NO.	48
Title	**Reproductive study of Schiff Base Derived from Methionine.**
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Abstract:

The aim of the study is to investigate the role of new schiff base derived from methionine in preventing the disturbances in sperm viability and reproductive efficiency which is induceed by sodium nitrite toxicity in rats (Ratus norvegicus). Certain parameters were measured like, sperm concentration, motility, Live sperm, Testosterone hormones. Moreover birth number, birth weight and fertility percent . The new schiff base causes significant increase in sperm concentration,motility, Live sperm, Testosterone hormones, birth number, birth weight and fertility percent in the $NaNO_2$ with synthesized compound group as compared with $NaNO_2$ group.

Keywords: Schiff base ,MDJ, Sodium nitrite, Biochemical tests, Antioxidant.

Introduction:

Sodium nitrite widespread use in the food industry contributes to the potential health risk if not handled cautiously and causes decreased whole blood viscosity. A growing body of evidence indicates the beneficial effect of sodium nitrite ,In medicine is used as antidote in the treatment of cyanide poisoning [1], and as a potential new treatment for peripheral vascular disease [2]. However, toxicity to humans and animals is well documented in nitrite over exposure [3].

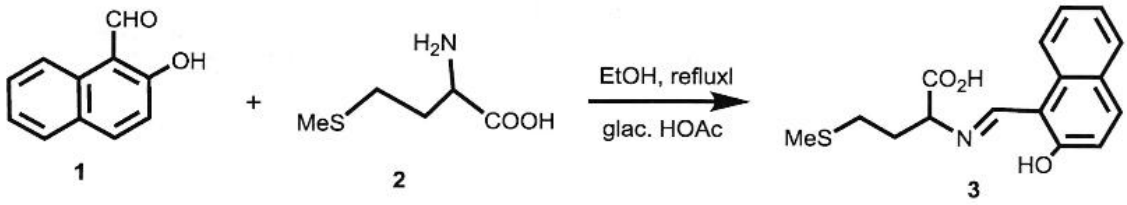
The role of oxidative stress as a causative agent of infertility has been well studied in human, the malonaldehyde increased in Patients with varicocele whereas superoxide dimutase, glutathione peroxidase and catalase are decreased in seminal fluid. These changes are associated with decreased sperm count, motility and increased percentage of abnormal sperms [4]

According to[5] Decreased sperm count, increased deformity in sperm and decreased serum testosterone are well known consequences of high altitude hypoxia.

Hydrogen peroxide induced oxidative stress in rat resulted in testicular degeneration and significant reduction in sperm viability, motility, count and rate of normal sperm [6]. Moreover, animal experimental data have shown reproductive toxicity associated with exposure to high levels of nitrate or nitrite [7]. Abnormalities in sperm-head and infertility in nitrite treated mice has been reported by [8].

Antioxidants are defined as substances that are capable of deactivating or stabilizing free radicals before they attack living cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being functions. In case of exposure to free radicals, from a variety of sources, has led organisms to develop a series of defense mechanisms [9]. Antioxidants like, vitamin C, vitamin E, and selenium, are intimately involved in the prevention of cellular damage which is the common pathway for a variety of diseases [10].

The aim of study is to investigate the role of new Schiff base derived from methionine in ameliorate the deleterious effects induce by sodium nitrite toxicity in rat.



Scheme 1. Synthesis of schiff base derivative of naphthalene conjugated L-methionine

Figure 1: Chemical structure of methionine derivative

Experimantal

Materials and Methods

Antioxidant in vivo.

Animals of the study

This study was carried out on 24 young male and female rats (Ratus norvegicus) weighing about 240- 250 gm. They were maintained at standard experimental condition, rats were housed in the animal house of College of Veterinary Medicine / University of Basrah, and they were kept under good hygienic conditions. The rats were (12-14) weeks of age with an average weight ranged between (230-250gm). Animals were placed in polypropylene cages (30 × 25 × 17 cm). The animals were maintained under laboratory conditions at temperature of (22-24 °C) and exposed to a photoperiod of 12 hrs light followed by 12 hrs of darkness.

