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**THE STUDY EFFECT OF SODIUM NITRITE ON BLOOD NITRIC OXIDE AND
HISTOPATHOLOGIC CHANGES ON DIFFERENT ARTERIES IN ADULT MALE
AND FEMALE RATS**

FATEMEH JUIBAR^{1*}, SAEED KHATAMSAZ², HOSSEIN FATTAHI³

¹-Young Researchers and Elites Club, Kazerun branch, Islamic Azad University, Kazerun, Iran

²-Department of Biology Branch Biology Islamic Azad University, Kazerun, Iran

³- Department of Microbiology, Kazerun branch, Islamic Azad University, Kazerun, Iran

***Corresponding Author: E Mail: dr.f.juibar@gmail.com**

ABSTRACT

Background: According to the consumption of large amount of fast-food in society and high levels of nitrite in fast-food and also due to the existence of nitrite in water, soil and ecosystem, is effective on human health. The aim of this study was to test the effect of sodium nitrite on aorta, carotid, and abdominal arteries in adult male and female rats.

Materials and Methods: 60 adult male and female wistar rats, divided into 3 groups of 10. They were examined for 60 day, and they were divided into 175 mg/kg.bw/day recipient group, 350 mg/kg.bw/day recipient group and control group. All the animals were sacrificed after 60 days of treatment. Specimens from areteris of rats were collected.

Samples were stained and Griess method was used to measure Ninitric oxide concentration . Ninitric oxide concentration in animal 's blood , in both sex with low and high recipient groups in comparing with control groups increased significantly ($P \leq 0.05$).

Results: Microscopic studies on different artery in high recipient group in both sex showed media layer changes comparing to control group. So in the high group media layer showed irregularity, and observed mass of lymphosite in media layer also thickness of media layer decreased in both sex high group comparing to the control.

Conclusions: Generally, the Results of this study indicate that consumption of sodium nitrite in various arteries in one sex and one artery in both sex are equal.

Keyword: Sodium nitrite, artery, rat, Nitric oxide

INTRODUCTION

Nitrite and nitrate are found in many food such a natural components or as Intentional additives. Some vegetables and cured meats have these compounds in diet, but they can also be found, in fish and dairy products. Nitrate and nitrite are added as preservatives to some food items to protect them from the growth of *Clostridium botulinum* or to enhance their colour and we can have a pink color of cured meat. (1).

Sodium nitrite use as a preservative in cooked meat and sausages. If we use of more than one type of such food, the percentage of nitrite content of the daily food consumption may be higher than the normal level. (2). some time organic nitrites and nitrates have been used clinically but the only inorganic nitrites of therapeutic are sodium nitrite (Heibashy and Abd El-Moneim, 1999). (2)

The presence of nitrates and nitrites in food has several effect that increas risk of gastrointestinal cancer and, methemoglobinemia in infants. (3)

We know nitric oxide synthesis independently in healthy tissues from L - arginine–NOS pathway. (4)

And anion nitrite (NO_2^-) is biochemical reservoir for nitric oxide (NO). Nitrite can

reduct to NO and it catalyzed by hemoglobin, myoglobin or other metal-containing enzymes and occurs at increasing rates under conditions of physiologic hypoxia or ischemia.(5)

Endothelial cells have various physical and chemical effect in circulating hormones, cytokines, drugs, and sub- stances released by sensory and autonomic nerves or platelets produce vasoactive relaxing substances and contracting substances that regulate vascular tone and permeability, hemostasis, angiogenesis, and inflammation (6).

The vascular endothelium cuse the balance between vasoconstriction and vasodilation (8).

