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The protective role of Quercetin in Mitigating MSG-induced nephrotoxicity and TNF-α in Male Albino Rats

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ABSTRACT

Background and Objective: This study aimed to evaluate the protective effect of quercetin against monosodium glutamate (MSG)-induced nephrotoxicity and oxidative stress in male albino rats.

Methods: The experiment was conducted in the Animal House of the Department of Life Sciences, College of Education for Pure Sciences, University of Anbar. Twenty adult male Sprague-Dawley rats (3–4 months old, weighing 200–250 g) were randomly assigned into four groups (n = 5). The control group received distilled water (5 mL/kg), the second group received quercetin (100 mg/kg), the third group received MSG (15 mg/kg), and the fourth group received MSG (15 mg/kg) followed by quercetin (100 mg/kg). All treatments were administered orally for 30 days. At the end of the experiment, serum urea and creatinine were measured to assess renal function, while malondialdehyde (MDA), total antioxidant capacity (T-AOC), and tumor necrosis factor-alpha (TNF-α) were evaluated as indicators of oxidative stress and inflammation.

Results: MSG administration significantly increased ($P \leq 0.05$) serum urea and creatinine levels, MDA concentration, and TNF-α levels, while significantly reducing T-AOC compared with the control group. In contrast, rats treated with quercetin following MSG exposure showed a significant reduction ($P \leq 0.05$) in urea, creatinine, MDA, and TNF-α levels, accompanied by a significant increase in T-AOC compared with the MSG-treated group.

Conclusions: Quercetin effectively attenuated MSG-induced renal dysfunction, oxidative stress, and inflammatory responses in male rats. These findings suggest that quercetin possesses Reno protective and antioxidant properties and may serve as a promising natural adjunct for reducing kidney toxicity associated with MSG exposure.

Introduction

Glutamate is the most prevalent non-essential amino acid in the body and comes in two primary forms: free glutamate and bound glutamate. Many foods, such as meat, fish, poultry, milk, and vegetables, naturally contain glutamate; the amount of free glutamate in vegetables is greater. Traditional sauces and spices are among the numerous prepared and processed meals that contain glutamate [1]. Originally originating in Asia, monosodium glutamate (MSG) has a unique flavour that is most prevalent there. Along with sweet, sour, salty, and bitter, it is regarded as the fifth fundamental flavour and is well-known in Western cultures[2].

One of the most popular food additives used worldwide for flavoring and enhancing food is monosodium glutamate. According to the US Food and Drug Administration (FDA), food makers are eager to continue using it despite the health issues it creates since it tastes delicious and is affordable [3]. While monosodium glutamate is frequently used as a food flavoring agent to enhance taste and appetite, studies have revealed that it can be toxic to laboratory animals, especially when used in high dosages, and to humans by negatively affecting the kidneys, liver, and testes [4].

Apoptosis, or programmed cell death, results from MSG's alteration of glucose metabolism, which lowers antioxidant defenses and damages DNA, proteins, lipids, and unsaturated fatty acids found in cell membranes [5]. Free radicals are increased as a result of mitochondrial action[6]. Monosodium glutamate (MSG) administration to neonatal mice has also been shown to harm certain brain areas that regulate neuroendocrine activities, including the arcuate nucleus and the ventromedial nucleus of the hypothalamus. Increased body weight, reduced motor activity, fat deposition, and growth hormone release are the results of this [7].

Medicinal and aromatic plants are becoming more and more popular worldwide. This illustrates the significance of these plants in both traditional and medical use. To make the most of these plants, biotechnology is being used in their development and seeding. Most people are looking to nature for a solution and using alternative medicine to avoid the negative effects that chemical medications and chemicals can have after using them [8].

