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The possible effects of sodium nitrite and sodium benzoate as food additives on the liver in male rats

EH Radwan¹, MM Elghazaly¹, H Kh Hussein², KK Abdel Aziz¹, AI Barakat¹

¹ Damanhour University, Faculty of Science, Zoology department, Egypt

² Alexandria University, Faculty of Science, Zoology department, Egypt

dr_eman_hashem@yahoo.com, eman.radwan@sci.dmn.edu.eg.

Abstract

Common food additives endorsed by Food and drug Organization "FDA" are utilized to preserve taste. The display think about examined the perilous impacts of sodium nitrite, sodium benzoate and their blend which utilized in fabricating of the food additives on a few biochemical parameters and histo-pathological examination in male rats. Male rats were divided into four groups; group I utilized as control, group II and III were treated orally with sodium nitrite NaNO₂ (80 mg/kg BW) and sodium benzoate (SB) (200 mg/kg BW), separately. Group IV was treated orally with the blend of sodium nitrite and sodium benzoate. Rats were managed their dosages each day for 8 weeks. It appeared that sodium nitrite, sodium benzoate and their blend (NaNO₂ and SB) initiated a critical increment within the serum levels of aspartate aminotransferase "AST", alanine aminotransferase "ALT". Antioxidant proteins (GSH, CAT) within the liver tissue recorded a decrease while, MDA recorded an increase action level within the tested groups. Over expression in p53 happened in exploratory groups which were treated by NaNO₂, SB and their blend. The present study concluded that the blend of food additives can actuate harmfulness within the liver of rats. In conclusion, it is noted that food additives induced hepatotoxicity within the liver. It diminished the antioxidant chemicals (GSH, CAT) and elevated the activity level of the MDA and increment tumor silencer quality p53 in liver tissue. Food additive substances caused changes in biochemical parameters (ALT, AST). The utilization of food additives must be diminished. The usage of the mixture of sodium nitrite and sodium benzoate induced changes in biochemical parameters and immune-histopathology.

Key words: Hepatotoxicity-P53- Sodium Nitrite- Sodium Benzoate- Food Additives- Male Rats.

Introduction

Food additives are utilized within the food industry in arrange to move forward the nourishment taste by protecting its flavor and avoiding it from souring [1]. Stafford [2] proposed that added substances are added to the nourishment to extend rack life. Soriano et al. [3] detailed that around 200 food added substances cause intense unfavorably susceptible responses. The food added substances are critical in hindering the development of bacteria causing conditions [4]. Certain food added substances cause hyperactivity in children [5]. Several public interest groups have drawn up lists of additives which consider generally to be avoided or to be treated with caution, especially by sensitive individuals [6]. Benzoic corrosive, benzoate and nitrites are nourishment added substances. Sodium benzoate and potassium nitrate are utilized broadly in nourishment conservation. Nitrites are utilized in cooked meats as additives [7-9]. Ingestion of nitrite compounds, defilement of drinking water with nitrates and utilization of nitrite-rich nourishment by newborn children are ordinarily credited with causing methemo- globinemia [10]. Mellor and Stafford [11] detailed that compounds such as N-nitrosodimethyl amine have been carcinogenic in a wide extend of creature species. Bremer et al. [12]; Alexander et al. [13]; Radwan [14]; Radwan [15]; Radwan [16] detailed that customers around the world utilize added substances and additives in their food.

Oxidative stress has been come about when pro-oxidants surpass the antioxidant capacity guaranteed that it may lead to genuine infection [17-23]. Oxidative stress has been recognized as a causative operator for



maladies due to a decrease of resistant capacities and the action of oxidative stretch biomarkers can be affected by sustenance [24-26]. Al-Shammari et al. [27]; Elghazaly et al. [28] depicted a relationship between the food added substances and resistant framework reactions. A decrease in resistant reaction has been watched in individuals who are in contact with food additives and flavors found in several foods.

