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# Effect of Marjoram Oil on the Clinicopathological, Cytogenetic and Histopathological Alterations Induced by Sodium Nitrite Toxicity in Rats

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**Abstract:** The present study aimed to investigate the potential effect of marjoram oil in alleviating hematological, serum biochemical, chromosomal and histopathological alterations induced by sodium nitrite toxicity in rats. For this purpose, forty adult male rats were divided into four groups (10 rats each);Group (1): received distilled water and served as a control, Group (2) was daily administered marjoram oil at a dose of 0.5 ml/kg b.wt.by stomach tube for 2 months, Group (3) was daily administered sodium nitrite at dose of 30 mg/ kg b.wt. by stomach tube for 2 months and Group (4) was concurrently administered sodium nitrite and marjoram oil with similar doses that mentioned before. The obtained results indicated that sodium nitrite possesses a deleterious effect on blood cytology, induce oxidative damage, hepato-renal dysfunction, chromosomal abnormalities and histopathological alterations. The administration of marjoram oil in conjugation with sodium nitrite minimized the hazard effects of sodium nitrite, it improved RBCs count, PCV, Hb concentration and WBCs count. It increased the level of serum total antioxidant capacity (TAC) and diminished the level of serum malondialdehyde (MDA). Moreover, it decreased the elevated activities of AST, ALT and ALP, cholesterol, BUN, creatinine and glucose concentration and elevated the values of total proteins and albumin in comparison to sodium nitrite group. In addition, marjoram oil ameliorated the genotoxic effect of sodium nitrite as it reduced the frequency of chromosomal aberrations and improved the histopathological changes in liver, kidney and spleen in comparison to sodium nitrite intoxicated group.

**Key words:** Marjoram Oil · Sodium Nitrite Toxicity · Chromosomal Aberrations · Hepatotoxicity · Histopathology

## INTRODUCTION

Sodium nitrite is an inorganic salt with wide spread applications in food industry as color fixative and preservative in meat and fish [1]. It is consumed in the manufacturing of azo dyes, nitroso compounds and other organic compounds [2]. Furthermore, sodium nitrite has many medical applications; it is used as a vasodilator, a bronchial dilator, an intestinal relaxant [3], post hemorrhagic cerebral vasospasm [4] and in myocardial infarction [5]. In veterinary medicine, it is used as an antiseptic by topical application to the teats of dairy cows after milking in order to prevent mastitis [2]. The toxic effects of nitrates and nitrites are well documented in mammalians including impairment of reproductive function [6], hepatotoxicity [7], dysregulation of inflammatory responses and tissue injury[8], growth retardation [9] and endocrine disturbance [10]. It inhibits a number of anti-tumor cytotoxic effector cell types as natural killer cells against pathogens and tumor cells [11]. Sodium nitrite exerts its effect by generation of free radicals that impair oxidant / antioxidant balance [12]. In such concern the role of antioxidants in reduction of sodium nitrites toxicity is well established [2].

Marjoram oil (Origanum majorana L.) is one of medicinal plants, which has been getting increasing attention lately. It is a member of the mint family, Lamiaceae. Traditionally, it is used as a folk remedy

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against cramps, depression, dizziness, gastrointestinal disorders, nervous headaches, paroxysmal coughs, asthma and rheumatism and as a diuretic [13, 14]. It has been shown to have antiviral, bactericidal, antiseptic and antifungal, antioxidant and antitumor [15].The essential oil obtained from marjoram has aromatic smell and contains high percentage of polyphenols and monoterpenes which are established as antioxidants [16]. The present study was designed to investigate the role of marjoram oil in modulating the hematological, biochemical, cytogenetic and histopathological changes induced by sodium nitrite toxicity in male albino rats.

## MATERIALS AND METHODS

**Animals:** Forty, male albino rats weighing about 100-120 g were used in the present study. They were obtained from the animal house, Faculty of Veterinary Medicine, Cairo University, fed on basal diet and watered *ad-libitum*. Rats were left for two weeks for acclimatization before starting the experiment.

**Chemicals:** Sodium nitrite (Sigma–Aldrich, St. Louis, MO) was applied as a freshly prepared solution and was given orally by stomach tube daily for 2 months at a dose of 30 mg/kg bodyweight as previously described by Isyaku and Joseph [17].

Marjoram oil (El-Captain Company) was applied as oil and given orally by stomach tube daily for 2 months at a dose of 0.5 ml/kg body weight [18].

