Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XX, Issue 4 - 2021, pag. 333 - 342

FOOD AND ENVIRONMENT SAFETY

ESTIMATION OF HEMATOPOITIC AND HISTOPATHOLOGICAL DISORDERS IN WISTAR RATS SUBCHRONOUSLY EXPOSED TO FOOD ADDITIVE E223 (NaMBS)

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Received 26th July 2021, accepted 20th December 2021

Abstract: *The objective of this study was to evaluate the effects of subchronic exposure to NaMBS (90 days), on the quantitative hemogram and the thymus histology spleen and stomach in Wistar rats. The study involved 24 female Wistar rats, receiving increasing concentrations of 0.25%, 1% and 4% of NaMBS in drinking water, for 90 days compared to the control group receiving drinking water without NaMBS. At the end of the experiment, the control and experimental rats were sacrificed, the blood and organs were removed and the hematological parameters were assayed. The thymus, spleen and stomach were used for histological study. In all cases a value p <0.05 was considered significant. The results obtained show that, the group receiving 4% of NaMBS showed a significant decrease in the number of red blood cells and hemoglobin (p< 0.05), an increase in the number of white blood cells (p< 0.01) and the number of platelets were observed. The doses of 1% and 4% cause statistically significant changes of the erythrocyte indices (MCV, MCHC, MCHC); and of the leukocyte formula (lymphocytes, monocytes, granulocytes), in comparison with the control group. Hyperplasia of the spleen and stomach in animals treated with 1% and 4% of NaMBS, was observed in comparison with those of control animals. In addition, histological examination revealed hyperplasia of the white pulp and inflammation of the spleen, in rats treated with 4% of NaMBS and inflammation of the gastric mucosa, in the groups receiving 1% and 4% of NaMBS dose. The results obtained indicate that, the subchronic ingestion of NaMBS above the ADI, seems to cause alterations in some hematological parameters such as splenic and gastric histo-alterations in Wistar rats.*
Abbreviations: *MCHC: Mean Corpuscular Hemoglobin Concentration, MCH: Mean Corpuscular Hemoglobin, MCV: Mean Corpuscular Volume, NaMBS: Sodium Metabisulfite, ADI: Acceptable Daily Intake.*

Keywords*: Hemogram, histophysiology, sodium metabisulfite, subchronic, toxicity, Wistar rat*

1. Introduction

Omnipresent in industrial food, additives are found in our plates in ever-increasing quantities. Since sea salt, the first chemical preservative for meat and fish since antiquity, the substances used by humans to protect or improve his food have multiplied [1].

Sulphites are used in food as additives and can also be found in some medicines [2,3].The European Union has classified them as preservatives and assigned them an "E" code ranging from E 220 to E 228 [4,5].

Sulfites are the result of the fermentation process, and therefore they form naturally in foods such as garlic and onions [6]. Sulphites are generated *in vivo* by the

DOI: https://doi.org/10.4316/fens.2021.036

degradation of sulfur amino acids, such as methionine and cysteine. They can also be generated by neutrophils polynuclear [7, 8]. In addition, many mammalian cells produce sulfites from H_2S [9].

Similarly, sulfites are allergenic additives, to which the majority of asthmatics are sensitive [10]. The sulfites ADI (expressed as SO_2) is 0.7 mg / kg of body weight [11]. They are mainly metabolized in the liver by sulfite oxidase; to give sulfates [12], which are excreted in the urine [13]. This enzyme is present in the mitochondria and in most tissues [14]. The organs with the highest sulfite-oxidase activity are the liver, kidneys, and heart, while the lowest are the brain, spleen, lungs, and testes [14,15]. The sulfites toxicity in mammals has been studied by many researchers. These studies have described the sulfites toxicological effects on animal organs with *in-vivo* and *in-vitro* tests [16, 17, 18, 19, 20, 21, 22, 23].

Our previous work has shown that the subchronic intake of 1% and 4% NaMBS seems to alter immune function, biochemical: (calcium, uric acid, urea, creatinine, transaminases), hematological: (hemoglobin, red blood cells and white blood cells) and physiological parameters in the Wistar rat [24].

