0.05$ (Tukey's *post-hoc* test), d: significantly different from high salt + *O. majorana* + *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test).

4. Discussion

It has been demonstrated that high salt ingestion significantly elevates the body weight of rats, likely due to its effects on sodium levels, fluid retention, and appetite regulation. Increased sodium disrupts fluid balance, leading to water retention as the body attempts to maintain sodium homeostasis [38,39]. Additionally, high salt intake

promotes thirst and water consumption, which further contributes to weight gain [40]. Evidence also suggests that salt enhances appetite, potentially important to increased food intake and fat accumulation [41]. Chronic salt consumption alters lipid metabolism and affects hormones such as leptin and insulin, disrupting appetite regulation and promoting fat storage [42-44]. Furthermore, chronic high salt intake induces low-grade inflammation, which has been implicated in promoting fat storage and weight gain [45]. Alterations in the gut microbiota due to high salt intake may also impact digestion and nutrient absorption, influencing body weight [46]. At the cellular level, NaCl exposure can principal to intracellular fluid accumulation and tissue swelling, contributing to increased body weight [47]. Stress-related pathways, such as RAAS, are also activated by high salt consumption, promoting fat deposition, reducing energy expenditure, and increasing hypertension [43].

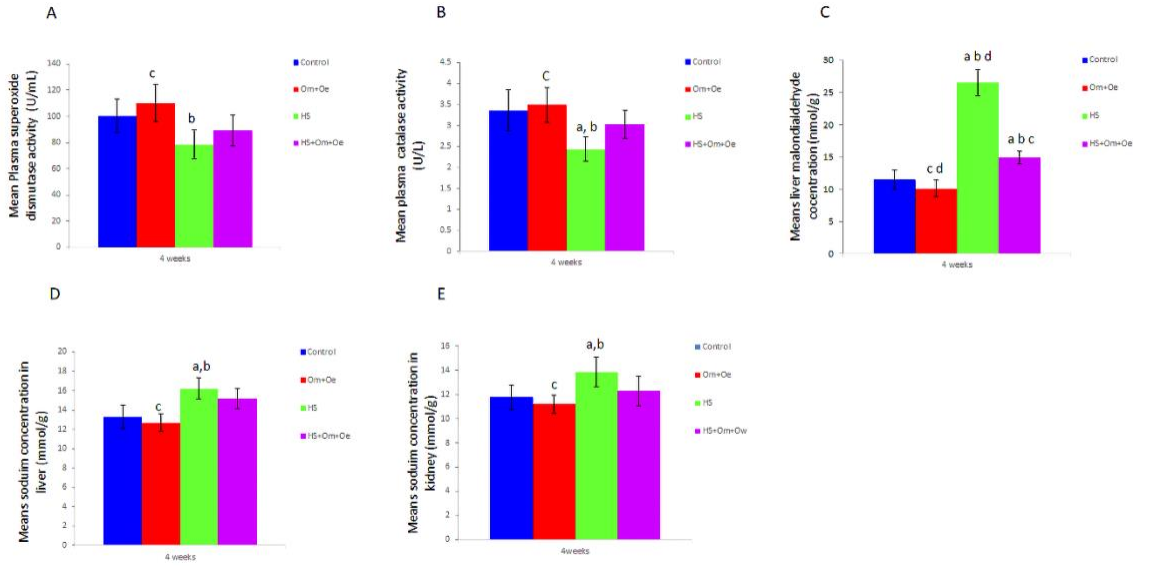

Fig. 3. Effects of high salt feeding for four weeks on plasma superoxide dismutase (A) and catalase (B) activities, and malondialdehyde (C) level, and sodium concentration in the liver (D) and kidney (E) of rats. Om: *O. majorana*, Oe: *O. europaea*; HS: high salt. Data are presented as means ± SD for five rats in each group. a: Significantly different from control group at $p<0.05$ (Tukey's *post-hoc* test), b: significantly different from *O. majorana* and *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test), c: significantly different from high salt group at $p<0.05$ (Tukey's *post-hoc* test), d: significantly different from high salt + *O. majorana* + *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test).

