

## Effect of sodium nitrite on sperm count in mature rats

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Sodium nitrite ( $\text{NaNO}_2$ ) is a common food additive used as a color fixative and preservative in meats and fish. It is also known as hypoxia inducible agent and hypoxia has been shown to affect testicular functions. The aim of the present study is to investigate the effect of  $\text{NaNO}_2$  on the sperm count in mature rats. Four-month-old male Wistar rats were intraperitoneally injected with  $\text{NaNO}_2$  at 50 mg/kg b.w. Treated animals were sacrificed at different time intervals (days 5, 10 and 20) following the administration. Testes and epididymides were sampled. Spermatozoa were isolated from both vasa deferentia and counted. Reduction in the sperm count was observed in all experimental groups after  $\text{NaNO}_2$  administration. The gonado-somatic index was elevated on the fifth day and returned within the normal range at later stages. Future studies would elucidate if  $\text{NaNO}_2$ -induced quantitative changes in the testicular structure are supported by histopathological findings.

*Key words:* sodium nitrite, rat sperm count

### Introduction

Humans are constantly exposed to  $\text{NaNO}_2$  through food and drinking water, with a minor contribution from air [5]. Sodium nitrite is known as E250 in the food industry. Other sources of  $\text{NaNO}_2$  are various industries including agricultural, chemical industry, textile processing industry, disinfectants, colouring agents, etc. [4]. In the circulation  $\text{NaNO}_2$  causes conversion of hemoglobin to methemoglobin, which is incapable of transporting oxygen to the body's tissues and organs and can cause hemic hypoxia. The widespread use of sodium nitrite in the food industry contributes to the potential health risk if not handled cautiously and arouses the necessity of studying its effects. There is evidence of developmental and reproductive toxicity of  $\text{NaNO}_2$  in experimental animal studies. However, available literature data for the impact of  $\text{NaNO}_2$  on the testis are insufficient. In this respect, the aim of the present study is to investigate the effect of  $\text{NaNO}_2$  on the sperm count in mature rats.

## Materials and Methods

The experiments were carried out on four-month-old male Wistar rats. The animals were divided into three  $\text{NaNO}_2$ -treated groups ( $n=15$  rats per group) and age-matched control group ( $n=16$ ). Rats were maintained in the institute's animal house in standard hard bottom polypropylene cages at  $23^\circ\text{C}\pm 2^\circ\text{C}$  and 12:12 h light/dark cycle with free access to laboratory chow and tap water throughout the study.

In brief,  $\text{NaNO}_2$  was injected intraperitoneally at 50 mg/kg body weight (1 ml dosing volume). Treated animals were sacrificed at different time intervals following the administration (days 5<sup>th</sup>, 10<sup>th</sup> and 20<sup>th</sup>) under light anesthesia. The control rats were injected with the same volume of distilled water. Testes and epididymides were sampled and weighed. Spermatozoa were isolated from both vasa deferentia and counted using Buerker's chamber. Data were statistically processed using Student's *t*-test.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

## Results and Discussion

Toxicity to humans and animals is documented in nitrite overexposure including impairment of reproductive function. Literature data for the effect of sodium nitrite on the male reproductive system are controversial. Several studies provide some evidence of testicular changes at the histopathological level in male rats, but the observed effects

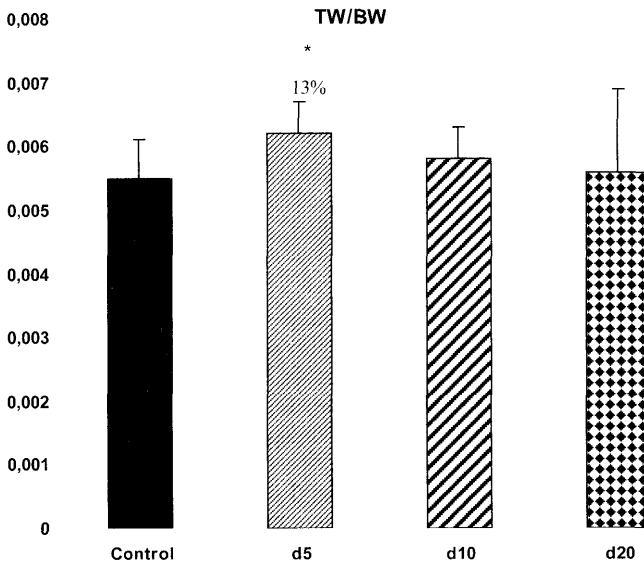


Fig. 1. Changes in rat gonado-somatic index (ratio of testis weight to body weight) at different time intervals after  $\text{NaNO}_2$  treatment. Data represent mean value  $\pm$  SDs (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