Although the hallmark of endothelial dysfunction is impairment of the endothelium-dependent vasodilation, other alterations (e.g., inflammation, increased lipoprotein oxidation, vascular smooth muscle proliferation and migration from the media to the intima, extra cellular matrix deposition or lysis, platelet activation, and thrombus formation) associated with this disorder have also been described. (7)

Also Nitric oxide is an omnipresent intercellular messenger in all vertebrates,

and can effect on modulating blood flow, thrombosis, and neural activity. Nitric oxide can be produced for 80 years by neurons in human brain without overt toxicity. Nitric oxide can react in pathology, reaction of Nitric oxide with superoxide ($O_2^{\cdot-}$) to form the much more powerful oxidant peroxynitrite ($ONOO^-$) is a key element in resolving the contrasting roles of NO in physiology and pathology. (9) Peroxynitrite ($ONOO^-$), the product of a reaction between nitric oxide and superoxide, is a potent and versatile oxidant implicated in a number of pathophysiological processes.(10)

every time Nitric oxide and superoxide collide, they form peroxynitrite. Nitric oxide enzyme is required to form peroxynitrite because no enzyme can possibly catalyze any reaction as fast.(9)

Although NO is reported to have many potentially toxic effects, many of them are more likely mediated by its oxidation products rather than Nitric oxide itself.(11, 9).

Aim

The research aimed to study the effect of sodium nitrite (food additives) as protective agent on aorta, carotid, and abdominal rat's arteries.

MATERIAL AND METHODS

Animals

Male and female wistar rats weighing between 200-220 gm were used. They were divided into two groups and allowed to acclimatize to the new environment conditions of our laboratory for one week before use.

Animals were kept in cages under proper environmental conditions at room temperature 25-27°C and 12 h light/dark cycle and fed with a commercial pellet diet. The animals had free access to water. The experiment continued for 60 days on which constant weight of diet was given for each rat. All the experiments were designed and conducted according to the ethical norms approved by the Ethical Committee of National Research Center.

60 male and female rats, divided into 6 groups of 10 as follows: Control group daily and freely used of Kazerun city water and standard laboratory food (healthy and natural diet) during the experiment, and any specific treatment was done. low dose treated group received low dose of sodium nitrite (175 mg/kg.bw/day) and another group with high dose of sodium nitrite (350 mg/kg.bw/ day) was absorbed through drinking water. Groups allowed to acclimatize to the new environment conditions of our laboratory for one week before use. At the first and after 60days of treatment with sodium nitrite,

the weight of the rats was measured using precise electronic scales.

Measurement of Nitric oxide concentration in blood:

The final products of Nitric oxide in vivo are nitrite and nitrate. The relative proportion of nitrite and nitrate is variable and cannot be predicted with certainty. Thus, the best index of total Nitric oxide production is the sum of both nitrite and nitrate. Nitric oxide was measured by means of the Griess Method. (Manabu Shiono, 2001) By the end of day the 60, after that Anesthesia and direct bloodletting from heart was performed and the level of Nitric oxide was determined ($\mu\text{M/L}$)

Histological studies

The arteries (aorta, carotid and abdominal) were removed and put them in formalin (31%). Then tissue sections were prepared for testing tissue changes. Staining samples with Hematoxilin Eozin and Mason Tri chrome method were reviewed microscope and Internal media was measured with Image tool software. Factors like morph metric and morphologic changes from arteries, was checked.

Statistical Analysis

Data are expressed as Mean \pm SE. Data were assessed by t-test P-values < 0.05 were considered statistically significant.

RESULT

The amount of water used by the rats

The experimental group consumed more water than the control group daily during the test period in both sex. But didnt have significant difference at level of $P \leq 0.05$

Changes in blood Nitric oxide concentration in different groups of male and female:

The average of male nitric oxide in low and high dose treated group Respectively were 306.63 ± 23.16 and 488.53 ± 28.25 micro mol and mean plasma concentrations of NOx in control group was 33.35 ± 4.30 micromol and The average of female nitric oxide in low and high dose treated group respectively were 250.87 ± 25.41 and 444.82 ± 27.10 micro mol and mean plasma concentrations of NOx in female control group was 27.77 ± 4.26 micromol (table 1).

