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Adverse Effect of Mixture of Food Additives on Some Biochemical Parameters in Male Albino Rats

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Abstract

The present study investigated the unsafe impacts of sodium nitrite, sodium benzoate and their mixture which is utilized in fabricating of the food on some biochemical parameters in male albino rats. Rats (40) were divided into four groups as follows: group 1 used as control, group II and III were treated orally with sodium nitrite $NaNO_2$ (80 mg/kg BW) and sodium benzoate (SB) (200 mg/kg BW), respectively. Group IV was treated orally with the mixture of sodium nitrite and sodium benzoate. Rats took their respective doses every day for 8 weeks. The obtained results showed that sodium nitrite, sodium benzoate and their mixture ($NaNO_2$ and SB) initiated diminish within in the activity of antioxidant enzymes (SOD, CAT and GSH) within kidney, while, MDA recorded a high significant activity level within the experimental groups. Urea and creatinine mean levels were expanded within plasma of the experimental rats. In the kidney sections edema appeared with mononuclear leukocyte cellular infiltration, shrinkage of glomeruli. The severity of these changes increased in the experimental group which treated by the mixture of sodium nitrite and sodium benzoate. Overexpression in p53 occurred in experimental groups which were treated by $NaNO_2$, SB and their mixture. The present study concluded that the mixture of food additives can induce toxicity in the kidney of rats. It is obvious that food additives induced nephrotoxicity within the kidney. It decreased the antioxidant enzymes (GSH, CAT and SOD) and increased MDA. Increase tumor suppressor gene p53 in kidney tissue. Food added substances caused changes in biochemical parameters as in creatinine and urea. The utilization of food additives must be decreased. The presence of more than one type of food additives on our food and the usage of the mixture of sodium nitrite and sodium benzoate initiated changes in biochemical parameters and immune-histopathological changes.

Keywords: Nephrotoxicity-P53- Oxidative Stress- Food Additives- Male Rats.

Introduction

Added substances and additives are utilized to keep the quality of the nourishment for a long period as well as to avoid the overabundance of the water within food [1]. Additives are antimicrobial agents [2], specialists such as salt, vinegar. They are utilized within the products such as greens dressings. Antioxidants including vitamin C are used in the foods containing high fats. Citric acid is used to prevent the flavor changes [3]. Flavor enhancers, such as monosodium glutamate (MSG) escalating the flavor of other compounds within the food [4]. Nitrite salts can respond with certain amines within the food to produce nitrosamines many of which are known carcinogens [5]. Food additives play a vital part in guaranteeing that the food supply remains the most secure. A major task of Food and Drug Administration "FDA" is to regulate the utilize of approved food additives. There's exceptionally small logical prove that the food additives are harmful at the levels at which they are utilized [6]. There were many studies were inspected in animal to assure that a number of food additives regularly used by the food industry which can have an antagonistic impact on the health [7]. Nitrate and nitrite have been utilized as nourishment added substances in numerous sorts of meat products [8, 9].

Nitrosamines can be shaped when dietary nitrites respond with secondary amines. This response is favored under acidic conditions and with heat. Cancer prevention agents such as erythorbate repress nitrosamine arrangement [10]. A few epidemiological thinks about have recommended a connect between dietary nitrates/nitrites, and the rate of cancer [11, 12]. The decay of food is due to the activity of enzymes upon food [13]. The food cannot stand up the activity of enzymes which may lead to food deterioration [14, 15]. Depending upon chemical additives these microorganism action and impede microorganism multiplication [6]. Abdelaziz et al. [16] expressed the added substances to the food are food additives to fix its taste. There has been an increment in public interest in this topic and there are signs that commercial interface have been impacted by consumer pressure controlling the circumstance by showcasing strategies [17]. Oxidation of a few portion of the included nitrite by proteins within the introductory step in curing may be dependable [18]. Nitrate and nitrite metabolism inside the body is essential for assessing conceivable dangers. The reduction of nitrite is promoted by lowering the pH in prepare that's improved by polyphenols and other specialists cancer prevention agents [19]. Russell and Roussel [20] illustrated that levels of blood of a few constituents may reflect the status of the animal physiology. Gladwin et al. [21] reported that for the preservation of fish, nitrite and nitrate had been used. During preparation of the food, nitrate is reduced to nitrite which is the major active ingredient in these salt mixtures. Hsu et al. [22] found that when nitrite is added to the food it interferes with the flavor.