Materials and Methods

The chemicals were purchased from El-jamhoureia Chemical Company, Alexandria, Egypt (Oxford laboratory, Mumbai, India). (a)- Sodium benzoate is the sodium salt of benzoic acid. It is an aromatic compound denoted by the chemical formula C₇H₅NaO₂, sodium benzoate is a white, odorless compound and is soluble in water. Sodium benzoate has antimicrobial characteristics and is typically used as a preservative in food products. (b)-Sodium nitrite is the inorganic compound with the chemical formula NaNO₂. It is a white crystalline powder that is very soluble in water.

Forty male albino rats weighing 160-180gm (8 weeks old) [29] were used throughout the experiment. They were obtained from the Animal Care Unit, Faculty of medicine, Alexandria University, Alexandria; Egypt. Animals acclimated to the laboratory environment for at least one week under standard housing conditions prior to the study initiation. Rats housed in a stainless steel cages and they maintained under controlled conditions in a room ventilated with fresh air. The animals were provided with water ad libitum. Drinking water changed daily and alternate day clearing and replacement of sawdust and droppings carried out. Rats were randomly distributed into four groups; each group contains 10 rats, room maintained at a relative humidity of 50-60%, room temperature of 22 ± 3 °C, and 12h light/dark cycle. Rats were fed standard laboratory pellets. The doses of sodium nitrite (NaNO₂) and sodium benzoate (SB) were used according to Kohn et al. [30] and Oyewole et al. [31]; respectively and dissolved in distilled water. At the end of the experiment, rats were anaesthetized with ether and sacrificed and livers were immediately removed, washed using saline solution (0.9%), and then were transferred to 70% alcohol. Blood samples were collected in test tubes containing heparin as an anticoagulant and placed immediately on ice. Blood was centrifuged for 20 min for separation of the plasma. The plasma was kept at -80°C until analyses of the tested parameters. The organs were minced and tissue perfuse with PBS (phosphate buffered saline) solution, pH 7.4, containing 0.16 mg/ml heparin to remove any blood cells and clots. 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 the activity of the alanine amino transaminase (ALT) was done by kinetic method of Schumann and Klauke [32].

Determination of the activity of the aspartate amino transaminase (AST) was done by colorimetric method of Schumann and Klauke [32]. Determination of the activity of the alkaline phosphatase (ALP) was done by Schumann and Klauke [32]. Determination of the total protein was done by using the method of Gornall *et al.* [33]. Determination of albumin was done by using the method of Doumas *et al.* [34]. Determination of the activity of malondialdehyde (MDA) was done by using the method of Tappel and Zalkin [35]. Determination of the activity of the glutathione (GSH) was done by Jollow *et al.* [36]. Determination of the activity of the catalase (CAT) was done by using the method of Luck [37]. Determination of the activity of the superoxide dismutase (SOD) was done by using the method of Mishra and Fridovich [38]. The immune-histochemical study for p53 was carried out on the liver of male rats' tissues, 5µm thick paraffin sections were cut, mounted onto positively charged slides [39]. The data was statistically treated by one-way ANOVA using 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 \pm standard error.

Results and Discussion

The mean values of the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the plasma of male rats which were treated orally with sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture for 8 weeks are represented in Table (1). In the treated groups II, III and IV, there were significant increase (p<0.05) in the activity of ALT, AST and ALP compared to control group



(group I). The treatment of treated group IV by the mixture of NaNO₂ and SB resulted in significant increase (p<0.05) in the mean values of ALT, AST and ALP when compared to other treated groups II and III which were treated by NaNO₂ and SB alone, respectively. In albumin levels reported an increase significantly (p<0.05) in the treated groups II, III and IV which treated by NaNO₂, SB alone and their mixture, respectively. The treatment of rats by the mixture of NaNO₂ and SB showed a significant increase (p<0.05) in bilirubin concentration when compared to the other treated groups II and III which treated by NaNO₂ and SB, respectively.