Another complementary study performed by El kadi et *al*, 2017[25], shows the effect of sodium metabisulphite in the genesis of an oxidative stress state, characterized by an overproduction of MDA (Malondialdehyde), a significant decrease in the antioxidant enzymes activities of (GPx, SOD, CAT): (Glutathione peroxidase, Superoxide dismutase, Catalase) and thiol groups in the stomach and the spleen of the Wistar rat.

In this work, we are interested in studying the effect of this synthetic preservative in doses (0.25%, 1%, 4%), that can be provided via the diet on certain haematological parameters and on the histological structure of certain target organs (thymus, spleen and stomach).

2. Materials and methods

2.1. Materials

2.1.1. Chemical material

The chosen test substance is sodium metabisulfite, which was procured from Biochem Chemopharma international, with a purity level of 95%. The substance appears as a very fine white powder with a pungent odor and easily soluble in water. The specifications of this product are listed in (Table 1).

2.1.2. Choice of the administered dose

The administration mode of NaMBS is in drinking water solution, to avoid partial destruction of thiamine in the diet by SO_2 before ingestion. The orally consumption of 0.25% NaMBS is equivalent to 72 mg/kg of SO_3^{-2}, with these data, the World Health Organization (WHO) has established an acceptable daily intake which is 0.7 mg/kg of body weight taking into account, the safety factor 100 [27].

The chosen doses in this study are based on the value of the ADI [28]. In addition, these doses were selected with reference to certain studies, which analyze the sulfites toxicity at high doses [29, 30].

2.1.3. Experimental groups and treatment

The experiments were carried out on 24 rats of Wistar strain provided by the breeding center of the Algiers Pasteur Institute. They are 8-week-old female rats, weighing an average (130.41±22.68gr), food and water were provided *ad libitum*.

The animals were divided into 4 lots at a rate of 6 rats per lot. Each lot received the test substance at different doses for 90 days, by varying the concentration of the prepared solution. The different lots were distributed as follows:

- 1st control lot: rats receiving water without NaMBS.
- 2nd lot: rats receiving a dose of 0.25% of NaMBS = ADI.
- 3rd lot: rats receiving a dose of 1% NaMBS = 4 times the ADI.
- 4th lot: rats receiving a dose of 4% NaMBS = 16 times the ADI.

Table 1
Sodium Metabisulfite E223 specifications, according to European Commission Directive 2008/84/EC [26]

Specifications	Specifications Biochem Chemopharma
Name of the product	Synthetic food preservative
Chemical name	Sodium metabisulfite
Synonyms	Disodium pentaoxodisulfate; Sodium pyrosulfite;
UE Reference Code	Sodium disulfite
CAS	E223
Einecs	7681-57-4
Chemical Formula	231-673-0
Molecular weight	$Na_2 S_2 O_5$
Purity ($Na_2S_2O_5$)	190.11
Insoluble material	Min 95%.
Heavy metals (expressed in Pb)	Max 0.005%
Chloride	Max 0.0005%
Iron	Max 0.005%
Arsenic	Max 0.0005%
	Max 0.0001

2.2. Methods

2.2.1. Hematological parameters

At the end of the experiment (D 90), the animals were anaesthetized under urethane, after 16 hours of fasting. Blood was collected by puncture in the abdominal aorta. The blood was collected in EDTA (Ethylen-Diamino-Tetracetic-Acid) tubes. The analyses of the haematological parameters were performed on the same day of the collection (MCV: Mean Corpuscular Volume, MCHC: Mean Corspuscular Haemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin, PLT: Platelets, LYM: Lymphocytes, MON: Monocytes, GRA: Granulocytes) using an ADVIA 60 CT (BAYER DC brand) automated system.

2.2.2. Histological parameters

From the first minutes after the animal's sacrifice, the organs (thymus, spleen and stomach) were carefully removed and rinsed with 0.9% of NaCl. Quickly, part of the thymus, spleen and stomach were immersed in the 10% buffered formalin solution for their fixation for histopathological examination. Tissue sections (3µm thick) were cut and stained with hematoxylin and eosin; and observed under an Olympus microscope (Zeiss, Axiostar plus, Oberkochen, Germany).

