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Histological and Molecular study for detection of *Inh-b* genes in Male rats treated with Monosodium Glutamate

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Abstract

Inhibin b play positive role in sperm counts this used for evaluation infertility of male by improved process of spermatogenesis. In order to investigate the effect of (MSG) on testis of adult male rats, the present study has been carried out at the College of Science, Al-Qadisiyah University. mRNA expression level of *inh-b* gene have been evaluated in testes tissues as well. 24 adult male rats (aged 60 days and weighted (180-200 g) have been used in the present study. Three groups of animals, 8 rats in each .First represent control: they injected daily a single dose distal water. Second (T1): injected daily a single dose of 20 mg/kg of monosodium glutamate. Third (T2): injected daily a single dose of 40 mg/kg of monosodium glutamate. After the experiment period end of 40 days animals dissected. Testis tissue of groups removed quickly, dipped in liquid nitrogen for RNA extraction and molecular study. Epididymis tissue removed for study the sperm changes. The result showed a significant decrease ($P<0.05$) in sperm concentration, sperm ratio, live sperm ratio and normal sperm ratio in the groups during the experimental period compared to control group .Quantification analysis results of gene expression, performed by real-time RT-PCR, revealed that treatment with MSG caused significant decrease of mRNA expression levels of *inh-b* gene during the study period. It can be concluded that treated with MSG effect in the reproductive efficiency of male rats. As well as its negative role in lower the expression level of *inh-b* gene, when used at the given dose and for 40 days.

Keywords: Monosodium Glutamate, testicular function, rats.

INTRODUCTION

Pathological processes considerably include the normal responses of body organs to abnormal environmental influences .There are many environmental chemicals, food additives and industrial pollutants have been embroiled in causing harmful effects [1]. Monosodium glutamate is type of food additive. MSG is harmless and that it should be kept on the "Generally Recognized as Safe" this reports by (FDA) [2].One of the common amino acids present in life and considered basic components of protein and peptide is Glutamate. Glutamate have two main sources; first one: it can be form by the body itself that play a sensitive function in body metabolism, second one : foods with high protein content such as meat, milk, fish & cheese or vegetable origins like tomato & mushrooms [3]. Monosodium glutamate generally classified as a type of preserve taste or taste enhancer [4]. In general, Monosodium glutamate was classified as a safe food additive which does not need to restrict the dose used in the day. However, improper misuse of this due to their abundant use or lack of warning signs on foodstuffs [5].Monosodium glutamate influences the appetite center by improves the palatability of meals and thus affects the increase in body weight [6]. Anyway monosodium glutamate stimulate the taste of food as a result, this leads to increased eating. Studies have shown it to be a toxic substance to humans and animals. Monosodium glutamate also has a toxic effect on the testis tissue it causes a decrease in sperm number and effect of sperm morphology in male rats [7]. Male infertility is the result of this effect lead to bleeding in the testicular tissue, degeneracy and lack of sperm cell numbers [8]. Monosodium glutamate also has a toxic effects of nervous tissue represented by brain cell damage, degeneracy of retinal, defect of endocrine also some pathological conditions such as a stroke occurs in the brain , psychological shock, epilepsy , neuropathic pain, anxiety, schizophrenia, parkinson's, Huntington's finally Alzheimer's disease [9] .

Several studies have demonstrated the toxic effect of glutamate on the reproductive system of male by studying its effect on male rats. The induces of oligospermia and a change in the shape of the sperm dependent on the dose [10]. On the other hand, it has also been reported that Monosodium glutamate causes degeneration, haemorrhage in testicular tissue [11][12].

In the recent years, concerns increased to the misuse and of the effect of toxins for glutamate, with few histological studies on testicular tissue of animals treated with monosodium glutamate, the present study came to investigation the affectivity of the reproductive capability of adult male rats by using monosodium glutamate.

MATERIALS AND METHODS:

Experimental protocol: Thirty male rats aged 56 days with a mean weight of (180-200 gram) divided into three groups (8 animals in each group). first :control injected single dose of distal water per day., T1: injected daily of 20 mg/kg of monosodium glutamate.,T2: a single dose of 40 mg/kg of monosodium glutamate is injected in the day. Rats were sacrificed at the end of experimental by using an anaesthetizing Ketamine and Xylazine (10 / 90, i.p.), tissue was removed for histological and molecular examination.