They were fed standard rat pellets and fresh clean water was provided (at libitum) throughout the experimental period. Animals were acclimitizated to the laboratory conditions for about 10 days before the application of experimental protocols.

Experimental Design of Antioxidant In Vivo.

After ten days of acclimatization young mal rat were divided into 4 groups (6 in each group) as following.

A- Control group: Rats were injected daily with Dimethyl sulfoxide [DMSO] intraperitoneally by 0.5ml for 21 days.

B- Second group: Rats were injected daily with $NaNO_2$ intraperitoneally by 0.5 ml at dose of 120 mg/kg. [1.5 mg/rat/day] for 21 days.

C- Third group: Rats were injected daily with 1/10 LD50 of synthesized compound methionine derivative (MDJ) intraperitoneally by 0.5 ml at dose of 47.7 mg/kg for 21 days.

D- Fourth group: Rats were injected daily with $NaNO_2$ intraperitoneally by 0.5 ml at dose of 120 mg/kg [1.5 mg/ rat/day] then after one hour the rats were injected with1/10 LD50 of synthesized compound methionine derivative (MDJ), intraperitoneally by 0.5 ml at dose of 47.7 mg/kg for 21 days.

At the end of experimental period (21 days) the rats were sacrificed under general anesthesia by placing them in tightly closed glass container which contain cotton soaked with chloroform as

inhaled anesthesia, After that the abdominal cavity was opened and a "Y" shaped cut the rat abdomen was done, Blood was collected via direct heart [11] by using 5ml disposable syringe. Blood samples were transferred into plain tubes centrifuged at (3000 rpm for 15 minutes) to obtain the serum which then transferred into numerous eppendorf tubes to use in analyses of different parameters and stored at - 4°C.

Testosterone hormone assay:

Principle of the test:

Testosterone is assayed by using mini VIDAS kit manufactured by Human Company for diagnostic and biochemical– Germany. This device has the ability to analyze 12 different samples simultaneously.

Reagent strip are used as standard, positive and negative control. Pipette tip-like disposable device, the solid phase receptacle (SPR), serve as solid phase as well as the pipette for the assay, the (SPR) is coated with antibodies. Reagent for the assay is in the sealed reagent strip an aliquot of the enrichment sample is placed in the reagent stripe and the sample is cycled in and out of the (SPR) for specific length of time. The antigens present in the sample will bind to the antibodies coating the interior of the (SPR). A fluorescent substrate, 4-methyl-umbelliferyl phosphate is introduced in the (SPR).enzyme remaining in the (SPR) wall will then catalyze conversion of the substrate to the fluorescent product, 4-methyl-umbelliferyl. The intensity of fluorescence is measured at 450 nm by the optical scanner in the VIDAS and is expressed in RFV (relative value of fluorescence). When the assay is completed, the results are analyzed automatically by the computer, a test value is generated and printed. The test value (TV)= sample RFV/standard FV. The result is negative if TV<0.05, the result is positive if TV≥0.05.A positive result must be confirmed following standard plating procedure using the remaining enrichment broth stored at 2-8 Co.

Seminal analysis

Sperm concentration ,dead spermatozoa percentage ,sperm motility are estimated by using Neubaure Hemocytometer chamber which is used in RBC and WBC count [12] and [13].

Fertility experiment

In this part of the experiment, 36 mature rats are used 24 female and 12 male. Females and males have been separated for the 16 days before the beginning of the experiment to insure that the females are not conceived. The rats divided to three groups as follow:

Control group: Males are injected I.P with 0.5ml DMSO.

Second group: Males are injected I.P with 120mg/kg $NaNO_2$

Third group: The male rats are injected I.P with 120 mg/kg $NaNO_2$, then after one hour for with 48mg/kg MDJ.

The experiment is lasted for 21 days.The mating duration was 10 days. One male were mate with two females, the females are separated in individual cages till the parturition once the female's rats are given birth, and the number of litters are calculated and weighted by a sensitive balance. The fertility percent are documented.