2.2.3. Statistical analysis The results are expressed as means and their standard error (X±ES). The comparison of means is performed using the t "Student" test. The differences are considered significant at $p < 0.05$.

3. Results and discussion

3.1. Hematological study

The results obtained reflect a certain form of hematopoiesis dysfunction, by a quantitative disturbance of the circulating blood. These results may lead us to suggest that, sulfites are capable of exerting continuous pressure on the hematopoiesis

and thereby cause hemopathy. According to our results, the analysis of the haemogram varied significantly (Fig.1).

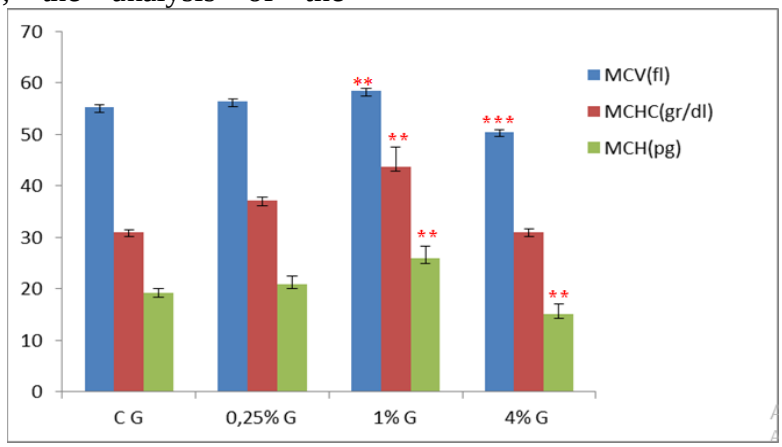

Fig 1 : Serum levels of MCV, MCHC and MCH of control and treatment groups
***** p<0.01 and ***p<.001:* significant difference from controls. CG: control group; 0.25% G: group receiving 0.25% NaMBS; 1%G: group receiving 1% NaMBS; 4%G: group receiving 4% NaMBS.**

The evaluation of erythrocyte indices: MCV, MCHC and MCH showed statistically significant changes, when compared to the control lot; MCV indicates the volume of red blood cells and, is used to diagnose and classify anemia. A MCV>94fl/L reflects a macrocytic anemia observed in vitamin B_{12} deficiency and hepatopathies [31]. Our study showed an increase in MCV value in the group treated with 1% NaMBS; and a decrease in the group receiving 4% of the test substance. The MCHC measures the quantity, more precisely the mass of hemoglobin contained in a red blood cell. The results obtained were correlated with that of the MCV. This parameter increased at a dose of 1% and decreased at a dose of 4%. The MCH corresponds to the hemoglobin charge per 100 ml of blood. It is the ratio of hemoglobin concentration to hematocrit. The MCH is significantly increased in 1% G (Fig.1).

Results indicate that the hemoleukocyte formula showed a decrease in lymphocytes; and an increase in monocytes and granulocytes in the group receiving the dose of 4% of NaMBS (Fig.2).

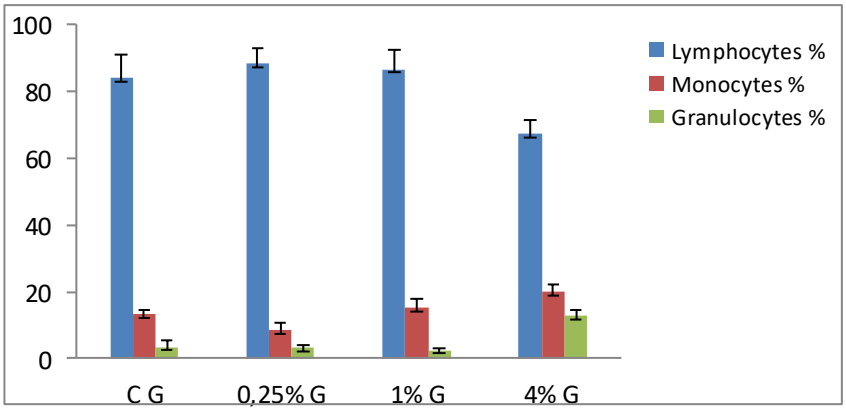

Fig 2 : Serum levels of lymphocytes, monocytes and granulocytes of control and treatment groups
***** p<0.01 and ***p<.001:* significant difference from controls. CG: control group; 0.25% G: group receiving 0.25% NaMBS; 1%G: group receiving 1% NaMBS; 4%G: group receiving 4% NaMBS.**