Table 1. Effects of high dietary salt intake on liver and kidney function tests and the protective effect of *O. majorana* (Om) and *O. europaea* (Oe) supplementation in male albino rats.

28 days	ALT (U/L)	AST (U/L)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	30.3 ± 4.74	85.5 ± 12.9	1.76 ± 0.28	0.48 ± 0.07
Om + Oe	28.3 ± 3.79 ^{c,d}	83.6 ± 12.5 ^{c}	1.60 ± 0.25 ^{c,d}	0.47 ± 0.06 ^{c}
HS	48.2 ± 6.44 ^{a,b,d}	114.5 ± 16.9 ^{a,b}	3.12 ± 0.46 ^{a,b,d}	0.65 ± 0.09 ^{a,b}
Om + Oe + HS	38.7 ± 5.38 ^{b,c}	103.2 ± 14.0	2.48 ± 0.35 ^{a,b,c}	0.53 ± 0.08

Data are presented as mean ± SD (n = 5). a: Significantly different from normal control group at $p<0.05$ (Tukey's *post-hoc* test), b: significantly different from *O. majorana* and *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test), c: significantly different from high salt group at $p<0.05$ (Tukey's *post-hoc* test), d: significantly different from high salt + *O. majorana* + *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test).

Table 2. Effects of high dietary salt intake on plasma testosterone, sperm parameters, and the protective effect of *O. majorana* (Om) and *O. europaea* (Oe) supplementation in male albino rats.

28 days	Testosterone (ng/dL)	Sperm count x 10^6/mL	Sperm motility (%)	Abnormal sperm morphology (%)
Control	26.3 ± 4.37	100.2 ± 15.0	93.0 ± 14.6	4.4 ± 0.54
Om + Oe	27.5 ± 4.39 [c]	103.4 ± 11.9 [c]	95.0 ± 13.8	3.20 ± 0.83 [c]
HS	19.8 ± 2.90 [b]	76.40 ± 10.8 [a,b]	74.2 ± 11.4	7.00 ± 2.54 [a,b]
Om + Oe + HS	23.1 ± 3.28	88.80 ± 10.7	80.0 ± 12.2	540 ± 0.54

Data are presented as mean ± SD (n = 5). a: Significantly different from normal control group at $p<0.05$ (Tukey's *post-hoc* test), b: significantly different from *O. majorana* and *O. europaea* extracts-treated group at $p<0.05$ (Tukey's *post-hoc* test), c: significantly different from high salt group at $p<0.05$ (Tukey's *post-hoc* test).

A high-salt diet has reflective effects on blood pressure, mainly through increased plasma sodium concentrations that lead to fluid retention, increase in blood volume, and heightened cardiac output [48]. The vascular effects of salt include increased stiffness and reduced relaxation of blood vessels, leading to elevated peripheral resistance and hypertension [49,50]. Dysregulation of the RAAS under chronic salt exposure raises angiotensin II levels, causing vasoconstriction and elevated systolic and diastolic blood pressure [51]. Salt also disrupts endothelial function, decreasing nitric oxide synthesis and raising oxidative stress, which exacerbates vascular dysfunction [52].