The outcome of Nitric oxide plasmatic density measurement showed that Nitric oxide level in animal's blood, in both sex with low and high dose recipient groups increased significantly at the level of $P \leq 0.05$ comparing with control groups.

The Comparison both sex

The average of NOx concentration between male and female control groups, also among the experimental groups received low dose (sodium nitrite), male and female, and the

male and female of experimental group receiving the highest dose, no significant difference was observed.

Measurement of tunica media layer (IMT) thickness in different arteries in male and female

In both sex ,group intake Sodium nitrite with high doses ,showed significant change in the average thickness of tunica media layer in male and female comparing to their control groups but in low dose there wasn't any significant difference with control group. (table 2, 3)

Photomicrographs of different arterias in different group of female rat

At the end of the experiment (the sixtieth day) arteries received 175 mg / kg made no change, and were such control group. But as we can see tunica medialayers of the artery in group which received 350 mg / kg, were slightly irregular, non-uniform and the average thickness of the middle layer also has declined in this group and had significant meaning with control group. Some accumulation of lymphocytes in the intimal layer indicates the exictance of inflammation.

DISCUSSION

Changes of blood NOx in different groups of male and female

The average of concentration of NOx in plasma in the groups receiving different

amounts of sodium nitrite had significantly difference with the control group. In a study in 2009 conducted by Karen and her colleagues reported that a solution of sodium nitrite in drinking water, increases nitrite and nitrate levels in plasma, heart and liver tissue. (12)

Effect of sex on NOx concentration

Average NOx concentration in the control group, does not reflect any significant difference between male and female. Watanabe T in 2000 reported that among men in different age groups there was no significant difference in the amount of NOx. (13) Peinado in 2007 reptred that the highest serum levels of NOx occur in men 41 to 50 years Although increased serum levels of NOx in women occurs only after menopause. (14). Since the present study, male rats and female rat were young and in a same age and according to studies, the most variations NOx levels occurs in old age and in menopause women. Compared to, NOx concentrations in serum in the different groups (control, minimum and maximum) of male and female data were not significant. And the results of previous study of Watanabe T in 2000 conform this result too.

Discussion on the results of the thickness of the tunica media layer of the artery

Nowadays measurements of tunica media layer (IMT) is a standard method for the detection of cardiovascular risk factors (15) In 2008 Ono reported that the Candesartan drug decreases the middle layer thickness of carotid artery and described that the decreases is due to the increased levels of Nitric oxide in the body . Nitric oxide (NO) through various disorders cause cell degeneration and destruction (especially in the nervous system (16). According to Ma's research in 2001, the primary reason vascular smooth muscle and endothelial layer damage under pathological conditions, is excessive production of Proxy nitrite in the vascular wall. (17)

In the present study, blood levels of Nitric oxide significantly increased and based on Ono and his coworkers data showed that Nitric oxide concentrations increase can reduce the thickness of the middle layer . And also induced cell death in the middle layer and mitochondria by nitric oxide and nitrite proxies can also reduce the thickness of the middle layer

Discussion on results of the microscopic changes of the arteries

The results of this study indicate that different doses of sodium nitrite in drinking water for 60 days in male and female rats caused changes in the middle layer.(17)

reported in his research that the main cause of Endo layers of epithelial and vascular smooth muscle damage under pathological conditions, is related to excessive production of Proxy in the vascular wall. (17) Nitric oxide toxic effects directly, not relatively large. But the reaction with the super oxide and formation of proxy nitrite the toxicity effect significantly increased. Proxy nitrite freely flows through the membrane lipid double layer of phospholipids and With many target molecules such as lipids, proteins and DNA reacts Which ultimately leads to cell death by necrosis or is apoptosis (18) Also, the exposure to huge amount of superoxide, hydrogen peroxide, nitric oxide and nitrite also a proxy nitrit can cause damage to DNA. (19). Proxy nitrite disrupt mitochondria cellular respiration and energy metabolism (non-activating cytochrome and NADH-COQ1) (20). Maneen in 2006, in his studies showed that proxy nitrite with Dpymrasation of f - actin impact on cerebral artery smooth muscle in rat. (21) The proxy nitrite can disable α -actin which is in cardiac myocytes. (9). The nitrite proxy disable the Ca^{2+} -ATPase in myocytes and vascular smooth muscle is Sarkoplasmy net. (22). Li (2004) reported that an endogenous proxy nitric can start apoptosis in cultured aortic smooth muscle cells. (18). proxy