Plants contain numerous biologically active compounds such as flavonoids, proteins, and terpenes. They also contain the pigment quercetin, which belongs to the flavonoid group and is found in many plants such as green tea, berries, apples, and onions. Quercetin is a common dietary antioxidant and has numerous health benefits, helping the body fight free radical damage associated with some chronic diseases such as heart disease, degenerative brain disorders, and certain types of cancer [9]. The current study aimed to evaluate the effectiveness of quercetin as a treatment for monosodium glutamate in male albino rats by examining different physiological conditions of nephrotoxicity in these rats.

Material and methods

The Experiment Design:

This research used a total of twenty male Sprague Dawley rats, each weighing between 200–250 g, aged between (3-4 months) , which were randomly divided into five groups (n = 5) based on similar body weights. Following a one-week acclimatization period, all animals received oral treatments via gastric gavage once daily for 30 days. The experimental groups were as follows:

- Group 1 (Control): Received 5 mL/kg of distilled water orally.
- Group 2 (Quercetin group): Received quercetin at a dose of 100 mg/kg in 2 mL of solution daily for 30 days.
- Group 3 (MSG group): Received monosodium glutamate (MSG) at a dose of 15 mg/kg in 2 mL of solution daily for 30 days.
- Group 4 (Monosodium Glutamate + Quercetin): Orally administered monosodium glutamate at a concentration of 15 mg/kg (2 mL) daily for one month, then left for 48 hours, followed by oral administration of quercetin at a concentration of 100 mg/kg (2 mL) daily for one month.

Blood Sample Collection:

At the end of the experimental period, animals were fasted for 24 hours. Anesthesia was induced via intraperitoneal injection of ketamine/xylazine at a dose of 0.1 mL/100 g body weight. Blood samples were collected directly from the heart using sterile 5 mL disposable syringes. The blood was transferred into plain tubes and centrifuged at 3000 rpm for 15 minutes. Serum was separated using micropipettes and stored at −20°C in labeled plastic tubes until biochemical analysis.

Assay of Oxidative Stress and Antioxidants:

Malondialdehyde (MDA) and Total antioxidant capacity (T-AOC) levels in the serum were assessed using commercially available

colorimetric assay kits (Elabscience, USA), following the manufacturer's instructions. Absorbance was measured using a spectrophotometer.

Measurement of the level of Tumor Necrosis Factor Alpha (TNF-α) in the blood serum:

Using an ELISA Microplate Reader and a pre-made test kit from the American company Elabscience (www.elabscience.com, **No:** E-EL-H0109), serum TNF-α levels were determined using the Sandwich ELISA technique.

Kidney function test:

The enzyme levels (creatinine, urea) were measured in blood serum using a ready-made test kit provided by the Spanish company Linear, using the UV enzymatic method on a Spectrophotometer.

Statistical Analysis:

Graph Pad Prism V.8.1 was used to statistically analyses the findings and determine whether there was a significant difference between the values of the treated groups. The ANOVA table was used for analysis of variance, and using fundamental statistical measurement techniques, the arithmetic mean and standard deviation were retrieved at a probability threshold of (P≤0.05). Post hoc comparisons were conducted using Duncan's multiple range test (10).

Result

Estimation of oxidative stress and antioxidants in the blood serum of male rats

Table 1 shows the effect of quercetin on oxidative stress. We notice a significant increase at the probability level (P≤ 0.05) in the activity of (MDA) malondialdehyde in the third group of male rats treated orally with (MSG) compared to the control group. Even though we see a dramatic decrease in the activity of (MDA) in the second group orally dosed with (quercetin) compared to the control group. Even though we see a dramatic decrease in (MDA) in the fourth group when compared to the third group dosed with (MSG), conversely, a dramatic decrease in the level of (MDA) is observed in the second group dosed orally with (quercetin).

Table 1 shows how quercetin affects total antioxidant capacity (T-AOC). Male rats in the second group showed a highly significant increase in total antioxidant capacity at the probability level (P≤ 0.05) when compared to the control group. However, the statistical analysis revealed a very sudden, significant decrease in the total antioxidant capacity of male rats from the third group at the probability level (P≤ 0.05) when compared to the control group. another hand that the antioxidant capacity of the fourth male rat group, which was given oral monosodium glutamate (MSG) plus quercetin, was considerably greater than that of the third group (MSG).