Study of semen parameters:

Semen Analysis: The spermatozoa total number was counted by using the slide chamber (haemocytometer), express number of sperm cells in millions/ml. The fluid of epididymis was diluted with Tris buffer solution to 0.5 ml, in order to determine sperm motility, which was expressed in percentage (%). Abnormal features of sperm morphology such as neck and middle piece defects ,tail and head defects; expressed as percentage (%) of morphologically abnormal sperm [13][14][15][16][17].

Analysis of the results of the molecular study:

Rat testicular RNA:

Testicular RNA was isolated from testes depending on the method mentioned by the TRIzol® reagent [18].

Statistical analysis

The mean ± standard error of the mean (SDM) was used to express the results. By multivariate ANOVA by Graph Pad Prism (SAS Institute, Inc., USA). Statistical significance was $P<0.05$. [19].

RESULT

Semen analysis:

Sperm concentration of all the groups show in (Table 1). Sperm motility was decreased in all the treatment groups, with Group T2 given 40 mg/kg MSG having the least % motility (53.8 ± 1.87, $p<0.05$). This same group had the highest number of dead sperm cells, as revealed by the lowest Life Death ratio of 48±6.7. All the treatment groups however had increased in cell death compared to the Control, but not as much as Group T2 animals treated with 60 mg/kg MSG.

Table (1) Shows the effect of treatment with MSG on Semen Parameters

Groups / Parameters	Control	T1(20mg/kg)	T2(40mg/kg)
Count*(10^6 /ml)	2.43 ±0.53 a	1.98± 0.87 b	1.44± 1.34 b
Motility	89.56± 0.46 A	72.4± 0.67 b	53.98 ± 1.87 b
Viability %	88.5 ±0.25 a	64.2 ±1.3 b	48.67±2.4 b
Morphology%	75± 0.5 a	65.9± 0.8 b	51.54± 2.41 b

• The results represent the mean ± standard error
• Different letters represent significantly lower than control (P<0.05).
• C : control
• T1: Treatment group with 20 mg / kg body weight of MSG.
• T2: Treatment group with 40 mg / kg body weight of MSG.

Molecular analysis:

A rt-Pcr method was used to analyze the Expression of ***inh-b*** gene, then compare the results with expression of the *Gadph* gen as a control. RNA was extracted from testes tissue by using TRIZOL reagent according to the protocol of manufacturer.

Relative quantification of target genes expression:

The ***inh-b*** gene expression was calculated using 2^-ΔΔCt livak and Schmittgen method.

Relative Quantities of Expression of *inh-b* in Testicular Tissue:

Results showed that the levels of gene expression of ***inh-b*** gene decreased with increased concentration. The results showed that the level of gene expression of the studied gene in the testis tissue there was a significant decrease P< 0.05 in T2 group when comparing the results with control . Figure (1)

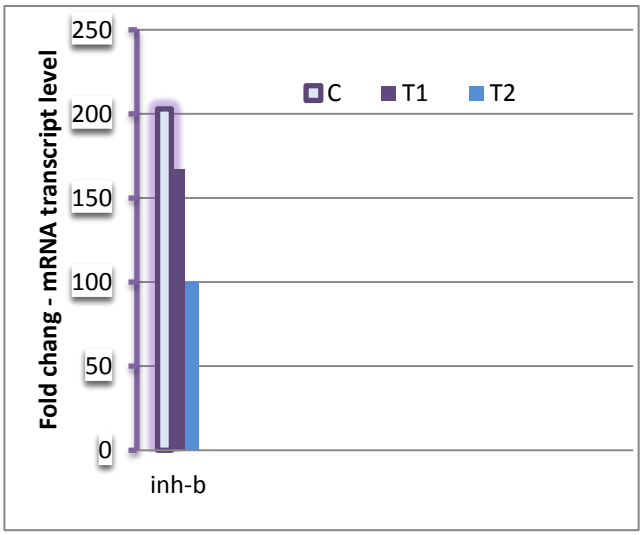

Figure (1) Fold change of ***Inh-b*** in testicular tissue
C : Control
T1: Treatment group with monosodium glutamate concentration (20mg / kg)
T2 :Treatment group with monosodium glutamate concentration (40mg / kg)

DISCUSSION:

Additives of most food act as flavor like MSG, it is widely used as flavor agent in whole world. Modern nutrition enables a continuous intake of the flavor enhancer, with resulting rise and accumulations of GA in blood [20]. Thus, the safety and toxicity of this food additive had become controversial because of the published data about its adverse reactions in people who usually eat foods containing it. [21].