Nitrite effects on the on or off of channel of ATP - potassium in cardiovascular system and interferes By different mechanisms in vascular relaxation. (9)

Since -based studies of proxy nitrite and nitric oxide by different mechanisms can affects on the cardiovascular system and starting process will cause cell death. Non-uniform state media layer in the experimental group of the males and females can be caused by the effects of nitric oxide and proxy nitrite on the cardiovascular system.

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Table 1: Measurement of Nitric oxide concentration in blood:

*There are Significant differences between the minimum and maximum dose in the experimental group compared with their control group but no significant difference were observed between two sex. (P ≤ 0.05)

Table 2: Measurement of interia media thickness in different arteries in male

*There are Significant differences between the high dose in the experimental group compared with their control group. (P ≤ 0.05)

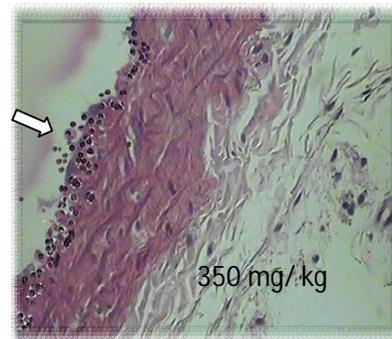
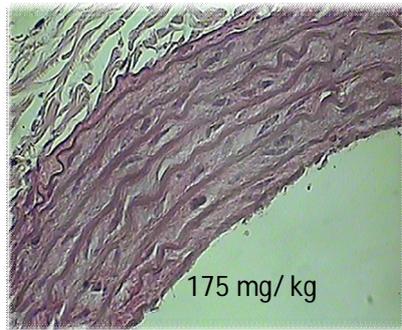
	Mean of NOx in female ($\bar{x} \pm SEM$) (μmol)	Mean of NOx in male ($\bar{x} \pm SEM$) (μmol)
control	27.77 ± 4.26	33.35 ± 4.30*
groups receiving 175mg/kg/daysodium nitrite	250.87 ± 25.41	360.63 ± 23.16*
groups receiving 350mg/kg/daysodium nitrite	444.82 ± 27.90	488.53 ± 28.25*

Table 3: Measurement of interia media thickness in different arteries in male

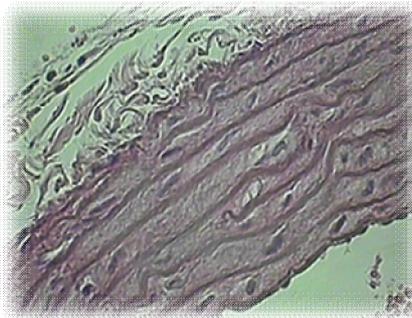
	Control(μm)male	groups receiving 175mg/kg/daysodium nitrite (μm)	groups receiving 350mg/kg/daysodium nitrite (μm)
Aorta artery	541.11±1.57	531.34±1.19	429.69±3.31*
Abdominal	403.11±2.11	411.17±1.80	321.22±1.09*
Karotid	391.46±4.42	387.10±3.23	311.27±2.24*

*There are Significant differences between the high dose in the experimental group compared with their control group. ($P \leq 0.05$)

