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Molecular Study of Some Genes in the Testicular Tissue of Male Wister Rats Treated with Monosodium Glutamate

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Abstract:

Food additive such as monosodium glutamate cause harmful effect for body. To investigate the effect of Monosodium Glutamate (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 E1f1ay, Ddx3y, Cyp17al and Cyp19 genes have been evaluated in testes tissue. 30 male Wister rats, aged (2 - 3) months, weighing between (200 and 250) grams. Three groups of animals 10 rats in each. First group as a control which injected daily a single dose of distal water. Second treated group (T1): injected daily a single dose of 40 mg/kg of monosodium glutamate. Third treated group (T2): injected daily a single dose of 60 mg/kg of monosodium glutamate. After the experiment period end of one month, the animals were dissected. Testis tissue of each group was 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 treated groups compared with 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 E1f1AY, Ddx3y, Cyp17al and Cyp19 genes during the study period. we can infer monosodium glutamate in high doses become harm for some reproductive system fertility parameters in 40 and 60 mg/kg doses because of oxidative stress which occur by it.

Keywords: Monosodium Glutamate, testis, gene expression, rats.

دراسة جزيئية لبعض الجينات في نسيج الخصى في ذكور جرذان وستر المعاملة بالكلوتاميت احادي الصوديوم

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الخلاصة

المواد الحافظة مثل كلوتاميت احادي الصوديوم يسبب تأثيرات ضارة للجسم . للتحقق من تأثير تأثير كلوتاميت احادي صوديوم في خصى ذكور الجرذان البيض، اجريت الدراسة في جامعة القادسية /كلية

العلوم . التعبير الجيني لـ E1f1ay , Ddx3y ,Cyp17al and Cyp19 قد قدر. 30 ذكر جرذ ابيض بعمر 2-3 شهر، وبوزن 200-250 غرام . وزعت على ثلاثة مجاميع كل مجموعة 10 جرذان . المجموعة الاولى كسيطرة حقنت يوميا بماء مقطر . المجموعة المعاملة الثانية استلمت حقنة واحدة من كلوتاميت احادي الصوديوم بجرعة 40 ملغم/ كغم يوميا. المجموعة المعاملة الثالثة استلمت حقنة من كلوتاميت احادي الصوديوم بجر عة 60 ملغم/كغم يوميا. بعد انتهاء فترة التجربة شهر . اخذت انسجة الخصى من كل مجموعة وغمرت بالنتروجين السائل لتسهيل عملية استخلاص RNA والتحليل الجزيئي. انسجة البربخ استخرجت لفحص تغيرات النطف. اظهرت النتائج انخفاض معنوي ($p<0.05$) في عدد النطف ، معدل النطف الميتة ، معدل النطف الفعالة والنسبة الطبيعية للنطف في الحيوانات المعاملة مقارنة بمجموعة السيطرة. نتائج التحليل الكمي لبيانات التعبير الجيني اظهرت ان كلوتاميت احادي الصوديوم يقلل بصورة معنوية التعبير الجيني للجينات E1f1ay , Ddx3y ,Cyp17al and Cyp19 خلال فترة التجربة. يمكننا ان نستنتج ان معاملة ذكور الجرذان بالكلوتاميت احادي الصوديوم بجرع عالية يصبح مضر لبعض معايير الخصوبة في جهاز التكاثر بالجرع المعطاة 40 و60 ملغم / كغم بسبب الجهد التأكسدي الذي يحدثه.

الكلمات المفتاحية:كلوتاميت احادي الصوديوم ، الخصى ، التعبير الجيني ، جرذان

Introduction

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, 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: it can be form by the body itself that play a sensitive function in body metabolism, second: foods with high protein content such as meat, milk, fish & cheese or vegetable origins like tomato & mushrooms (2).

Monosodium glutamate generally classified as a type of preserve taste or taste enhancer (3, 4). In general, Monosodium glutamate were classified as a safe food additive which do 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 stimulates 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). Monosodium glutamate can cause reproductive toxicity to male mice by damaging GnRH neurons (8). Male infertility is the result of this effect lead to bleeding in the testicular tissue, degeneracy and lack of sperm cell numbers (9). Monosodium glutamate also has a toxic effects on 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 (a psychiatric disorder characterized by multiple symptoms such as positive symptoms, negative symptoms, and cognitive symptoms (10), parkinson's and Alzheimer's disease (11) .also chronic glutamate abnormalities interact with acute stress to induce cognitive deficits, and resonate with gene x environment theories of schizophrenia (12).

Many studies have shown that sodium glutamate is related to male infertility through the degeneration and change of the number and morphology of sperm cells. (13,14). The induces of oligospermia and a change in the shape of the sperm dependent on the dose (15). On the other hand, it has also been reported that Monosodium glutamate causes degeneration, haemorrhage in testicular tissue (16,17).

On the other side Monosodium glutamate have effect on Y chromosome genes such as E1f1ay (encodes a protein related to eukaryotic translation initiation factor 1A (EIF1A) required for the binding of the 43S complex (a 40S subunit, eIF2/GTP/Met-tRNAi and eIF3) to the 5' end of capped RNA) and Ddx3y (responsible of RNA metabolism neuronal regulation, apoptosis, and cell cycle progression) (18) and on cytochrome P450 superfamily Cyp17a1gene (steroid biosynthesis steroid 17 α – hydroxylase) and Cyp19 gene (steroid biosynthesis aromatase) (19).