Statistical Analysis

The results of the present three experiments were analyzed by univalent analysis of variance (ANOVA) by using computerized SPSS (Statistical Packages for the Social Sciences) V.13

program. $P<0.05$ was considered to be the limit of significance. The data were expressed as mean ± standard deviation (mean ± SD). Least significant difference test (LSD) was used to test the difference between groups .

Results and Discussion:

The effects of sodium nitrite and MDJ on sperm viability parameters, birth weight and testosterone level.

The results show a reduction in all sperm viability, average birth weight of litters and testosterone treated rats compared with control.

The results confirmed histologically where that $NaNO_2$ causes a testicular degeneration , degeneration of spermatogenesis tissue, an absence of sperms, necrosis of spermatogenesis tissue, and necrosis of connective tissue.The present results are in agreement with the results of [14]. It has been reported that Oxidative stress plays a key role in the pathogenesis of infertility in human [15].

The resulting hypoxia from nitrite induced methemoglobinemia may contribute to the adverse effects of sodium nitrite on sperm quality and quantity.

It has been reported that hypoxia leads to the germinal epithelium degeneration and reduction of spermatogenesis in rats [16]. In addition to that, the effects of chronic hypobaric hypoxia on spermatogenesis may be partially related to changes in the hypophysis-gonadal axis, as FSH rises initially due to spermatogenic damage [17].

According to [18] as significant increases in an oxidative stress with increased nitrite dose causes an increasing in NO from a variety of stresses which decreases testosterone secretion.

Depending on the results it seems that the $NaNO_2$ acts on litter weights and does not on litter's size .The results shows a significant reduction in offspring's body weight rather than offspring's number compared with the control groups. Injection of MDJ to the treated rats increased offspring's body weight significantly and it is close to the normal litter size body weight compared to the control body weight. No significant effect is observed on offspring's number due to MDJ injection compared with the control offspring's number. It seems that fertility percentage has affected by $NaNO_2$ injection and becomes less compared with the control fertility percentage.

The injection of MDJ retained the fertility percentage to its normal compared to the control fertility percentage.

The ability of MDJ to improve the fertility is due to the MDJ potential antioxidants, in general, scavenge and suppress the formation of ROS, or oppose their actions. These ameliorating effects of MDJ and its role in rehabilitating the sperm viability and testosterone might be due to its ability to scavenge the radicals.

Table 1 : The Effect of NaNO2 and MDJ on sperm concentration, motility, live sperm and Testosterone in male rats.

Parameters / Groups	Sperm Concentration n x 10^6/mm^3	Sperm Motility %	Live sperm% sperm	Testesteron ng/ml
Control DMSO 0.5ml	145.6 ±16.9 a	86 ± 3.6 a	73 ±2 a	1.53 ± 0.45 c
NaNo2 120mg/kg	45.5 ± 8.4 b	4.7 ±1.7 b	12.7 ±2.5 b	0.50 ±0.2 d
MDJ 48mg/kg	147.0 ±13.3 a	87.7 ±1.7 a	74.7 ±1.2 a	4.15 ± 0.61 a
NaNo2 + MDJ	148.6 ±12.6 a	85.2 ±3.5 a	74 ±2.1 a	3.12 ± 0.90 b
LSD	81.25	60.2	60.2	1.02

DMSO= Dimethyl sulfoxide , NaNo2 = sodium nitrite , MDJ= methionine derivative

Table 2 : Effects of NaNO2 and MDJ on, birth number, birth weight and fertility % in male rats mate with female for 10 days.

Parameters / Groups	NO. of female	NO. delivered female	Average NO. of Litters	Average Birth weight of litters gm	Fertility percent %
Control DMSO 0.5ml	4	4	10 ± 2.02 a	6.7 ± 0.8 a	100
NaNO2 120mg/kg	4	3	8.1 ± 1.2 a	5.0 ± 1.2 b	75
NaNO2 + MDJ48mg/kg	4	4	9.5 ± 2.1 a	6.2 ±0.9 a	100

DMSO= Dimethyl sulfoxide , NaNo2 = sodium nitrite , MDJ= methionine derivative

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