Lipid oxidation depends on chemical composition of meat. The oxidation of the lipid can be decreased or repressed by utilizing of antioxidants in meat and thus the product quality and shelf-life can be progressed [23]. The additives which are allowed within food are considered to be without potential unfavorable impacts. The unfavorable impacts of nitrite have been detailed counting the hindrance of intestinal retention in rats [24]. Responsive oxygen species "ROS" lead to oxidative harm which is produced persistently by the mitochondrial respiratory chain. Sorbic acid and benzoic acids are known to function as both prooxidants [25, 26]. Schwimmer et al. [27] recommended that SB may moreover affect the cells with oxidative stress. The treatment of lymphocytes with SB comes about in critical changes in the membrane ultrastructure. Mamur et al. [28] concentered on the poisonous impacts of sodium benzoate in mammalian cell systems. It is reported that utilization of certain blends of artificial food colors and SB preservative are related with increments in the hyperactivity in the children. Hazan et al. [29] and Mccann et al. [30] stated that hyperactivity in children is initiated by using the artificial colors in their diet. Sodium benzoate and potassium sorbate were utilized in cosmetics and they are used in the food of the children. Food and Agriculture Organization (FAO) and World Health Organization (WHO) assessed and set up the satisfactory acceptable daily intake (ADI) expressed in milligrams per kilogram of body weight per day (mg/kg, body weight/day). Benzoic acid and its calcium, potassium and sodium salts ADI were 0-5 mg/kg body weight [31].

Materials and Methods

The chemicals were purchased from El-Jomhoreia Chemical Company, Alexandria, Egypt (Oxford laboratory, Mumbai, India). Sodium benzoate is the sodium salt of benzoic acid and Sodium nitrite is the inorganic compound. 40 Male albino rats weighing 160–180g [32] were used throughout the experimental period. They were brought from the animal care unit, Faculty of medicine, Alexandria University, Egypt. Animals acclimated to the laboratory environment for at least one week under standard housing conditions prior to study initiation. Rats housed in a stainless steel cages and they were maintained under controlled conditions in a room ventilated with fresh and filtered air. The animal provided water ad libitum. Rats were randomly distributed into four groups; each group contains 10 animals. The room temperature of 22 ± 3 ºC, and 12-h light/dark cycle, rats were fed standard laboratory pellets.

The doses of sodium nitrite ($NaNo_2$) and sodium benzoate (SB) were chosen according to Kohn et al. [33] and Oyewole et al. [34]; respectively and dissolved in distilled water. At the end of the experimental period animals were anaesthetized with ether and sacrificed and kidneys were immediately removed, washed using saline solution (0.9%), then transferred to 70% alcohol. The collected blood samples were put in test tubes containing heparin as an anticoagulant and placed immediately on ice. The gathered blood was centrifuged

for 20 min for separation of plasma. The plasma was kept at -80ºc. The organs were minced and tissue perfuse with PBS (phosphate buffered saline) solution, pH 7.4. Homogenize the tissue in 5-10 ml cold buffer (50 mm potassium phosphate, pH 7.5) per gram tissue. Centrifuge at 3000 g for 15 minutes. Remove the supernatant for assay and store on ice. Determination of mean level of urea was done by enzymatic colorimetric method according to the method of Fawcett and Scott [35]. Determination of the mean level of creatinine was done by kinetic method according to the method of Fabiny et al. [36]. Determination of the mean activity level of malondialdehyde (MDA) was done by colorimetric method according to the method of Tappel and Zalkin [37]. Determination of the mean activity level of glutathione (GSH) by colorimetric method was done according to Jollow et al. [38]. Determination of the mean activity level of catalase (CAT) was done by using the method of Luck [39]. Determination of the mean activity level of superoxide dismutase (SOD) was done by using the method of Mishra and Fridovich [40].

Histological studies:

Kidneys were chosen in the present study for the histological examination was carried out according to Drury et al. [41]. Sections were examined for hiso-pathological changes. The immunohistochemical study for p53 was carried out on the kidney of rats tissues. Five µm thick paraffin sections were cut, mounted onto positively charged slides [42]. One-way ANOVA was used, SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with ($p<0.05$) considered to be expressed statistically significant. The results were expressed as mean value ± standard error.