**Experimental Design:** Rats were randomly divided into four main groups with ten rats each: Group (1): received distilled water and served as a control. Group (2) was daily administered marjoram oil at a dose of 0.5 ml/kg b.wt. by stomach tube for 2 months. Group (3) was daily administered sodium nitrite at dose of 30 mg/ kg b.wt. by stomach tube for 2 months. Group (4) was concurrently administered sodium nitrite and marjoram oil with similar doses that mentioned before. At the end of the experimental period, two blood samples were collected. The First one was anticoagulated by di-potassium salt of ethylene diaminetetra-acetic acid (EDTA) and used for evaluating hemogram. The second part was collected in a clean centrifuge tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non hemolysed supernatant serum was harvested for biochemical studies. Bone marrow samples from the both femur of each rat (5/group) were collected for cytogenetic analysis. Tissue specimens were collected from liver, kidney and spleen for histopathological examination.

**Hematological Studies:** Total erythrocyte and leukocyte counts were done using an improved Neubauer hemocytometer. Packed cell volume (PCV %) was estimated by microhematocrit technique. Hemoglobin concentration was colorimetrically determined using cyanmethemoglobin method. Differential leukocytic count was performed on Giemsa stained blood smears [19].

**Biochemical Studies:** Serum samples were prepared to assay the following biochemical studies;

Alanine (ALT) and aspartate (AST) amino transferases and alkaline phoasphatase (ALP) activities were performed according to the Reitman and Frankel [20] and Tietz [21], respectively. Serum total cholesterol was determined according to Allain *et al*. [22]. Serum total proteins were determined according to the biuret method after Weichselbaun [23], serum albumin after Dumas and Biggs [24] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. Blood glucose level was determined as described by Trinder [25]. Blood urea nitrogen was determined by an enzymatic method after Tabacco *et al*. [26] and serum creatinine was assayed using the method described by Fabiny and Eringhausen [27]. The previous biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA. Serum malondialdehyde (MDA) was determined as previously described by Draper and Hadley [28] and total antioxidant capacity (TAC) was determined according to Koracevic *et al*. [29] using bio-doiagnostic kits.

**Chromosomal Aberrations:** Chromosome preparation from bone marrow cells of rats was done according to Adler [30]. Rats were injected (i.p.) with colchicine

Table 1: Grading scheme for focal and multifocal hepatic lesions:

| Severity | Proportion of liver affected | Grade | Quantifiable finding |
|---|---|---|---|
| Marginal or minimal | Very small amount | 1 | 1-2 foci |
| Slight or few | Small amount | 2 | 3-6 foci |
| Moderate or several | Medium amount | 3 | 7-12 foci |
| Marked or many | Large amount | 4 | >12 foci |
| Severe | Very large amount | 5 | Diffuse |

(4mg/kg), 1.5 h before sacrifice. Both femurs were dissected out and bone marrow cells were collected from both femurs by flushing in normal saline. Cells were centrifuged at 600 rpm for 10 min and then hypotonic solution KCl (0.075M) were added for 25min. Cells were recentrifuged and fixed in acetomethanol (acetic acid: methanol, 1:3 v/v). Centrifugation and fixation were repeated five times at an interval of 20min. The cells were resuspended in a small volume of fixative, dropped onto chilled slides and allowed to dry. The following day, the slides were stained with freshly prepared 2% Giemsa stain for 3-5min. and washed in distilled water to remove excess stain. Fifty metaphases per each rat were screened for chromosomal abnormalities.

**Histopathological Examination and Lesion Scoring:** Tissue specimens were collected from liver, kidney and spleen preserved in 10% neutral buffered formalin, dehydrated in different grades of alcohol, cleared in xylene, embedding in paraffin, sectioned with microtome at 5µ thickness and finally stained with hematoxylin and eosin (H&E) according to Bancroft *et al*. [31]. Hepatic lesion scoring was performed according to Hardisty and Eustis [32] and Derelanko [33].

**Statistical Analysis:** The data were given as individual values and as mean ± standard deviation.

Comparisons between the means of various groups were analyzed using one way ANOVA as described by Snedecor and Cochran [34].