Hyperleukocytosis may be the result of the immune system activation in response to infection, inflammation, and necrosis [32], other situations may also cause changes in white blood cell counts (WBCs) such as, emotional stress and dehydration [33].
These results correspond to the work carried out by Etlik et *al* , 1997 [34], which indicates a statistically significant increase, in the number of leukocytes in 7 rats, exposed to (26.6 mg/m^3) of sulphur dioxide 1 h/d for 45 days. Moreover, Till et *al*, 1972 [35], detected hyperleukocytosis in *Wistar* rats receiving 6% of NaMBS for 56 days.

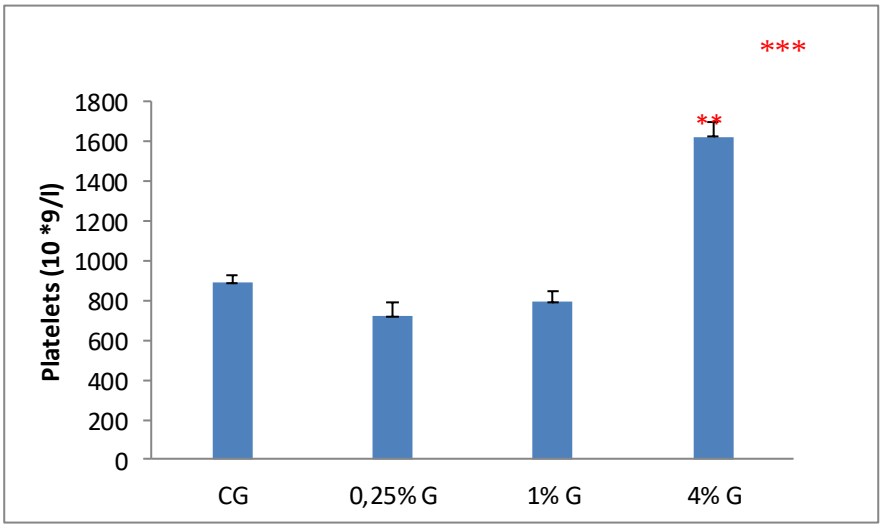

Fig 3 : Serum levels of platelets of control and treatment groups
***** p<0.01 and ***p<.001:* significant difference from controls. CG: control group; 0.25% G: group receiving 0.25% NaMBS; 1%G: group receiving 1% NaMBS; 4%G: group receiving 4% NaMBS.**

The platelet count revealed a very significant increase in 4% G (Fig.3), which can be explained by a bone marrow disorder. Similarly, this increase indicates a hypercoagulability risk. It has been shown that the bone marrow can be a target for certain toxins that can destroy its cells, resulting in a decrease in the number of red blood cells [36].
Our results are in agreement with those carried out by Gunnison et *al* , 1981 [37],who demonstrated that rats fed with a diet containing 6% of NaMBS for 21 days, became severely anemic, and this was due to the destruction of cyanocobalamin (B_{12} vitamin), in the diet or colon. Likewise, Roberts and Buidinsky, 2000 [36] carried out a study in *Wistar* rats, receiving high dietary levels of NaMBS (0%-8 %) for 10-56 days. Anaemia was produced at the 2% dose. Several inhalation studies show the oxidative effects on erythrocytes of sulfur dioxide exposure. According to Dikmenoglu et *al* ,1991 [38], an increase in lipid peroxidation of erythrocytes was reported in 12 guinea pigs, exposed to 26.6 mg/m^{3}of sulphur dioxide for 30 days. Our results are not similar to those of Etlik et *al*, 1997 [34], which indicate a significant increase in methemoglobin and sulfhemoglobin ratios, lipid peroxidation and increased erythrocyte fragility, as well as a statistically significant increase in erythrocyte count, hematocrit and hemoglobin in 7 rats exposed to 26.6 mg/m^3 of sulfur dioxide 1 h/d for 45 days.