Results and Discussion

The effect of sodium nitrite ($NaNO_2$), sodium benzoate (SB) and their mixture on the plasma protein, urea and creatinine are illustrated in table (1). Table (1) shows the mean values of total protein, urea and creatinine of the treated groups II, III and IV. The data recorded a significant decrease ($p<0.05$) in the protein concentrations in treated groups II, III and IV compared to control group of rats. The mean values of the concentrations of urea and creatinine recorded a significant increase ($p<0.05$) in the treated group II, III and IV compared to group one. In group IV, there was a significant increase in urea concentration than the treated groups II and III.

Table (1): Mean values ± S.E. of plasma total protein, urea and creatinine of male rats treated orally with sodium nitrite ($NaNO_2$), sodium benzoate (SB) and their mixture for 8 weeks.

Parameters	Control group I	$NaNO_2$ group II	SB, group III	$NaNO_2$+SB group IV
Total protein (g/dl)	11.5 ± 1.11 ^{b}	8.35 ± 0.48 ^{a}	7.1 ± 0.45 ^{a}	8.47 ± 0.45 ^{a}
Urea (mg/dl)	22 ± 0.8 ^{a}	43.2 ± 1.25 ^{b}	47.7 ± 2.27 ^{b}	74.25 ± 2.21 ^{c}
Creatinine (mg/dl)	0.18 ± 0.005 ^{a}	0.6 ± 0.008 ^{b}	0.67 ± 0.009 ^{b}	0.7 ± 0.008 ^{b}

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p< 0.05$.

In the current study, the treatment of male albino rats by $NaNO_2$, SB and their mixture were represented in table (2), the concentrations of MDA were increased significantly ($p<0.05$) in the treated groups II, III and IV. While, the mean values of the activities of GSH, CAT and SOD were showed a significant decrease ($p<0.05$) compared to the control rats. The mean glutathione activity level in the treated group IV which was treated by the mixture of $NaNO_2$ and SB reported a significant decrease ($p<0.05$) when compared to the treated groups II and III which were treated by $NaNO_2$ and SB alone, respectively.

Table (2): Mean values ± S.E. of kidney oxidative stress markers malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) of male rats treated orally with sodium nitrite ($NaNO_2$), sodium benzoate (SB) and their mixture for 8 weeks.

Oxidative stress Markers	Control group I	$NaNO_2$ group II	SB, group III	$NaNO_2$+SB,group IV
MDA (nmol/g.tissue)	4.4 ± 0.42 ^{a}	9.2 ± 0.28 $^{b'c}$	8.2 ± 0.28 ^{b}	12 ± 0.41 ^{c}
GSH (mg/g.tissue)	43 ± 1.4 ^{c}	21.5 ± 0.7 ^{b}	23.5 ± 0.6 ^{b}	12 ± 1.2 ^{a}
CAT (U/g.tissue)	911 ± 4.2 ^{d}	882 ± 5.1 ^{c}	588 ± 2.4 ^{b}	186 ± 4.5 ^{a}
SOD (U/g.tissue)	53.2 ± 2.4 ^{d}	32 ± 1.7 ^{c}	23.4 ± 2.8 ^{b}	9.5 ± 2.1 ^{a}

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p< 0.05$. MDA=malondialdehyde, GSH=gultathione, CAT= catalase, SOD=superoxide dismutase.

Histopathological observations of the kidney

Fig. 1 (A & B) showed the control group I and observed that normal cortical architecture with normal glomerulus, proximal convoluted tubules and distal convoluted tubules. Medulla region shows collecting tubules and loop of Henle represented by descending limb and ascending limb. The histopathological observations of nano2-treated group II showed peri-glomerular edema with few mononuclear leukocytic cellular infiltration and inter-tubular mononuclear leukocytic cellular infiltration, note shrinkage of glomeruli and degeneration of the lining epithelium of renal tubules in association with pyknotic nucleus of renal tubules epithelium. Medulla region showing necrosis of the lining epithelium of renal tubules with the presence of eosinophilic hyaline casts in the lumen of some tubules (Fig. 2, A & B). Fig. 3 (A & B) showed cellular vacuolation, shrunken in glomeruli tuft with capsular space and cellular infiltration. Medulla region illustrates necrosis of the lining epithelium of renal tubules with the presence of eosinophilic hyaline casts in the lumen of some tubule in SB-treated group III. Fig. 4 (A & B) of nano2 and SB mixture-treated group IV illustrated deformation in normal architecture of kidney tissues, shrinkage of glomeruli.