Table (1): Mean values of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and albumin of male rats treated orally with sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture for 8 weeks:

Groups				
parameters	Control, Group I	NaNO ₂ , Group II	SB, Group III	NaNO ₂ + SB
				Group IV
ALT (U/mL)	22.2 ± 2.21 ª	49.5 ± 1.70 ^{b'C}	48.5 ± 3.69 b	54.5 ± 2.64 ^c
AST (U/ml)	81 ± 2.16 ª	127.25 ± 6.07 b	117 ± 6.45 b	210.7± 7.47 ^c
ALP (IU/L)	114 ± 5.2 ª	149 ± 8.04 ^b	140 ± 9.46 ^b	256.75 ± 5.5 °
Albumin (g/dl)	1.01 ± 0.029 ª	3.2 ± 0.014 b	2.9 ± 0.052 b	3.3 ±0.032 b
Bilirubin (mg/dl)	0.12 ± 0.005 ª	0.30 ± 0.004 ^b	0.25 ± 0.004 b	0.54±0.003 ^c

Mean value are expressed as means \pm S.E. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, p < 0.05. ALT=alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase The mean activities of malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and super oxidase dismutase (SOD) were measured in albino rats which treated orally with sodium nitrite, sodium benzoate and their mixture for 8 weeks.

The data recoded in Table 2, indicated that there was an increase significantly (p<0.05) in the concentration of MDA in the treated groups II, III and IV when compared to the control group I. The treatment of male rats in group IV by the mixture of two food additives NaNO₂ and SB showed a significant increase (p<0.05) when compared to the treated groups II and III. The mean values of the concentrations of GSH and CAT and SOD resulted in a significant decrease (p<0.05) in the treated groups II, III and IV which were treated by NaNO₂, SB and their mixture, respectively.



Table (2): Mean value \pm S.E. of plasma oxidative stress markers malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) of male rats treated orally with sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture for 8 weeks:

Oxidative stress Markers	Control group I	NaNO ₂ , group II	SB group III	NaNO2+SB group IV
MDA (nmol/ml)	1.2 ± 0.14 ª	3.6 ± 0.28 b	4.04 ± 0.07 b	8.25 ± 0.07 °
GSH (mg/dl)	25 ± 1.41 ^b	2.53 ± 0.05 ª	3.15 ± 0.07 ^a	2.35 ± 0.35 ª
CAT (U/L)	288.5 ± 9.1 b	122 ± 2.8 ª	117.5 ± 0.7 ª	103 ± 4.24 ª
SOD (U/L)	44.1± 1.4 ^d	21± 0.7 b	33.2 ± 1.8 ^c	13.2 ± 0.7 ª

Mean values are expressed as means \pm S.E. 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. In the present study, table (3) showed the mean values of the activities of liver antioxidant enzymes GSH, CAT and SOD as well as the malondialdeyde (MDA) concentration were reported for the experimental groups I, II, III and IV. The treated groups which were treated by SB and the mixture of NaNO₂ and SB, respectively showed a significant increase in MDA (p<0.05) when compared to the control rats. The activities of GSH, CAT and SOD in treated groups II, III and IV showed a significant decrease (p<0.05) when compared to control group I. The treated groups II, III and IV reported significant differences in the concentrations of CAT and SOD when compared to each other. In contrast, the concentrations of GSH in the treated groups II and III have shown non-significant changes when compared to each other.

Table (3): Mean values \pm S.E. of liver oxidative stress markers malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) of male rats treated orally with sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture for 8 weeks:

Oxidative stress markers	Control group I	NaNO₂ group II	SB group III	NaNO2+SB group IV
MDA (nmol/g.tissue)	5.6 ± 0.28 ^a	6.6 ± 0.28 ^a	9.5 ± 0.70 ^b	16.35 ± 0.4
GSH (mg/g.tissue)	13.5 ± 0.7 °	6.65 ± 0.9 ^b	8.4 ± 0.7 b	1.4 ± 0.5 ª
CAT (U/g.tissue)	772.5 ± 10.6 ^d	162.5 ± 3.5 b	403.5 ± 9.1 °	127.5 ± 6.3 ª
SOD (U/g.tissue)	55.3 ± 1.4 ^d	22 ± 0.7 b	31.7 ± 1.8 [°]	12.2 ± 1.9 ª



Values are expressed as means \pm S.E. 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.