## RESULTS AND DISCUSSION

**Hematological Results:** Statistical analysis of hematological indices is illustrated in Table (2). Erythrogram mean values of different experimental groups, in comparison to those of control group revealed the presence of macrocytic hypochromic anemia in sodium nitrite administered group which determined by significant decreases in erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV%), mean corpuscular hemoglobin concentration (MCHC) and a significant increase in mean corpuscular volume (MCV) values. The developed anemia may be referred to the toxic effect of sodium nitrite on bone marrow, spleen and liver [35]. Sodium nitrite induces oxidative damage and free radical generation that stimulates oxidation of ferrous ions in oxyhemoglobin to form methemoglobin [36, 37] and erythrocyte lysis [38]. On the other hand, rats concurrently administered sodium nitrite with marjoram oils showed significant improvement in the previous parameters compared to sodium nitrite administered group. Marjoram oil contains essential oils that inhibit lipid peroxidation (LPO) in the membranes of erythrocytes that resulted in increasing membrane resistance to spontaneous hemolysis, decreasing membrane microviscosity, maintenance of their integrity and functional activity [39].

Data of leukogram revealed a significant decrease in the total leukocytic count of sodium nitrite administered group in comparable to control group. The recorded leukopenia was associated with lymphopenia which reflects the immunosuppressive effect of sodium nitrite [40, 41]. Our results are supported histopathologically where the white pulp of spleen showed focal necrosis and depletion of lymphoid element (Fig.5-a). Whereas, co-administration of marjoram oil with sodium nitrite improved the total leukocytic count which may be due to immunostimulatory activity of marjoram oil as a result of lymphoblastic activation and decreased the necrotic reaction of lymphoid elements [42].

**Serum Biochemical Evaluation:** Statistical analysis of different serum biochemical values are illustrated in Table (3).

Results of oxidative stress biomarkers revealed a remarkable increase in the values of serum malondialdehyde (MDA) with a significant decrease in the serum total antioxidant capacity (TAC) of sodium nitrite administered group compared to control group. The present perturbations in oxidative markers of sodium nitrite rats could be attributed to the oxidative cytotoxicity and detrimental effect of nitrite [43]. However, a significant reduction in the level of lipid peroxidation (MDA) with a significant increase in the total antioxidant capacity was observed in rats concomitantly administered sodium nitrite and marjoram oil compared to sodium nitrite administered group. The previous findings reflect the antioxidant effect of marjoram oil which is in accordance with Hossain *et al*. [44] and Babili *et al*. [45].

Regarding hepato-renal markers, statistical analysis of our data clarify the adverse effect of sodium nitrite on hepatic and renal functions. Sodium nitrite intoxicated rats (Group 3) exhibited a significant increase in the activities of serum AST, ALT and ALP enzymes in comparison to control rats (Group 1). The increased activities could be attributed to the toxic effect of nitroso-compounds, formed in the acidic environment of the stomach, causing severe hepatic necrosis [46]. The pervious results are supported histopathologicaly as liver of sodium nitrite

administered group showed sever hepatocellular necrosis and vacuolization with mononuclear cell infiltration (Fig.3-d).

Moreover, significant hypercholesterolemia was recorded in sodium nitrite administered group compared to control group. This elevation may be due to the mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids [47].

Means values of serum total proteins, albumin and A/G were significantly lowered in sodium nitrite administered group compared to control group. The resultant hypoproteinemia with hypoalbuminemia may be attributed to the effects of sodium nitrite on the liver either through inhibiting oxidative phosphorylation process and hence the availability of the energy source of protein synthesis [48] or through the necrotic changes especially of the plasma membrane [49].

Compared to control group, a significant increase in serum glucose level was observed in sodium nitrite intoxicated rats (group 3). This finding suggests nitrite-stimulation of gluconeogenesis and glucose shift from tissue to blood or an impairment of glucose mobilization [50]. Furthermore, nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia [51]. The obtained results go in parallel with those reported on the hyperglycemic effect of sodium nitrite in rats [52,53].

Sodium nitrite intoxicated group exhibited a remarkable increase in the values of blood urea nitrogen and serum creatinine in comparison to control group. These findings reflect nephrotoxic effect of sodium nitrite on the kidney [54]. Sodium nitrite may react with amines of the foods in the stomach and produce nitrosamines and free radicals which may increase lipid peroxidation leading to oxidative stress and can be harmful to different organs including kidney [55]. Our results are in agreement with those obtained by Eman and Fahmy [56] and correlated with the results of the histopathological findings of the kidney of sodium nitrite administered rats (Fig.4-d).