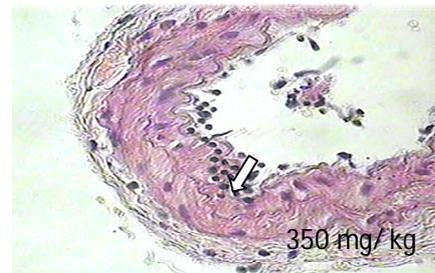
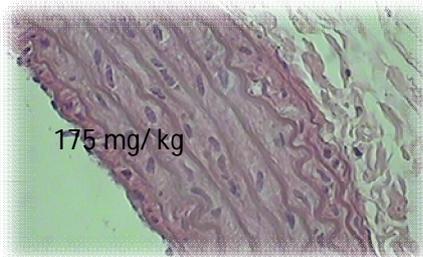
	Control(μm) female	groups receiving 175mg/kg/daysodium nitrite (μm)	groups receiving 350mg/kg/daysodium nitrite (μm)
Aorta artery	541.42 \pm 2.45	514.17 \pm 1.75	419.69 \pm 3.31
Abdominal	425.41 \pm 2.46	413.17 \pm 2.91	331.25 \pm 4.49
Karotid	391.46 \pm 2.13	380.17 \pm 2.23	290.22 \pm 1.61



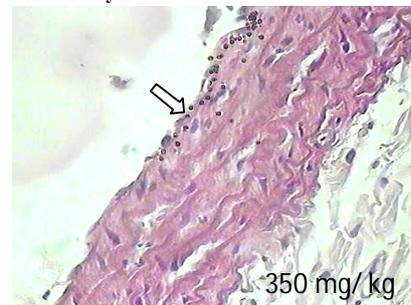
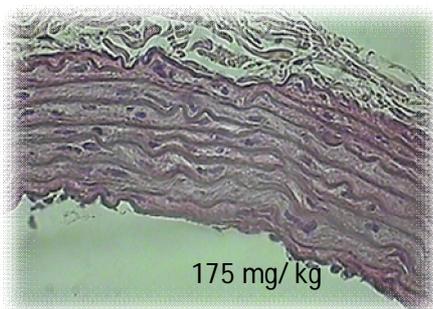
The results of light microscope studies of aorta artery in male rats



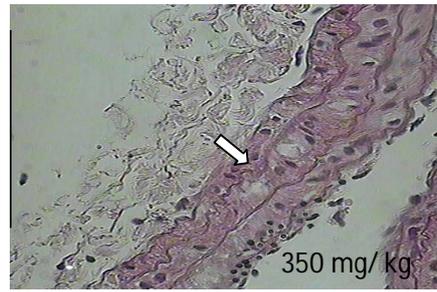
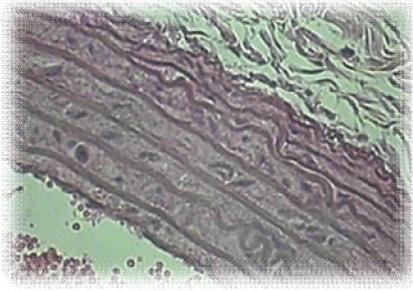
The results of light microscope studies of abdominal aorta in male rats



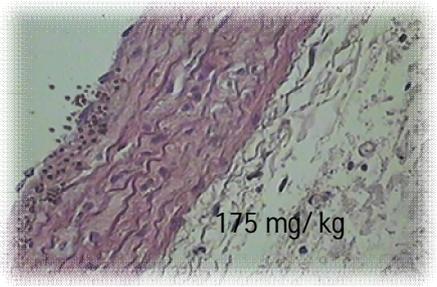
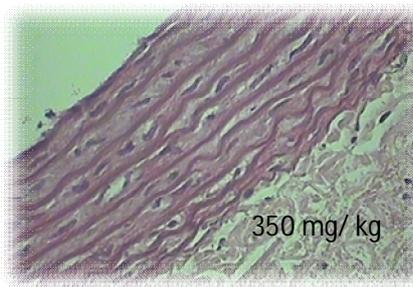
The results of light microscope studies of carotid artery in male rats



The results of light microscopic studies of arteries in the different artery in male group



The results of light microscope studies of abdominal aorta in male rats



The results of light microscope studies of carotid artery in male rats