Histo-pathological observations of liver:

Fig. 1 (A & B) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein were observed in the control group as well as, the portal area was observed by the bile duct and portal vein.

Fig. 2 (A, B, C and D) showed increase in the number of hepatocytes, congested central vein, portal area fibrosis infiltrated with leukocytes, apoptotic hepatocytes, increase number of binucleated hepatocytes, dilation of central vein and disappear of normal blood sinusoid in NaNO₂-treated group II.

Fig. 3 (A, B, C and D) illustrated SB-treated group III and showed foamy area, hepatocytes vacuolation, necrosis, disappear of central vein and increase number of bi-nucleated of hepatocytes.

Fig. 4 (A, B, C and D) represented the treated group IV which treated by the mixture of NaNO₂ and sodium benzoate (SB) and showed that completely disappear of normal architecture of liver tissues, vacuolation, degenerated hepatocytes with pyknotic nuclei, karyolysis, hemorrhage in portal vein and lymphocytes aggregation.





Fig. (1): Photomicrograph of liver sections (A) control group showing normal architecture of liver tissue with radically arranged hepatic strands, central vein (C.V), endothelial cell (E), hepatocytes (arrow), blood sinusoid (S). (B) Portal area of control group showing portal vein and bile ductile (arrow) (H. & E., X: 400).





Fig. (2): Photomicrograph of liver sections (A, B, C and D) of group II treated by NaNO₂ showing an increase in number of hepatocytes (arrow), abnormal of central vein (head arrow), congested central vein (dotted arrow), hemorrhage in portal area (star), degenerated hepatocytes with pyknotic nuclei (circle) and disappear of normal blood sinus oid (S). (H. & E., X: 400).





Fig.(3): Photomicrograph of liver sections (A, B, C and D) of group III treated by SB showing foamy area (circle), dilation of blood sinusoids (dotted arrow), disappear of normal central vein (CV) and an increase in number of bi-nucleated of hepatocytes (arrow). (H. & E., X: 400).





Fig. (4): Photomicrograph of liver sections (A, B, C and D) of group IV treated by mixture of NaNO₂ and sodium benzoate (SB) showing completely disappear of normal architecture of liver tissues, vacuolation (arrow), cellular infiltration (star), karyolysis (head arrow), necrosis (circle). (H. & E., X: 400).



Effect of sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture on tumor suppressor gene (p53) of liver of rats: The results of expression tumor suppressor gene p53 in liver of male rats which treated by NaNO₂ and SB and their mixture were recorded in table 4 and Fig. 5. In cytoplasmic expression of p53 in liver, the mean values of p53 levels in treated group which treated by NaNO₂, sodium benzoate and their mixture were showed significant increase (P<0.05) when compared to control rats.

The treated group IV which treated by the mixture of two food additives NaNO₂ and SB was reported a significant increase (p<0.05) when compared to treated groups II and III. In nuclear expression, treatment of male albino rats by NaNO₂ and sodium benzoate alone, respectively was shown a significant increase as well as the group IV which were treated by the mixture of NaNO₂ and SB recorded significant increase (p<0.05) compared to group I, II and III.

Table 4: Effect of sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture on tumor suppressor gene (p53) of liver organ

Parameters	Control group I	NaNO₂ group II	SB group III	NaNO ₂ +SB group IV
Liver p53 Cytoplasmic(%)	2.8± 0.5ª	4.9± 0.2 ^b	5± 0.18 ^b	5.5± 0.13°
Liver Nuclear(%)	0.42± 0.03ª	0.81± 0.03 ^b	0.86± 0.02 ^c	0.92± 0.06 ^d



Fig 5: Effect of sodium nitrite (NaNO₂), sodium benzoate (SB) and their mixture on tumor suppressor gene (p53) of liver organ.