Marjoram oil administered rats (Group 2) showed no significant changes in the tested biochemical parameters in comparison to control group. On the other hand, co-administration of marjoram oil and sodium nitrite improved the previous serum biochemical values compared to the values of sodium nitrite administered rats (Group 3). There was a remarkable decrease in the activities of ALT, AST and ALP, cholesterol, glucose, BUN and creatinine values and a significant increase in the values of serum total proteins and albumin.

**Cytogenetic Analysis:** Results of chromosomal aberrations of different experimental groups are illustrated in Table (4). The observed chromosomal aberrations were categorized into structural and numerical aberrations. Structural aberrations were in the form of break, gap, deletion, fragment, centromeric attenuation and ring. Numerical aberrations were in the form of peridiploidy (hypoploidy and hyperpolidy) (Fig.1,2).

Cytogenetic analysis of different aberrant cells revealed no significant changes in the frequency of total chromosomal aberrations in the bone marrow cells of rats administered marjoram oil (7.0± 1.41) in comparable to control group (7.5 ± 0.58). However a remarkable increase in the frequency of total chromosomal aberrations was recorded in sodium nitrite intoxicated group (22.75 ± 3.96) compared to control group (7.5 ± 0.58). The production of chromosomal aberrations is a complex cellular process and chromosome changes observed at the metaphase are the end products of a chain of events initiated by molecular lesions in the DNA and they are considered as the appropriate of genotoxic potential of chemicals [57]. Therefore, our results reflect the mutagenic effect of sodium nitrite. It generates nitric oxide (free radicals) which cause DNA damage through inhibition of its synthesis and cell cycle arrest [58]. These findings are in accordance with Donia *et al*. [59] who recorded significant increases in the percentage of chromosomal aberrations in the bone marrow of male rats administered sodium nitrite at a dose of 46.66 mg/kg. On the other hand, a significant reduction in the previous aberrations was recorded in the group of rats concurrently administered sodium nitrite with marjoram oil (14.00±1.83) in comparable to sodium nitrite administered group (22.75 ± 3.96).The reduction in chromosomal aberrations may be attributed to the antioxidant effect of marjoram oil, scavenging of free radicals and suppression of oxidative DNA damage [60]. Marjoram oil contains terpenes, polyphenols, phenolic glycosides, phenolic derivatives, flavonoids, tannins, sitosterol and essential oil. The high potential of phenolic components to scavenger radicals might be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups [61].

**Histopathological Alterations:** Lesions score and histopathological alterations in different treated groups were summarized and illustrated in Table (5) and Figures (3,4,5).

**Liver:** Various histological alterations indicating hepatotoxicity were observed in sodium nitrites administered group, the most prominent alterations were

Table 2: Means values of Hematological indices of different experimental groups.

| Parameters | (Units) | Group 1 (Control) | Group 2 (Marjoram oil) | Group 3 (Sod. nitrite) | Group 4 (Sod. nitrite +Marjoram oil) |
|---|---|---|---|---|---|
| PCV | (%) | 39.75 ± 2.22[a] | 40.0 ± 1.83 [a] | 31.0 ± 2.16 [c] | 37.5 ± 1.29 [b] |
| Hb | (g/dL) | 13.7 ± 1.11 [a] | 14.25 ± 0.70 [a] | 8.48 ± 1.63 [c] | 12.5 ± 1.39 [b] |
| RBCs | ($\times10^6$ /µl) | 6.78 ± 0.45 [a] | 6.96 ± 0.75 [a] | 4.87 ± 0.44 [c] | 5.93 ± 0.38 [b] |
| MCV | (fl) | 58.6 ± 2.03 [b] | 57.8 ± 3.58 [b] | 64.00 ± 4.58 [a] | 63.4 ± 1.87 [ab] |
| MCHC | (%) | 34.5 ± 1.01 [a] | 35.6 ± 0.27 [a] | 27.36 ± 4.89[b] | 33.4 ± 2.84 [a] |
| TLC | ($\times10^3$/µl) | 9.05 ± 1.27 [a] | 9.39 ± 0.75 [a] | 7.13 ± 1.44[b] | 8.41 ± 1.41[ab] |
| Lymp. | ($\times10^3$/µl) | 5.28 ± 0.62 [a] | 5.47± 0.49 [a] | 2.96 ± 0.64 [c] | 4.13 ± 0.62 [b] |
| Neut. | ($\times10^3$/µl) | 3.31 ± 0.52 [a] | 3.53 ± 0.41 [a] | 3.73 ± 0.78 [a] | 3.82 ± 0.75 [a] |
| Mono. | ($\times10^3$/µl) | 0.23 ± 0.08 [a] | 0.21 ± 0.05 [a] | 0.25 ± 0.06 [a] | 0.25 ± 0.07 [a] |
| Esino. | ($\times10^3$/µl) | 0.23 ± 0.08 [a] | 0.25 ± 0.07 [a] | 0.19 ± 0.06 [a] | 0.21 ± 0.06 [a] |

Values represent means ± SD

Means with different superscripts (a, b and c) within the same row are significantly different at P <0.05.