3.2. Histological observation

3.2.1. Thymic parenchyma

The different doses of NaMBS do not cause any histological alteration of the

thymic parenchyma compared to the control group (Fig .4).

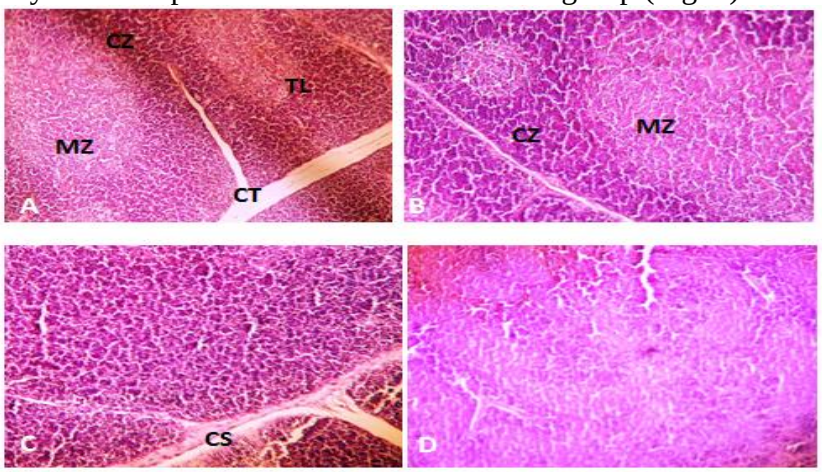

Fig .4: Histological sections of the thymus of control and experimental *Wistar* rats (G×10).
A: control; B: 0.25% of NaMBS; C: 1% of NaMBS; D: 4% of NaMBS
CZ : cortical zone (cortex); MZ : medullary zone (medulla); CT: conjunctive tissue; CS : conjunctive septum; TL: thymic lobule.

3.2.2. Splenic parenchyma

Administration of the 0.25% and 1% NaMBS doses during the 90 days didn't cause any changes or alterations in the tissue architecture of the spleen. However, rats's splenic tissue receiving 4% of NaMBS showed white pulp hyperplasia and polymorphic inflammatory infiltrates (Fig.5).

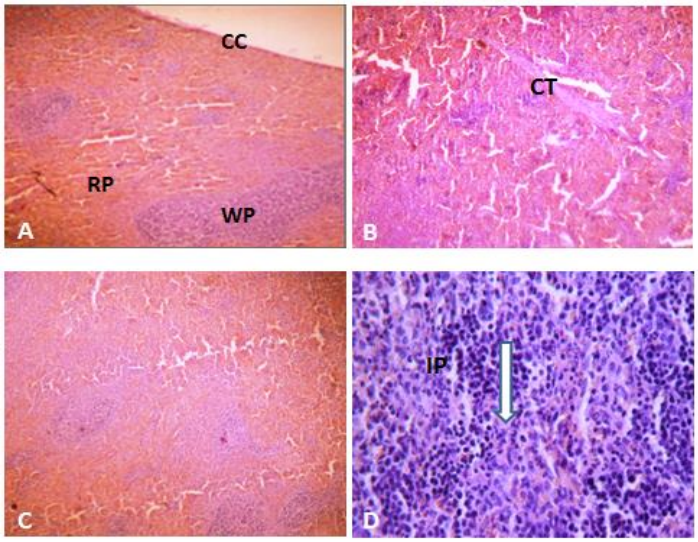

Fig.5: Histological sections of the spleen of control and experimental *Wistar* rats

A: Control (G×10); B: 0.25% of NaMBS (G×10); C: 1% of NaMBS (G×10); D: 4% of NaMBS (G×40).
CC: conjunctive capsule; RP: red pulp; WP: white pulp; CT: conjunctive tract with trabecular vein; IP: inflammatory process.

3.2.3. Gastric parenchyma

Histological examination confirms the presence in the gastric mucosa of the small inflammatory areas of animals treated with 1% of NaMBS. At the highest dose (4%), we will find the same lesions described above but with more inflammatory foci (Fig.6).