Fig. (6): Photomicrograph of liver sections; (A) control group I of male rats showing weakly stain of 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).



Many of food added substances have been progressively recognized as possibly dangerous components for human being such as hepatotoxicity and nephrotoxicity [40, 28, 19-23]. Receptive nitrogen species created by introduction to nitrite is considered one of the foremost critical causes of carcinogenesis through its response with body tissues and activating lipid peroxidation, DNA injuries, chemical inactivation and harm of diverse organs [41]. The oxidative stress pointer is lipid peroxidation which might be credited to the oxidative cytotoxicity of nitrite [42, 43, 19-23].

The activity levels of ALT, AST within the serum of rats which were treated by NaNO₂ that's credited to the harmful impact of nitros-compounds which were shaped within the acidic environment of the stomach and causing extreme hepatic corruption. NaNO₂ has shown to form carcinogens [44]. Abdelaziz et al. [45] examined the impact of the additives on the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in rats which were treated for 35 days and found that, there was an increment in their values essentially concurring to the separate hepatic rot which happened within the liver tissue. The biochemical examination offer assistance to distinguish the target organs of the poisonous quality [44]. The science comes about in connection to liver work and sodium nitrite treated bunches within the display consider concur with Hassan et al. [46] they examined that the increment within the activity of AST and ALT chemicals within the serum of NaNO₂-treated rats can be credited to the arrangement of over 300 cytotoxic N-nitrosocompounds within the acidic environment of the stomach as well as a result of combination of sodium nitrite with secondary amines in the food, causing severe hepatic and renal necrosis.

Anemia induced hypoxic injury to hepatocytes cause the enzyme leakage [47]. The food additives are fundamental for nourishment capacity whereas, they can provide rise to certain unfavorable wellbeing issues as well as cause diverse sensitivities and hyperactivity within the a few individuals who are touchy to particular chemicals [48]. The food containing added substances can cause asthma and dermatitis. A few of the known added substances and additives are as takes after; benzoates can trigger the hypersensitivities such as skin rashes and asthma [49]. The day by day admissions of food additives as NaNO₂ and SB, shows an increment in ALT, AST and MDA when compared to control rats. These comes about are in agreement with EI-Wakf et al. [50]; Radwan et al. [19]; Radwan et al. [20]; Radwan et al. [21]; Radwan et al. [22, 23] examined the activity of a few liver chemicals which were utilized as vital biomarkers for location of hepatotoxic nature of diverse drugs. Serum hepatic marker chemicals (ALT, AST) were evaluated when hepatotoxicity was happened. The liver is the foremost sensitive organ to pre-oxidative damage. The more severe liver damages have the higher release of the liver enzymes, so an increase in serum level of ALT, AST and ALP as observed in groups induced with the food additives may reflect the damage of liver cells and cellular degeneration and destruction in this organ as well as the increase in the activities of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis.

When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream [51]. The rises of AST and ALT activity show the utilization of amino acids for the oxidation or for gluconeogenesis and are utilized to decide liver harm. The expanded levels of serum protein such as AST and ALT show the expanded penetrability and rot of hepatocytes [52]. Farombi and Onyema [53] expressed that SB admissions seem to harm the liver, thus discharging the ALT which will lead to liver harm. This increment may moreover be clarified by the free radical generation which responds with polyunsaturated fatty acids of cell film driving to impedance of mitochondrial and plasma layers coming about in protein spillage. The comes about of the current ponder apparently concur with the reports of Onyema et al. [54]; Elghazaly et al. [28], they watched that the action of serum ALT expanded in male rats which supplemented SB due to the finding that SB actuated oxidative stress within the liver. In this way, it can be concluded that SB may be hepatotoxic, consequently ought to be maintained a strategic distance from amid the treatment of liver disarranges [55]. The increase of ALT may be a hazard figure for coronary heart malady [56].