Table 3: Means values of some serum biochemical parameters of different experimental groups.

| Parameters | Units | Group 1 (Control) | Group 2 (Marjoram oil) | Group 3 (Sod. nitrite) | Group 4 (Sod. nitrite +Marjoram oil) |
|---|---|---|---|---|---|
| TAC | mmol/L | 2.36 ± 0.30 [a] | 2.50 ± 0.42 [a] | 1.30 ± 0.25[c] | 1.84 ± 0.34 [b] |
| MDA | nmol/ml | 7.71 ± 0.47 [c] | 6.90 ± 0.50 [c] | 9.43 ± 0.74 [a] | 8.33 ± 0.29[b] |
| ALT | (IU/L) | 24.25±1.71[c] | 25.70 ± 2.27[bc] | 49.05 ± 2.66[a] | 29.30 ± 2.56[b] |
| AST | (IU/L) | 81.2 ± 2.29[c] | 78.5 ±3.42[c] | 104.85 ± 4.09[a] | 89.35 ± 2.93[b] |
| ALP | (IU/L) | 28.55 ± 3.55[c] | 27.45 ± 2.20[c] | 38.15 ± 1.73[a] | 33.7 ±2.53[b] |
| Choles. | (mg/dl) | 124.15 ± 3.28[c] | 121.50 ± 3.42[c] | 157.55 ± 3.59 [a] | 133.15 ± 2.60[b] |
| T. proteins | (g/dl) | 6.84 ± 0.32[ab] | 7.43 ± 0.64[a] | 5.65 ± 0.57 [c] | 6.54 ± 0.33[b] |
| Albumin | (g/dl) | 4.60 ± 0.37[a] | 4.65± 0.34[a] | 3.38 ± 0.35[b] | 4.15 ± 0.47 [a] |
| Globulins | (g/dl) | 2.27 ± 0.46 [a] | 2.78± 0.69 [a] | 2.24 ± 0.07 [a] | 2.39± 0.56 [a] |
| A/G Ratio | | 2.06 ± 0.19[a] | 1.77 ± 0.44 [a] | 1.48 ± 0.37[b] | 1.86 ± 0.71[ab] |
| Glucose | (mg/dl) | 102.60 ± 4.31[c] | 98.78 ± 1.85[c] | 200.80 ± 5.19[a] | 118.00 ± 4.32[b] |
| BUN | (mg/dl) | 23.54 ± 1.96[b] | 24.59 ± 1.80[b] | 35.75 ± 2.30[a] | 26.80 ± 2.38[b] |
| Creatinine | (mg/dl) | 1.15 ± 0.20[b] | 1.22 ± 0.19 [b] | 1.65 ± 0.17[a] | 1.35 ± 0.15[b] |

Values represent means ± SD

Means with different superscripts (a, b and c) within the same row are significantly different at P <0.05.

Table 4: Means values of the frequency of chromosomal aberrations of different experimental groups.