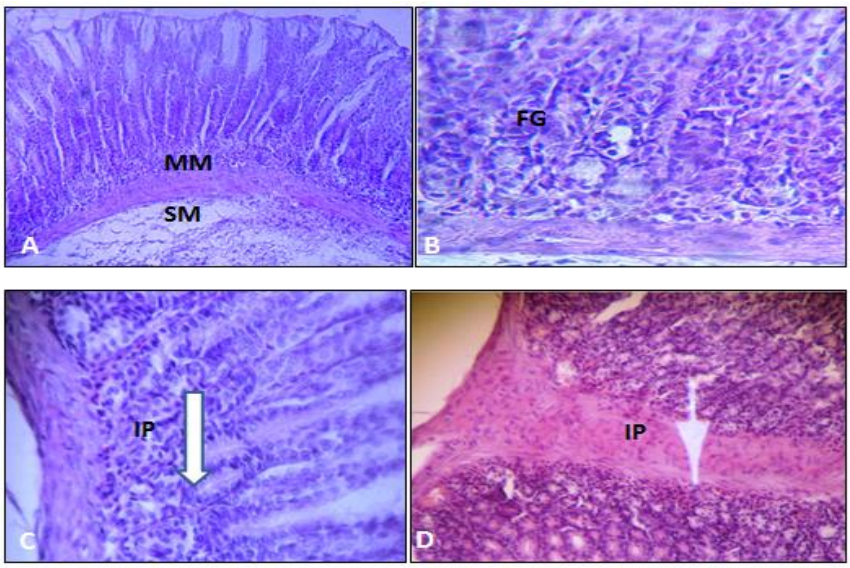

Fig.6: Histological sections of the gastric mucosa of control and experimental *Wistar* rats

A: control (G×10); B: 0.25% of NaMBS (G×40); C: 1% of NaMBS (G×40); D: 4% of NaMBS (G×40). MM: muscular mucosa; SM: submucosa with vessels; FG : fundic glands; IP : inflammatory process.

The effect of NaMBS on biological parameters was considered as an indicator of tissue damage. The histological study didn't reveal any tissue damage of thymus. On the other hand, inflammatory areas with white pulp hyperplasia (due to hyperfunction) were observed in the spleen of treated rats with 4% of NaMBS. In addition, inflammation of the stomach in the 1% and 4% dose groups were observed. These tissue disturbances reflect the appearance of an imbalance between the organism's antioxidant and pro-oxidant systems in favor of the latter. This imbalance confronts the organism to super exposure to reactive oxygen species, which always manifests itself by a decrease in tissue levels of free radical scavengers coumpounds such as, glutathione [39]. Thus, free radicals can be diffused in the cytoplasm and across membranes to attack cellular components [40]. The attack of organic components cells (lipids, proteins, carbohydrates), allows the rapid transmission of free radicals and triggers severe pathologies up to animal death [41]. This confirms our results, particularly with regard to the inflammatory states observed in the splenic and gastric parenchyma of the animals. Moreover, the sulfite oxidase (SOX) activity in mammalian tissues shows a very significant difference in the same species. For example, the liver, kidney and heart tissues have high SOX activity, while the brain, spleen and testes have very low activity [14, 15].

Several researchers have intensively studied the gastrotoxic effect of sulfites. Beems et *al* , 1982 [42], showed that, administration of 6% of sodium metabisulfite in male *Wistar* rats for 12 weeks caused hyperplasia of the fundic

mucosa glands. Similarly, Till et *al* , 1972 [35] concluded that, the incorporation of NaMBS (≥1%) in the diet of *Wistar* rats for 8 weeks caused glandular hyperplasia, hemorrhage, ulceration and stomach necrosis with inflammation of stomach. According to Hui et *al* , 1989[28], gastric lesions are noted in both (normal and sulfite oxidase-deficient) receiving 350 mg/kg bw/day of sulfur dioxide equivalent for 3-5 weeks; gastric lesions are more severe and more frequent in sulfite oxidase deficient rats.

4. Conclusion

In conclusion, the toxicity of NaMBS is dose-dependent, including all damage appearing with the highest dose (above the ADI). All these results suggest that NaMBS has a very high toxic potential, because it disrupts some important biological functions such as hematopoiesis and gastric and splenic histological structure.

5. Acknowledgments

The authors declare that they have no conflicts of interest in relation to this article.

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