Figure 4

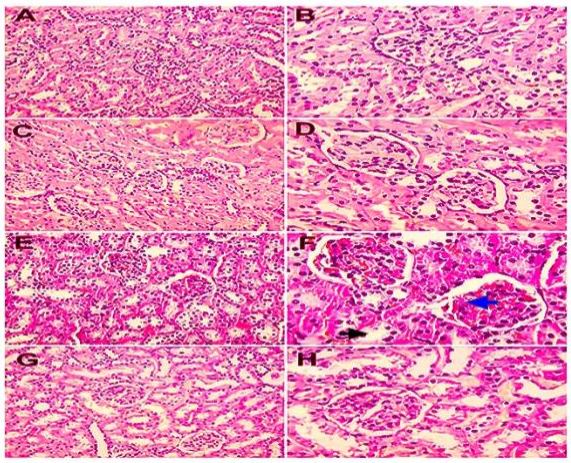

Figure 5

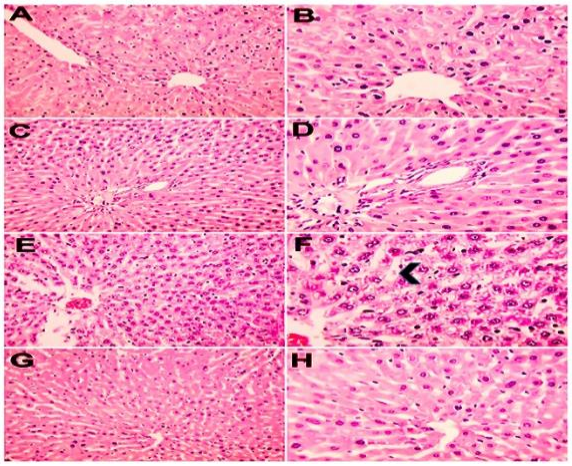

Fig. 4. Histological sections of the rat kidney after 4 weeks of treatment, show a section in the cortex of the control (A & B) rat with a regular structure. The marjoram and olive extracts-treated group (C & D) is more or less similar to the control group. The high salt-fed group (E &F) shows marked affection for the renal tubules through glomeruli congestion (blue arrow), signs of tubular cell lining vacuolation (black arrow), and intratubular hemorrhage. The marjoram and olive extracts + high salt-fed group (G & H) shows minimal affection for the renal tubules with almost normal glomeruli and tubules (X 200: H & E section A, C, E & G; X 400: H & E section B, D, F & H).

Fig. 5. Histological sections of the rat liver after 4 weeks of treatment, show a section in the liver of the control (A & B) rat with the typical arrangement of the hepatocytes around the central veins. The marjoram and olive extracts-treated group (C & D) is more or less similar to the control group. The high salt-fed group (E &F) shows marked affection for the hepatocytes, which appear with vacuolated cytoplasm (black arrowhead) and distorted arrangement. The marjoram and olive extracts + high salt-fed group (G & H) shows minimal affection for liver parenchyma with more or less standard structure (X 200: H & E section A, C, E & G; X 400: H & E section B, D, F & H).

Oxidative stress is a major result of high salt consumption, as indicated by increased plasma malondialdehyde (MDA) levels, a marker of LPO [53]. The production of reactive oxygen species (ROS), including superoxide anions and hydroxyl radicals, leads to damage in cellular structures like lipids, proteins, and DNA [53,54]. MDA reacts with cellular components to form advanced glycation end products (AGEs), which further lead to tissue dysfunction [54]. The mechanisms underlying increased ROS production include monocyte activation, NADPH oxidase pathway dysregulation, mitochondrial dysfunction, and inflammation [55]. Pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-33, further amplify ROS production and tissue damage [56]. These processes also reduce activities of antioxidant enzyme, such as CAT and superoxide SOD, which impair the body's ability to neutralize ROS [57,58]. Prolonged salt consumption leads to a suppression of antioxidant defense mechanisms, exacerbating oxidative damage over time [21,59].