| Parameters | Group 1 (Control) | Group 2 (Marjoram oil) | Group 3(Sod. nitrite) | Group 4 (Sod. nitrite +Marjoram oil) |
|---|---|---|---|---|
| Frequency of metaphase cells with chromosomal aberrations | | | | |
| Structural aberrations | | | | |
| Break | 0.75 ± 0.50[b] | 0.50 ± 0.58[b] | 2.75 ± 0.96[a] | 1.25 ± 0.96[b] |
| Gap | 0.50 ± 0.58[c] | 0.50 ± 0.58[c] | 2.25 ± 0.50[a] | 1.50 ± 0.58[b] |
| Deletion | 1.00 ± 0.82[c] | 1.50 ± 0.58[c] | 5.25 ± 1.71[a] | 3.00 ± 0.82[b] |
| Fragment | 1.25 ± 0.50[b] | 0.50 ± 0.58[c] | 2.50 ± 0.58[a] | 1.50 ± 0.50[b] |
| CA | 1.50 ± 0.58[b] | 1.25 ± 0.50[b] | 3.75 ± 0.96[a] | 2.00 ± 0.82[b] |
| Ring | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] | 1.00 ± 0.00[a] | 0.25 ± 0.50[b] |
| TSA | 5.25 ± 1.41[c] | 4.25 ± 0.96 [c] | 17.00 ± 3.74 [a] | 10.00 ± 0.20[b] |
| Numerical aberrations | | | | |
| Hypoploidy | 2.00 ± 0.82 [b] | 1.75 ± 0.96 [b] | 4.00 ± 0.82[a] | 2.25 ± 0.96 [b] |
| Hyperploidy | 0.75 ± 0.50 [a] | 1.00 ± 0.82 [a] | 1.75 ± 0.50 [a] | 1.75 ± 0.96 [a] |
| Peridiploidy | 2.75 ± 0.50 [b] | 2.75 ± 1.71 [b] | 5.75 ± 0.50 [a] | 4.00 ± 1.41[ab] |
| TCA | 7.50 ± 0.58[c] | 7.00 ± 1.41[c] | 22.75± 3.69[a] | 14.00 ±1.83 [b] |

CA: Centromeric attenuation, TSA: Total structural aberrations, TCA: Total chromosomal aberrations

Means with different superscripts (a, b and c) within the same row are significantly different at P<0.05.

Table 5: Histopatological lesion score in different experimental groups:

| Lesions | Group 3 (Sod. nitrite) | Group 4 (Sod. nitr. +Marjoram oil) |
|---|---|---|
| | Liver | |
| Binucleated hepatocytes | 3 | 1 |
| sinusoidal dilatation and congestion | 2 | 1 |
| Hepatocellular necrosis | 3 | 1 |
| Hepatocellular vacuolation | 3 | 2 |
| Mononuclear cell aggregation | 2 | 1 |
| | Kidney | |
| Tubular degeneration | 3 | 1 |
| Tubular necrosis | 2 | 1 |
| Spleen | | |
| Lymphoid depletion | 2 | 1 |
| Lymphoblastic activation | 0 | 1 |

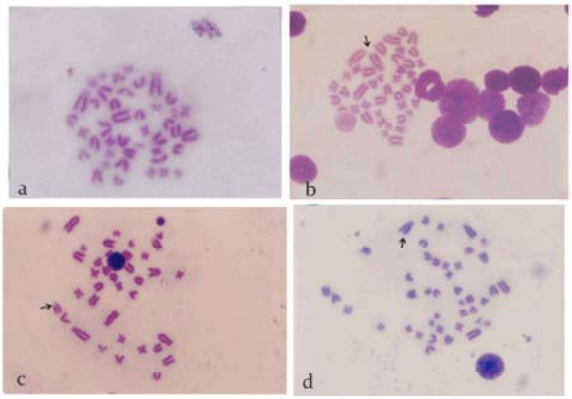


Fig. 1: a: Normal spread metaphase of rat bone marrow cells 2n=42. b: Metaphase spread of sodium nitrite administered rat showing break. c: Metaphase spread of sodium nitrite administered rat showing gap d: Metaphase spread of sodium nitrite administered rat showing deletion.

increased number of binucleated hepatocytes associated with hepatocytomegally, karyomegally and anisokaryosis (Fig.3-a). These hepatocellular alterations may be attributed to toxic injury of sodium nitrite on hepatocytes as previous studies demonstrated that the increased occurrence of binucleated hepatocytes hypothesized as an index of the severity of hepatic illness and that binuclearity may be interpreted as a cell state that is more capable of responding to a major demand for protein synthesis [62]and the binucleated hepatocytes may be a reactive cell response to liver injury [63] while hepatocytomegally was considered as spontaneous lesion in aging rat and was also observed in hepatocytes after treatment of mice liver with phenobarbitone [64, 65].