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 Wister rats, aged (2 -3) months, weighing between (200 and 250) grams. The rats were preserved in accustomed laboratory circumstances (25 °C and 60±10% humidity), rats are allowed freely to ordinary rat chow and water (20). Divided into three groups (10 animals to each group). first: control injected single dose of distal water per day., T1: injected daily single dose of 40 mg/kg of monosodium glutamate.T2: a single dose of 60 mg/kg of monosodium glutamate is injected in the day. After the experiment period end of one month, Rats were sacrificed 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. (21, 22, 23, 24, 25)

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 (26).

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 $P<0.05$ (27)

Result

Semen analysis:

The concentration of sperm in each group is shown in Table 1. All treatment groups showed a reduction in sperm motility, with G3 receiving 60 mg/kg MSG exhibiting the lowest percentage of motility. The group that had the lowest Life Death ratio also had the largest amount of dead sperm cells. While there was an increase in cell death in all treatment groups as compared to the healthy group, it was not as high as in Group G3, which received 60 mg/kg of MSG therapy.

(Table 1.) Effect of MSG on the semen activities

Groups / Parameters	Control	T1(40mg/kg)	T2(60mg/kg)
Count*(10^6 /ml)	2.58 ±0.45 a	1.88± 0.91 B	1.65± 1.46 B
Motility	88.56± 0.5 A	73.4± 0.82 b	55.9 ± 2.06 B
Viability %	87.5 ±0.3 a	60 ±2.3 b	49±2.4 b

Morphology %	75± 0.5 a	65.9± 0.8 b	52.5± 1.31 B

•The mean ± SE is shown in the findings.

•Letters that differ from the control indicate substantially lower values (P<0.05.(

•C: command

•T1: MSG at a dose of 40 mg per kilogram of body weight.

•

T2: MSG dosage of 60 mg/kg body weight in the treatment group.

Molecular analysis:

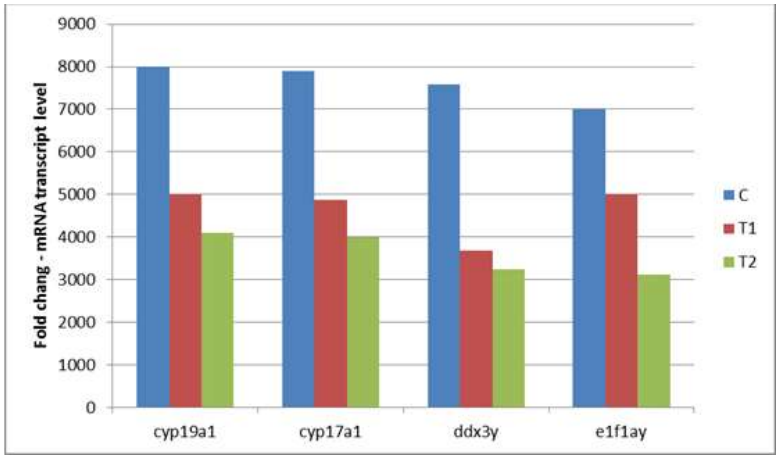
The gene expression of the (E1f1ay, Ddx3y, Cyp17a1, Cyp19a1) was analyzed using the rt-Pcr technique. The findings were then compared to the expression of the Gadph gene, which was used as a reference. TRIZOL reagent was used to extract RNA from testicular tissue in accordance with the manufacturer's procedure.

The target genes expression:

The 2^-ΔΔCt livak and Schmittgen technique was used to determine the gene expression of target genes.

Expression of target genes:

The results demonstrated that as concentration rose, so did gene expression for E1f1y, Ddx3y, Cyp17a1, and Cyp19a1. When comparing the T2 group's findings with those of the control group, they revealed a substantial drop in gene expression of the investigated genes at (P<0.05) in the testis tissue.



(Figure 1.) the gene expression E1f1ay, Ddx3y, Cyp17a1, Cyp19a1 in testicular tissue

C: Control

T1 (G2): treated group with monosodium glutamate (40mg / kg)

T2 (G3): treated group with monosodium glutamate (60mg / kg)

Discussion:

MSG is a commonly used food additive in processed food to enhance palatability. Modern nutrition enables a continuous intake of the flavor enhancer, with resulting rise and accumulations of GA in blood (28). 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. (29)

Effect of MSG on testicular changes in some parameters of the sperm

Sperm counting is the most sensitive tests for spermatogenesis, where they reveal the important steps to produce the sperm, and thus affecting fertility (30), and the results showed that MSG cause a significant decrease in sperm number in the experiment period, so it is cytotoxic to the sperm. 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 spermatogenic cells until the end of the 30 days. This is may be due to the influence on the stem cells. The T2 group was the lowest in sperm count , this may be due to that the spermatogenic cells 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 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. (31). Previous researches have been evidencing the existence of functional glutamate transporters and receptors in testis of rats (32, 33), 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 (34) 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 (35). The defects of the characteristics sperm and infertility in male may be due to oxidative stress (36). The results of the current study have been identical with other studies which proved the defect in the fertility of rat treated with glutamate (37).

Tezcan et al (38) explained that 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. 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 (39).

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., (40), 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. 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.

Effect of MSG on Relative Quantities of Gene Expression in Testicular tissue.

The results of the study showed a significant decrease in the expression levels of Cyp19a1, Cyp17a1, E1f1ay, Ddx3y in the tissue of the testis during the studied period, this may be due to the effect of MSG on the testicular lipid hormone. 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 (41). 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 (42), 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 (43, 44). 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 (45).

We can conclude that the increases in the dose concentration of MSG can affect and decrease some sperm parameters and decreased the gene expression of some genes in testis.

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