Chronic salt intake stimulates the sympathetic nervous system, causing hypertension, which increases the risk of cardiovascular diseases such as hypertension, heart failure, stroke, and kidney disorder [60, 61]. It exerts detrimental effects on multiple organs. In the kidneys, it increases MDA levels, promoting inflammation, fibrosis, and glomerular damage [62]. This results in reduced filtration efficiency and elevated plasma urea and createnine concentrations [63,64]. Prolonged exposure to high salt levels leads to glomerulosclerosis and reduced renal blood flow, impairing waste elimination and increasing intra-glomerular pressure reducing the efficiency of the kidneys in filtering waste products [63,65]. In the liver, high salt intake disrupts hepatocyte integrity through oxidative stress, LPO, and mitochondrial impairment, resulting in vacuolation and impaired metabolic functions [66,67]. Hepatocyte damage is further aggravated by inflammation, and immune response, which interferes with the liver's detoxification and protein synthesis abilities [68-70]. Chronic high salt intake disrupts cellular osmotic balance, causing hepatocyte swelling, membrane damage, and the release of AST and ALT enzymes [66,71]. It also induces mitochondrial damage and endoplasmic reticulum dysfunction, leading to protein misfolding, hepatocyte death, and enzyme leakage [72].

Figure 6

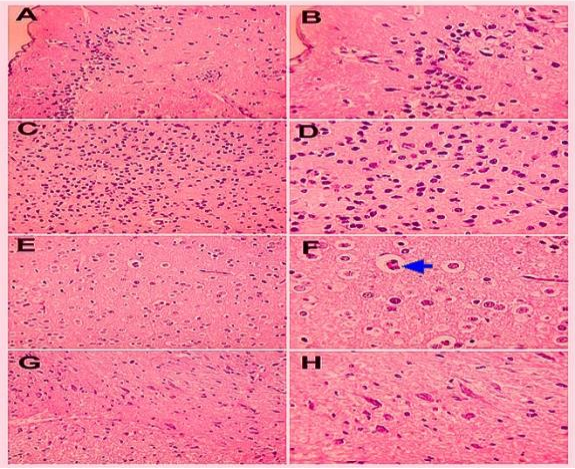

Figure 7

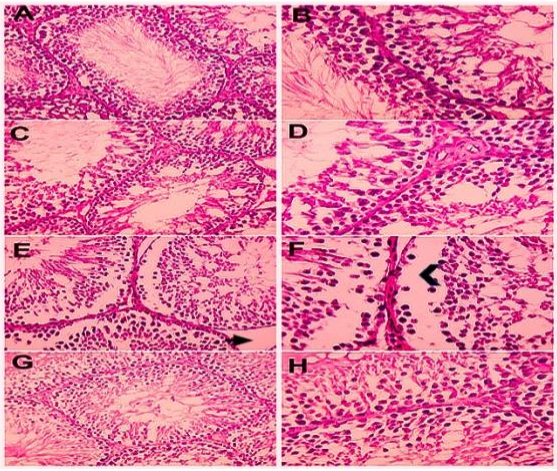

Fig. 6. Histological sections of the rat brain after 4 weeks of treatment, show a section in the cerebral cortex of the control (A & B) rat with a standard structure. The marjoram and olive extracts-treated group (C & D) is more or less similar to the control group. The high salt-fed group (E &F) shows marked affection for the brain cells with signs of cell atrophy and surrounded vacuolation (blue arrow). The marjoram and olive extracts + high salt-fed group (G & H) shows almost normal cerebral cortex nerve cells (X 200: H & E section A, C, E & G; X 400: H & E section B, D, F & H).

Fig. 7. Histological sections of rat testis after 4 weeks of treatment, show a section in the testis of a control (A & B) rat with the standard structure of the seminiferous tubules with regular germinal epithelium arrangement. The marjoram and olive extracts-treated group (C & D) is more or less similar to the control group. The high salt-fed group (E &F) shows marked affection for the germinal epithelium through wide separation of the cells (black arrow) and diminished spermatids in the lumen (black arrowhead). The marjoram and olive extracts + high salt-fed group

(G & H) shows minimal affection for the renal tubules with almost normal seminiferous tubules (X 200: H & E section A, C, E & G; X 400: H & E section B, D, F & H).