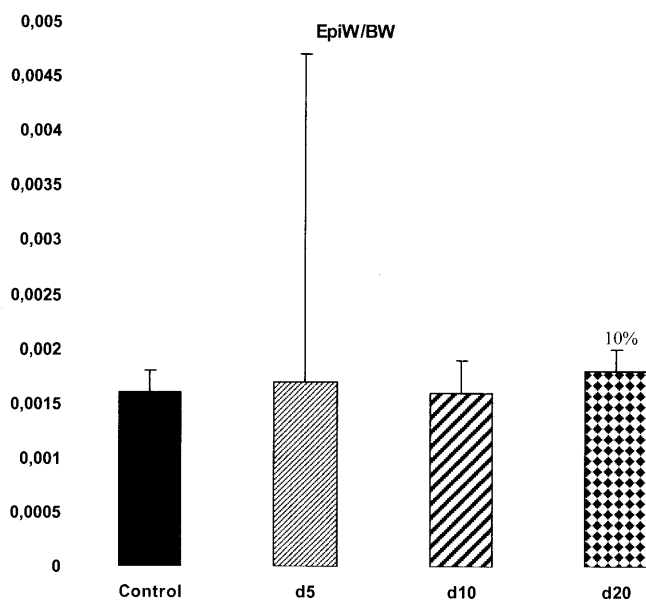


Fig. 2. Changes in rat epididymal index (ratio of epididymal weight to body weight) at different time intervals after NaNO<sub>2</sub> treatment. Data represent mean value ± SDs (\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001).

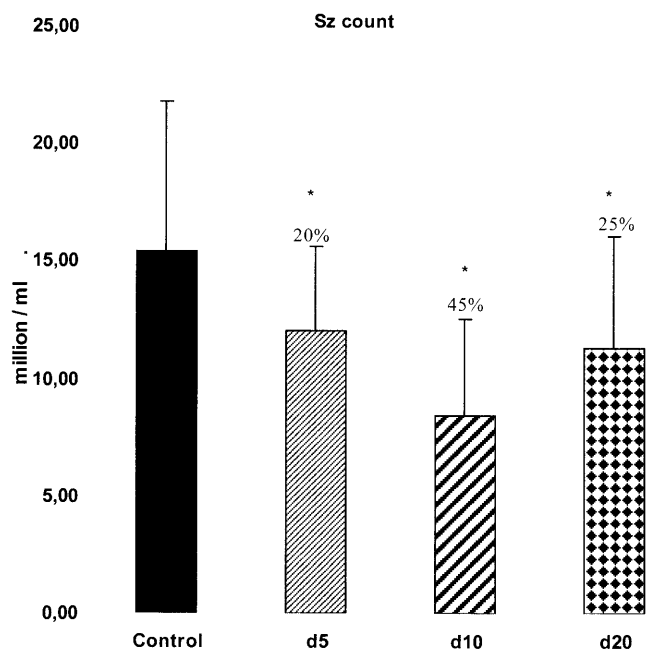


Fig. 3. Changes in rat sperm count at different time intervals after NaNO<sub>2</sub> treatment. Data represent mean value ± SDs (\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001). Sz count – spermatozoa count.

could not be confidently attributed to  $\text{NaNO}_2$  exposure [3]. Sperm-head abnormalities after treatment of differentiating spermatogonia are reported in mice [1]. In contrast, no evidence of testicular pathology is identified in animals subjected to a 3-day regimen of  $\text{NaNO}_2$  injections [2].

In the present study we report a decrease in the rat sperm count after different periods of sodium nitrite treatment. The gonado-somatic index (ratio testicular weight to body weight) was elevated on the fifth day (13%) and returned within the normal range at later stages (Fig. 1) whereas epididymal index (ratio epididymal weight to body weight) remained at normal values in all investigated periods (Fig. 2). Elevated gonado-somatic index in our study could be due to increased testicular fluid volume which may be associated with vascular permeability and disturbances of water-salt balance. Hormonal changes resulting from dysfunction in the hypothalamo-hypophysial-gonadal axis could be another possible cause for the elevated index. Our data also suggest that the testis is more sensitive to the effect of  $\text{NaNO}_2$  in the first days after administration in comparison to epididymis.

We can conclude that  $\text{NaNO}_2$  affects some weight indices and sperm count in mature rats. Our future work would elucidate if  $\text{NaNO}_2$  administration provokes changes in the testicular morphology and morphometric parameters.

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