The impacts of food additives might to be credited to the cytotoxic impact of N-nitrosocompounds in renal tubular cells and the changes within the limit of tubular reabsorption, renal blood stream and glomerular filtration rate. The concentration of proteins, bilirubin and albumin within the serum can be utilized as pointers



for the state of the liver and can be utilized to distinguish between distinctive sorts of liver harm [57]. Minimizing poisons in your count calories is an critical step toward upgrading your wellbeing and bringing down your hazard of illness. Prompt impacts may incorporate alter in vitality level, and changes in mental concentration, behavior and safe reaction. Long-term impacts may increment your hazard of cancer, cardiovascular infection and other degenerative conditions [58, 19-23]. Abdelaziz et al. [45] examined the impact of nourishment added substances on the grown-up pale skinned person rats were weighing (100-120 gm) and found that there was an increment within the egg whites level and a diminish within the add up to protein concentration.

Kalantari and Salehi [44] illustrated that there was an inhibitory impact of NaNO₂ on the biosynthesis of protein and proposed a incitement of the thyroid and the adrenal organs by NaNO₂ which can lead to expanded rate of free amino acids, and diminished protein turnover [27]. Lau et al. [1] recorded that there were decreases in add up to protein and phospholipids in affiliation with expanded lipid peroxidation and NO levels may be due to the expanded peroxidative degradation of the fundamentally critical myelin phospholipids within the brain of NaNO₂ inebriated rats and the blend of SB and sodium nitrite. This diminishment may be ascribed to the restraint of oxidative Phosphorylation [41].

Blood serum protein may be a decently labile biochemical framework, accurately reflecting the condition of the living being and the changes which happen to the living being beneath impact of inner and outside components [59]. The current examination illustrated that the day by day admissions of SB and NaNO₂ displayed an increment in bilirubin when compared to control rats. Wawrzyniak [60] detailed that the lifted levels of bilirubin (hyper-bilirubenimia) are watched in infections of the liver due to expanded generation, erythrocyte haemolysis, and diminished take-up by the liver and diminished conjugation. Oxidation items might cause pulverization of the film structure with resulting erythrocyte haemolysis.

The fatal level of sodium nitrite is within the run of 22 to 23 milligrams per kilogram of body weight. Lower dosages of sodium nitrite have caused intense methemoglobinemia, especially in newborn children, coming about from change of nitrate to nitrite after consumption [61]. Geha et al. [62]; Elghazaly et al.[28] found that there were a decrease in the serum total bilirubin and albumin of the treated rats after administration of sodium benzoate as well as discussed this reduction according to synthetic function of liver was altered by SB, so albumin and bilirubin levels were decreased. Sequeira et al. [63] characterized the cancer prevention agents are any substance that when display delays or avoids oxidation of cell substance like proteins, lipid. Vinodini et al. [64]; Radwan et al. [19]; Radwan et al. [20]; Radwan et al. [21]; Radwan et al. [22,23] discussed the lipid peroxidation might be elevated due to the increment within the blood glutamate and glutamine which are detailed to support lipogenesis. Within the liver of rats, glutamine corruption yields glutamate which at that point experiences oxidative deamination to create ammonium particles, keto-glutarate and NADH. Thus, the expanded level of glutamine might too start lipid peroxidation by changing the redox potential of the cell. It diminished the antioxidant chemicals (GSH, CAT) and expanded MDA and increment tumor silencer quality p53 in liver tissue. Food additive substances caused changes in biochemical parameters (ALT, AST).

Conclusions

The utilization of food additives added substances must be diminished. The nearness of more than one sort of food additives on our food and the usage of the mixture of sodium nitrite and sodium benzoate induced changes in biochemical parameters and immune-histopathology.

The authors declare that there is no conflict of interest.

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