The brain is also vulnerable to high salt exposure, with evidence of neuronal atrophy and vacuolation in brain cells due to hypernatremia-induced osmotic stress and inflammation [73,74]. Pro-inflammatory cytokines, such as IL-4, IL-6, and TNF-α, lead to neuronal damage and impaired hippocampal neurogenesis [75,76]. Oxidative stress impairs mitochondrial function and synaptic integrity, resulting in impairments in learning, memory, and executive tasks [77-81]. A high-salt diet also compromises the blood-brain barrier, allowing harmful substances to infiltrate the brain and exacerbate neurodegenerative processes [82-84]. Loss of structural integrity in brain cells can manifest deficits in learning, memory, and executive functions [85,86].

In the male reproductive system, high salt intake reduces testosterone levels and impairs sperm quality, including motility and morphology [21,87]. Oxidative stress in testicular tissue disrupts Leydig and Sertoli cell function, primary to reduced testosterone production and abnormal spermatogenesis [88-92]. Increased ROS production damages sperm DNA and plasma membranes, resulting in reduced fertility potential [93]. Sodium-induced osmotic stress disrupts the germinal epithelium, further impairing sperm development [94,95].

The administration of *O. majorana* and *O. europaea* extracts demonstrated significant protective effects against the detrimental impacts of high salt intake. These extracts, rich in flavonoids, polyphenols, and terpenoids, exhibited strong antioxidant and anti-inflammatory properties [96-98]. They reduced oxidative stress by neutralizing ROS and enhancing antioxidant enzyme activities, protecting tissues from LPO and structural damage [99-101]. Both extracts also restored metabolic and hormonal functions, improving nutrient absorption and utilization [102,103].

In the kidneys, these extracts reduced sodium-induced inflammation and fibrosis, preserving nephron integrity and enhancing waste elimination [104-110]. In the liver, they protected hepatocytes from LPO and oxidative stress, maintaining cellular architecture and metabolic functions [111-116]. Neuroprotective effects included reduced neuroinflammation, oxidative damage, and vacuolation, improving cognitive function and preserving neuronal integrity [117-121]. In the reproductive system, *O. majorana* restored testosterone levels and protected the germinal epithelium, while *O. europaea* improved sperm quality and reduced morphological abnormalities through its antioxidant properties [122-124].

Overall, *O. majorana* and *O. europaea* extracts offer promising therapeutic potential to counteract the negative impacts of chronic high salt intake on body weight, organ function, and oxidative stress. Additional studies is necessary to explain their molecular processes and assess their prolonged efficacy in mitigating salt-induced damage.

5. Conclusion

In conclusion, the investigation provides compelling evidence that high salt intake contributes to a range of physiological and biochemical alterations, including weight gain, hypertension, oxidative stress, organ damage, and impaired reproductive health. The study also highlights the potential of *O. majorana* and *O. europaea* extracts as therapeutic agents that can mitigate these harmful effects. By enhancing antioxidant defense mechanisms and reducing inflammation, these extracts offer a promising strategy to counteracting the harmful consequences of chronic salt consumption on various organs and systems. Additional research is required to clarify the basic molecular mechanisms and to confirm the extended-term efficacy of these extracts in preventing salt-induced damage.

Credit authorship contribution statement

Mohamed Gaber Shalan: conducted experimental design, all testing procedures and measurements, statistical analysis, and wrote the manuscript.

Ethics statements

The experimental procedures were evaluated and authorized by the Research Ethics Committee of the Faculty of Science at Arish University under approval number "ARU001". Our techniques adhere to the 8th edition guide for the care and use of laboratory animals, ensuring rigorous ethical standards.

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Data availability statement

The data will be made available upon request.

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work presented in this paper.

List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CAT	Catalase
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FSH	Follicle-stimulating hormone
HS	High salt
IL	Interleukin
LH	Luteinizing hormone
MDA	Malondialdehyde
LPO	Lipid peroxidation
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
No	Nitric oxide
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase

TNF-α Tumor necrosis factor-alpha

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