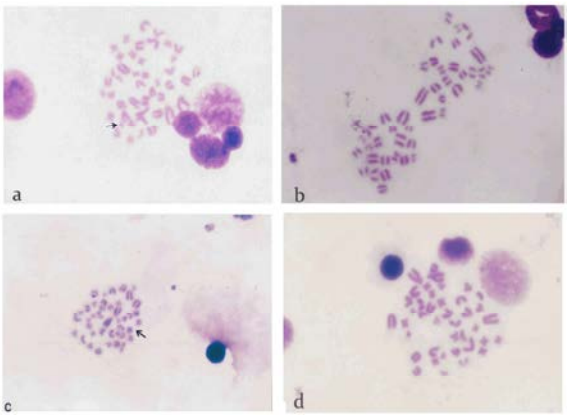


Fig. 2: a: Metaphase spread of sodium nitrite administered rat showing fragment. b: Metaphase spread of sodium nitrite administered rat showing centromeric attenuation. c: Metaphase spread of sodium nitrite administered rat showing ring. d: Metaphase spread of sodium nitrite administered rat showing hypoploidy

The centrilobular area showing marked sinusoidal dilatation with sporadic cell necrosis and kupffer cell activation (Fig.3-b);the portal area showing marked bile duct hyperplasia, increased number of bile ductules, oval cell hyperplasia, portal congestion and mononuclear cells infiltration (Fig.3-c).The lesions were associated with periportal hepatocellular vacuolation and necrosis (Fig.3-d). Another study hypothesized the sodium nitrite inducing vasodilation via nitricoxide (NO) activation of the cyclic guanosine monophosphate (cGMP) signaling pathway in vascular smooth muscle cells [66]. Periportal hepatocellular vacuolation and necrosis were observed (Fig.3-d). Similar findings were described by Zeenat and Phil [67]. On contrast; these histopathological alterations were alleviated in rats concurrently administered sodium nitrite with marjoram oil where liver sections of this group showed relative decrease in the number of binucleated hepatocytes (Fig.3-e),focal mononuclear aggregation (Fig.3-f), hyperplasia of bile ducts with sporadic hepatocellular necrosis (Fig.4-a). Mild oval cell proliferation in the portal area and dilatation of sinusoids were observed in individual hepatic lobules within centrilobular area (Fig.4-b). No significant histological hepatic alterations have been detected in marjoram oil administered group.

**Kidneys:** The microscopic examination revealed massive necrobiotic changes of renal tubules that ranged from vacuolization and swelling to necrosis of epithelial lining

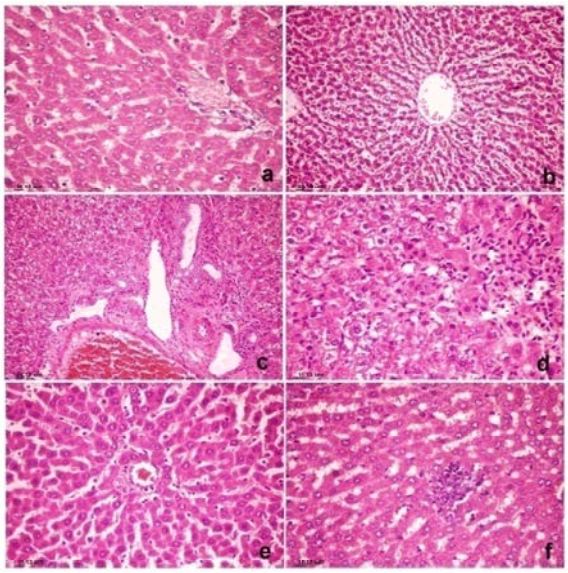


Fig. 3: a) Liver of sodium nitrite administered group showing increased number of binucleated hepatocytes with hepatocytomegally. b) Liver of sodium nitrite administered group showing marked sinusoidal dilatation associated with kupffer cell activation. c) Liver of sodium nitrite administered group showing bile duct hyperplasia with newly formed ductules, oval cell hyperplasia and portal mononuclear cell infiltration. d) Liver of sodium nitrite administered group showing sever hepatocellular necrosis and vacuolization with mononuclear cell infiltration. e) Liver of sodium nitrite+ marjoram administered group showing decreased binucleated hepatocytes numbers with apparently normal hepatocytes. f) Liver of sodium nitrite+marjoram administered group showing focal mononuclear cell aggregation.

the proximal convoluted tubules and pars rectae (Fig.4-c) with formation of hyaline droplets of the epithelial lining the distal convoluted tubules (Fig.4-d) associated with accumulation of proteinaceous eosinophilc cast in collecting tubular lumina (Fig.4-e). All renal alterations are attributed to sodium nitrite inducing hypoxia with the subsequent free radical formation that inducing tissue changes including renal tissue [55, 68]. On the other hand, the renal lesions were few and restricted to vacuolization of renal tubular epithelium in rats co-administered sodium nitrite with marjoram oil (Fig.4-f) with no difference in the lesion distribution in cortex and medulla. No histological renal changes could be detected in marjoram oil administered group.