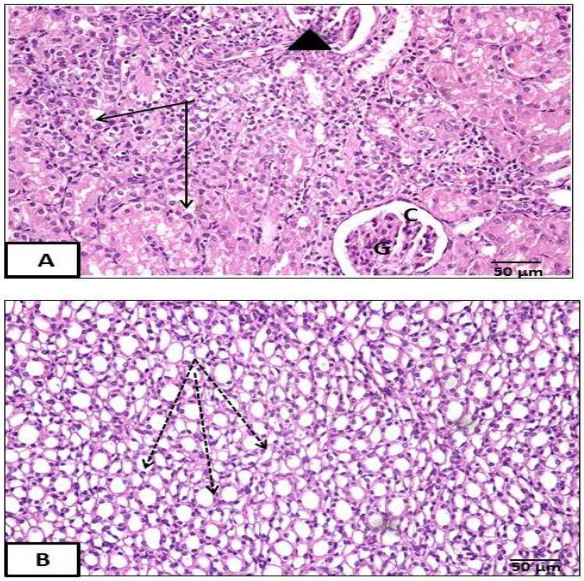

Fig (1): Photomicrograph of kidney sections of control group I (A) Cortex region showing normal cortical architecture with normal glomerulus (G), proximal convoluted tubules (black arrow) and distal convoluted tubules (head arrow).(B) Medulla region showing collecting tubules (C) and loop of henle represented by descending limb (arrow) and ascending limb (head arrow) (H. & E., X: 400).

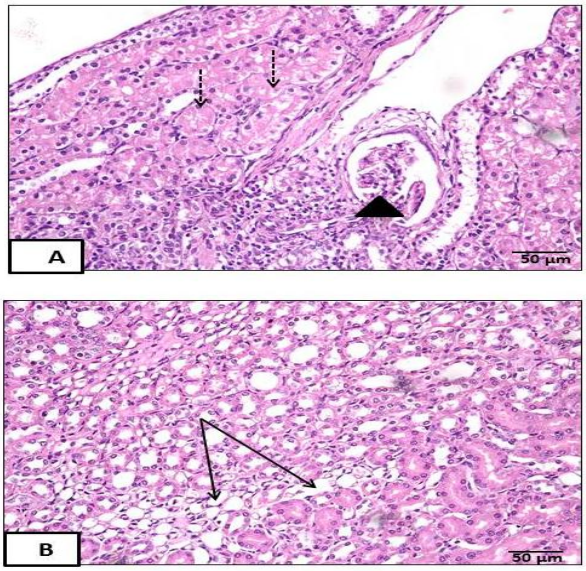

Fig. (2): Photomicrograph of kidney sections of group II treated by $NaNO_2$ (A) Cortex region showing periglomerular edema with few mononuclear leukocytic cellular infiltration and inter-tubular mononuclear leukocytic cellular infiltration (head arrow), note shrinkage of glomeruli and degeneration of the lining epithelium of renal tubules in association with pyknotic nucleus of renal tubules epithelium (dotted arrow). (B) Medulla region showing necrosis of the lining epithelium of renal tubules with the presence of eosinophilic hyaline casts in the lumen of some tubules (arrow) (H. & E., X: 400).

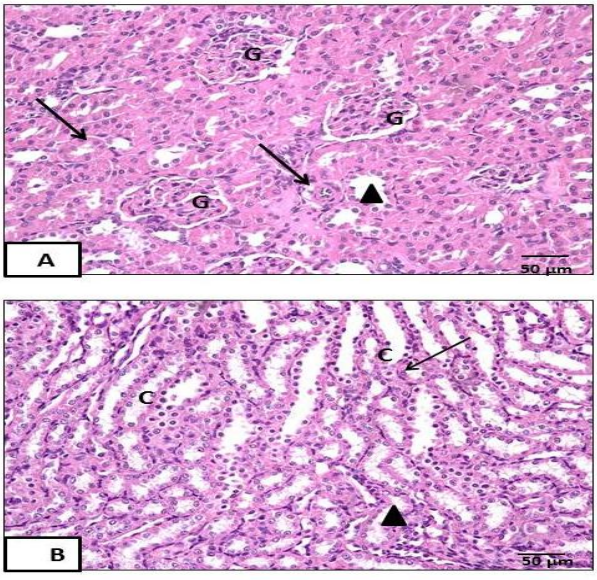

Fig (3) :Photomicrograph of kidney sections of group III treated by sodium benzoate (SB) (A) Cortex region showing cellular vacuolation (arrow), shrunken in glomeruli tuft (G) with space (C) and cellular infiltration (head arrow). (B) Medulla region illustrates necrosis of the lining epithelium of renal tubules with the presence of eosinophilic hyaline casts in the lumen of some tubules (dotted arrow) (H. & E., X: 400).