1-Effect of MSG on some parameters of the sperm

The infertility in the world affects about 15% of all couples. Inhibin-b very important element for evaluation of male infertility by the effect on spermatogenesis. The results showed that MSG cause a significant decrease in sperm number in the experiment period ,so it is cytotoxic to the sperm [22]. Sperm counting is the most sensitive tests for spermatogenesis, where they reveal the important steps to produce the sperm, and thus affecting fertility. The T2 group was the lowest in sperm count was, this may be due to that the spermatogone is the most sensitive to the toxicity of MSG, another cells can be damaged and affecting the function of the epididymis may be another possibility of deficiency. The period of cycle of sperm formation in rats about 52 to 60 days, our results indicate that most affected cells are the spermatids, spermatocytes, and spermatogonia. There is a decrease in number of cells until the end of the 30 days. This is may be due to the influence on the stem cells.

The experimentation of the experimental animals with MSG concentration resulted in a clear reduction in the parameters of the sperm under study. The results showed that the morbidity decreased in both groups for both periods compared with control group. , testis is the target organ of glutamate. Receptors of Glutamate are found in many tissues such as hypothalamus, thymus, kidneys, endocrine system, ovary, etc [23]. Previous research have been evidence the existence of functional glutamate transporters and receptors in testis of rats [24][25] as well as in mice, one of the reasons is the effect of glutamate directly by glutamate receptors and transporters on the epithelial cells of the seminiferous tubules. The second reason have been proven by other studies [26] which confirmed that there are neurotoxin effects of MSG on the function of hypothalamus–pituitary–gonadal system. Since these studies indicated that the monosodium glutamate toxicity due to change in the inner membrane of mitochondria, this lead to a lack of GSH levels in mitochondria and high production level of H2O2 by the electron transmission chain in the mitochondria [27]. The defects of the characteristics sperm and infertility in male may be due to oxidative stress [28]. The results of the current study have been identical with other studies which proved the defect in the fertility of rat treated with glutamate [29].The effect of monosodium glutamate by receptors and on the cells of epithelial of the seminiferous tubules may be one of the mechanisms . The presence of free radicals in testes of rat injected with monosodium glutamate resulting from high levels of malondialdehyde, it is a marker of oxidative stress and it is also considered as a product of the prostaglandin metabolism [30].One study that was consistent with the results of our study, indicated that the spermatozoa exposure to free radicals as a result of height concentration of fatty acids in the plasma membrane of sperm and a very low concentration of cytoplasmic antioxidants [31].

Increasing level of lipid peroxidation may be is one of the important reason for damage of testicular tissues, the results of the study agree with Aitken *et al*., [32] where he confirmed that the increase in lipid peroxidation due to oxidative damage the nucleic acid of sperms, change the functions of membrane, lack of mobility and this leads to an effect in the development of sperm. Or production of free radicals as a result of monosodium glutamate toxicity effects on some physiological and biochemical

parameters of spermatozoa might be due to the lack of effectiveness of reproductive system of male rat.

2- **Effect of MSG on Relative Quantities of Expression of *inh-b* gene in Testicular tissue**.

Inh-b level it is very important marker for spermatogenesis and function of testes.

The results of the study showed a significant decrease in the expression levels of *inh-b gene* in the tissue of the testis during the studied period, this may be due to the effect of MSG on the testicular lipid hormone . About the molecular results detected in this study, the histological results went hand in hand with DNA results and supported them. Analysis of RNA samples showed marked decrease in the RNA expression in comparison to control group.The main function of p53 is to regulate metabolic defiance like the regulation of glucose stability, substrate oxidation, safety of mitochondria, autophagy and apoptosis. This has a role in regulating proliferation balance and growth with the availability of essential nutrient conversely ,a decline metabolic stress-induced damage [33]. Decrease in gene expression may be due to existence of free radicals, which are always produced during normal metabolism , where low levels of ROS can reduce reproductive susceptibility , high level of oxidative stress can damage nucleic acid, proteins and participation in aging, cardiac disease and cancer [34]. These findings could be interpreted by (ROS) fundamental. One of the main reasons of the decline in gene expression is the damage of DNA due to the formation of free radicals of various types as a result of treatment with monosodium glutamate. As in the process of mitochondrial oxidative phosphorylation, ROS are produced endogenously or they might arise from interactions with exogenous stimulus. Oxidative stress occurs when reactive oxygen species inhibit cellular antioxidant systems. Studies have shown that oxidative stress has a direct or indirect effect in a damage of nucleic acids and this contributes in carcinogenesis [35] [36]. The effects of MSG extracts on protein levels could be attributed to inhibition of RNA polymerase at the level of transcription, resulting in reduced gene expression, leading to reduced protein synthesis.

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