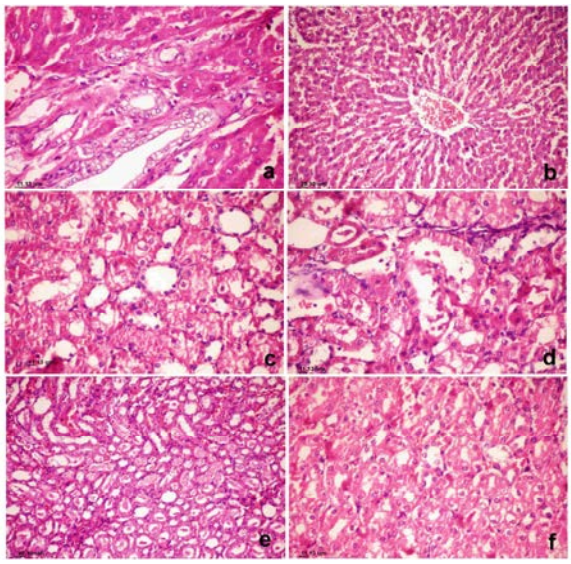


Fig. 4: a) Liver of sodium nitrite+marjoram administered group showing bile duct hyperplasia with presence of newly formed ductules associated with periportal individual hepatocellualr necrosis. b) Liver of sodium nitrite+marjoram administered group showing mild sinusoidal dilatation. c) Kidney of sodium nitrite administered group showing vacuolation, swelling and necrosis of renal tubular epithelium. d) Kidney of sodium nitrite administered group showing necrosis with presence of hyaline droplets in the lumen of renal tubules. e) Kidney of sodium nitrite administered group showing massive accumulation of protein casts in the lumina of collecting tubules. f) Kidney of sodium nitrite +marjoram administered group showing mild vacuolar degeneration of renal tubular epithelium.

**Spleen:** Concerning spleen, the white pulp of sodium nitrite administered rats showed focal necrosis and depletion of lymphoid element (Fig.5-a) comprising the lymphoid follicles that showing atrophy while the red pulp showed extra medullary hematopoiesis represented by megakaryocytosis (Fig.5-b) and proliferation of erythroid element in splenic sinuses (Fig.5-c). While spleen of rats concurrently administered sodium nitrite with marjoram oil showed necrosis and depletion of individual lymphoid elements (Fig.5-d) but with slight atrophy of the lymphoid follicle size and mild lymphoblastic activation within lymphoid follicle and relative decrease in the number of megakaryoctes in the red pulp (Fig.5-e). No splenic histological alterations have been observed in the marjoram oil administered group.

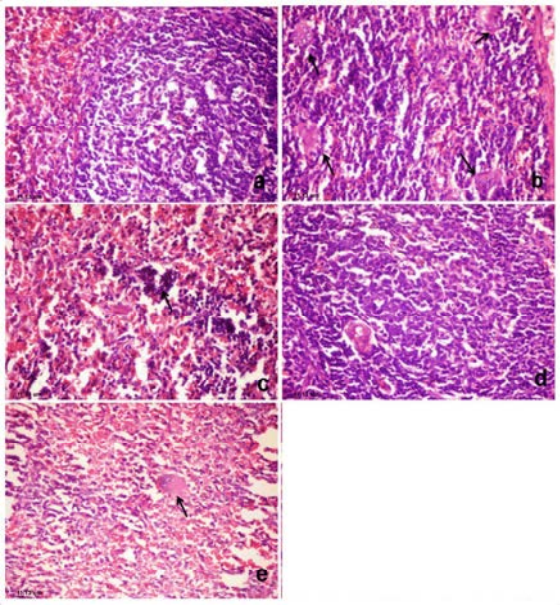


Fig. 5: a) Spleen of sodium nitrite administered group showing lymphoid depletion with necrosis of lymphoid elements. b) Spleen of sodium nitrite administered group showing megakarycytosis (arrow). c) Spleen of sodium administered group showing aggregation of erythroid elements in splenic red pulp (arrow). d) Spleen of sodium nitrite+marjoram administered group showing necrosis of lymphoid elements associated with mild lymphoblastic activation. e) Spleen of sodium nitrite+marjoram administered group showing decreased number of megakaryocytes in splenic red pulp (arrow).

From the achieved results, it could be concluded that the marjoram oil possesses a conspicuous modulating effects against the adverse effect of sodium nitrite. It improved its hematological, biochemical, cytological and histopathological perturbations.

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