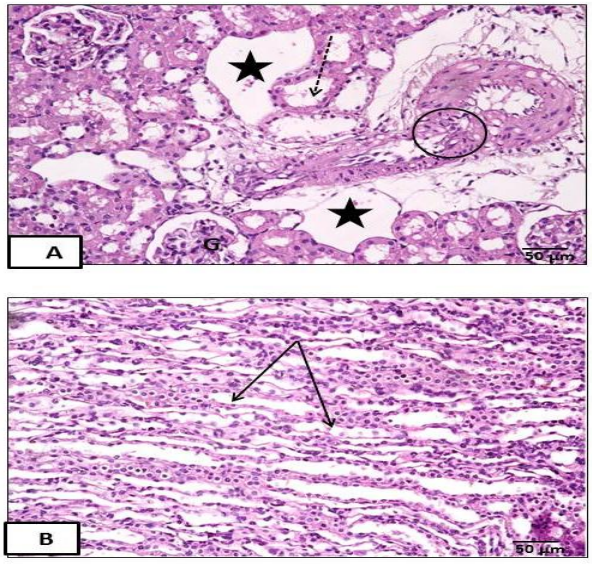

Fig (4): Photomicrograph of kidney sections group IV treated by mixture of $NaNO_2$ and SB (A) Cortex region showing deformation in normal architecture of kidney tissues (circle), shrinkage of glomeruli (G), cellular vacuolation (star) and degeneration of the lining epithelium of renal tubules in association with pyknotic nucleus of renal tubules (dotted arrow). (B) Medulla region showing degeneration of the lining epithelium of some proximal and distal convoluted tubules (H. & E., X: 400).

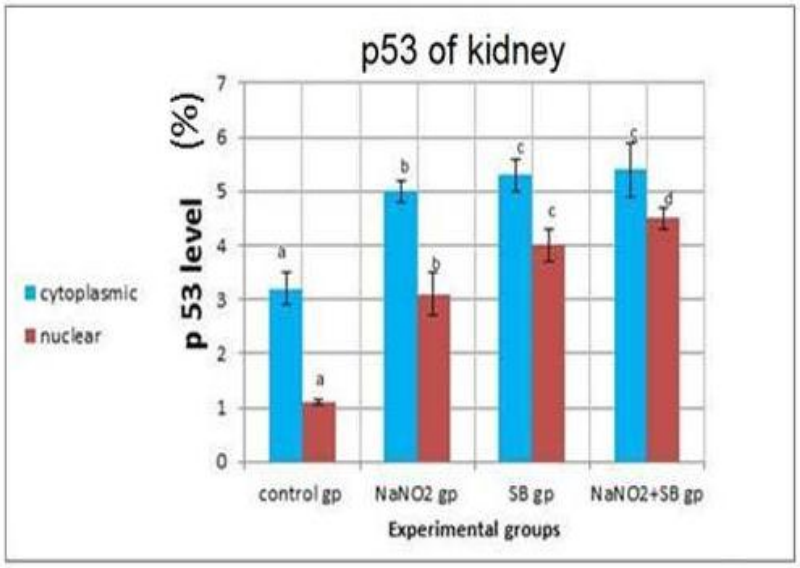

Fig.5 : Tumor suppressor gene (p53) of the kidney of the four experimented groups.

The mean values of cytoplasmic expression of p53 in kidney of male rats groups II, III and IV were showed significant increase ($p<0.05$) compared to control group. In nuclear expression of p53 in kidney, the mean values of treated groups II, III and IV, reported a significant increase compared to group I ($p<0.05$). In the control group, there was a weak stain of p53 (fig. 5) on the kidney tissues. While, in the treated groups II and III which treated by $NaNO_2$ and Sodium benzoate; respectively illustrating strong stain of p53.The treatment of male rats by the combination of $NaNO_2$ and SB showing over expression of p53 by photomicrograph examination (fig. 5).

Table (3): Mean values ± S.E. of tumor suppressor gene (p53) in the kidney of male rats treated orally with sodium nitrite ($NaNO_2$), sodium benzoate (SB) and their mixture for 8 weeks

Parameters	Control group I	$NaNO_2$group II	SB group III	$NaNO_2$+SB group IV
Kidney,p53 cytoplasmic (%)	3.2 ± 0.3^{a}	5 ± 0.2^{b}	5.3 ± 0.3^{c}	5.4 ± 0.5^{c}
Kidney, p53 Nuclear (%)	1.1 ± 0.05^{a}	3.1 ± 0.4^{b}	4 ± 0.3^{c}	4.5 ± 0.2^{d}

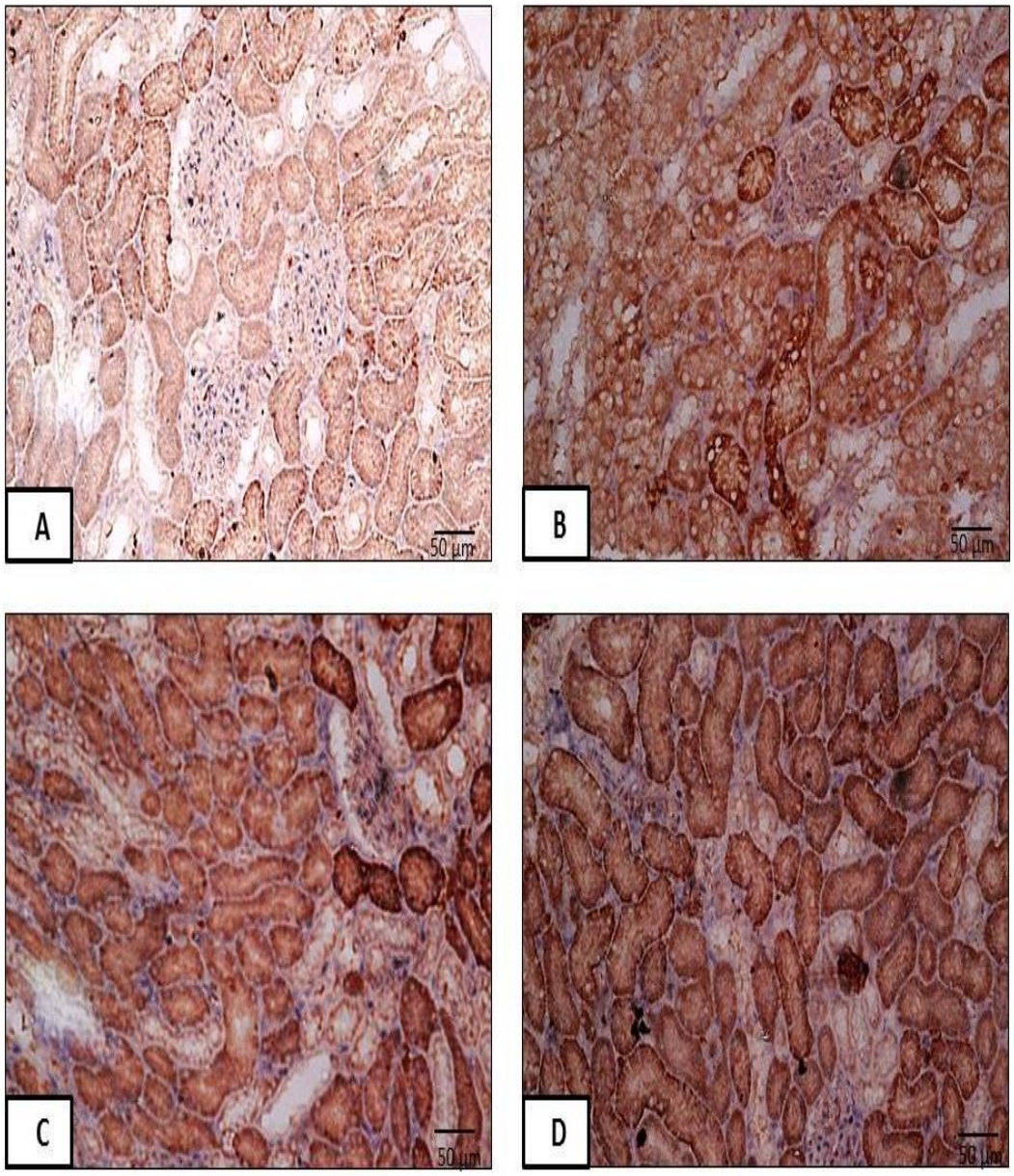

Fig (6): Photomicrograph of kidney sections (A) control group I showing weakly stain of tumor suppressor gene p53, (B) group II treated by nano2 and (C) group III treated by SB showing strong stain of p53 (D) group IV treated by the mixture of sodium nitrite and sodium benzoate illustrating over expression of tumor suppressor gene (p53).

Numerous of food additives have been progressively known as possibly perilous components for human health [43]. Reactive nitrogen species are delivered by presentation to nitrite cause carcinogenesis [44]. The oxidative stress pointer is lipid peroxidation which can be credited to the oxidative cytotoxicity of nitrite [45, 46]. Laimer et al. [47] examined the side effect of food added substances as $NaNO_2$ on the white albino rats and observed that there is an increase in the body weight of treated group. Mozes et al. [48] discussed that food additives may induce an increase in energy intake. The added nano2 has exceptionally powerless carcinogenic potential, especially within the squamous epithelium of the forestomach. Additionally, in combination with other chemicals, nano2 has also been appeared to make carcinogens [49].The biochemical investigation might offer assistance to distinguish the target organs of the harmfulness and the common wellbeing status. It gives an early caution flag in pushed life form [50]. The

food additives and preservatives can cause allergies [51]. The foods containing additives can cause asthma and rashes. Benzoates can cause asthma and brain damage [30].

The outcomes of the current consideration is in agreement with Hassan et al. [52] who recorded that there was an increment within the levels of creatinine and urea in the plasma of the experimental animal in response to $NaNO_2$ treatment as food added substances. This rise was agreeing to suggest an impedance of kidney functions. These impacts seem moreover be ascribed to the cytotoxic effect of N-nitroso-compounds in renal tubular cells [53, 54]. Impacts of food additives may be harmful to the immune response [55]. Abdelaziz et al. [61] examined the impact of food added substances on the adult albino rats and found that there was a diminish within the total protein concentration. Kalantari and Salehi [49] illustrated that there was an inhibitory impact of $NaNO_2$ on the biosynthesis of protein. These data suggest an incitement of the thyroid and the adrenal glands by $NaNo_2$ which can lead to the protein amalgamation and diminished protein turnover. People are altogether uncovered to nourishment added substances as a constituent of numerous nourishments and drinks expended each day. Lau et al. [17] watched that there were diminishments in total protein may be due to the expanded peroxidative degradation of the structurally important myelin phospholipids in the brain. This diminishment may be credited to the inhibition of oxidative phosphorylation process [44]. Blood serum protein is a fairly labile biochemical system reflecting the condition of the organism [56]. Lower dosages of sodium nitrite have caused intense methemoglobinemia, particularly in infants [57]. Geha et al. [58] found that there were a diminish in the serum total bilirubin of the treated animals after administration of sodium benzoate. Sequeira et al. [59] defined the antioxidants are any substance that when present at low concentration significantly delays the oxidation of cell content like proteins and DNA.

The present investigation revealed that oral administration of $NaNo_2$ suppressed the activity of catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) in the kidney. These comes about concurred with those detailed by Hassan [43]. Vinodini et al. [60], Radwan [61], Radwan et al. [62-65] discussed the lipid peroxidation could be increment within the blood glutamate and glutamine which are detailed to support lipogenesis. The blood glutamate and glutamine Within the liver of rodent, glutamine debasement yields glutamate which at that point experiences oxidative deamination to deliver ammonium particles, α-ketoglutarate and NADH. Subsequently, the expanded level of glutamine might too start lipid peroxidation by changing the redox potential of the cell.

Conclusions

In conclusion, it is clear that food additives induced nephrotoxicity in the kidney. It decreased the antioxidant enzymes (GSH, CAT and SOD) and increased MDA. Increase tumor suppressor gene p53 in kidney tissue. Food additives caused changes in biochemical parameters as in creatinine and urea.

Recommendation: The usage of food additives must be reduced. The presence of more than one type of food additives on our food and the usage of the mixture of sodium nitrite and sodium benzoate induced changes in biochemical parameters and immunohistopathological changes.

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