Table (1): The effect of quercetin on the levels of the (MDA) and (T-AOC) in male rats treated with monosodium glutamate

		Control	Quer.	MSG	MSG + Quer.
MDA nmol/ml	Mean	28.50 [a]	28.45 [b]	65.26 [c]	31.54 [a]
	± SD	±2.561	±2.680	±3.652	±3.778
T- AOC µ mol/ml	Mean	49.29 [a]	60.03 [b]	31.96 [c]	47.52 [a]
	± SD	±3.001	±3.265	±2.760	±2.778
Mean values within a column not sharing a common superscript letter (a, b, and c) were significantly different, p < 0.05.					

Effect of monosodium glutamate on the level of TNF-α in the serum of male rats treated with quercetin

The third group of male rats showed a very significant level of TNF-α (56.98±3.705) mI/pg at the probability level (P≤ 0.05) compared to the control group (33.45±2.547) mI/pg. , as shown in Figure 3. While the second group showed a significant reduction in the level of TNF-α at the

probability level (P≤ 0.05) (28.83±3.230) mI/pg compared to the third group. On the other hand, we observe a remarkable reduction in the level of α-TNF in the fourth group (37.15±2.778) mI/pg

when compared to the third group, which was treated with an MSG dose.

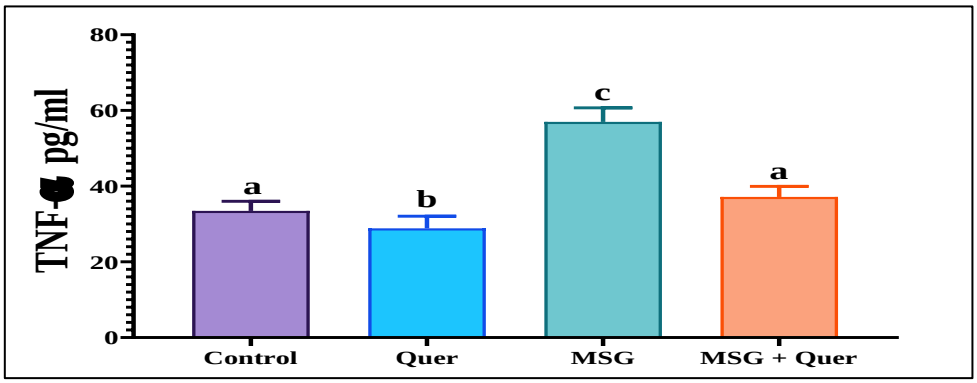

Fig .1 The effect of quercetin on the level of TNF-α in male rats treated with monosodium glutamate.
-Values are expressed as means ±SD; n=5 for each treatment group; means in each column with different superscript letters are significantly different ($p<0.05$).

Effect of monosodium glutamate on the level of kidney enzymes function in the serum of male rats treated with quercetin

Figures 2 and 3 present the findings about renal function. In the third group of male rats given monosodium glutamate (MSG) orally, the serum levels of both urea and creatinine were significantly higher at the probability level ($P\leq 0.05$) with 32.44 ±1.433dL/mg and 0.044±0.841 dL/mg, respectively, than in the control group (15.86±2.035) and (0.432±0.032), respectively.

However, the results of statistical analysis of the current study did not present any difference at the probability level ($P\leq 0.05$) for urea and creatinine among male rats that were treated with quercetin in the second group compared to the control. The result of the statistical test indicated a significant decline at the probability level ($P\leq 0.05$) of both urea and creatinine in male rats' blood serum in Group 4 to (15.25±1.543) dL/mg and (0.370±0.030) dL/mg, respectively, from the third group.

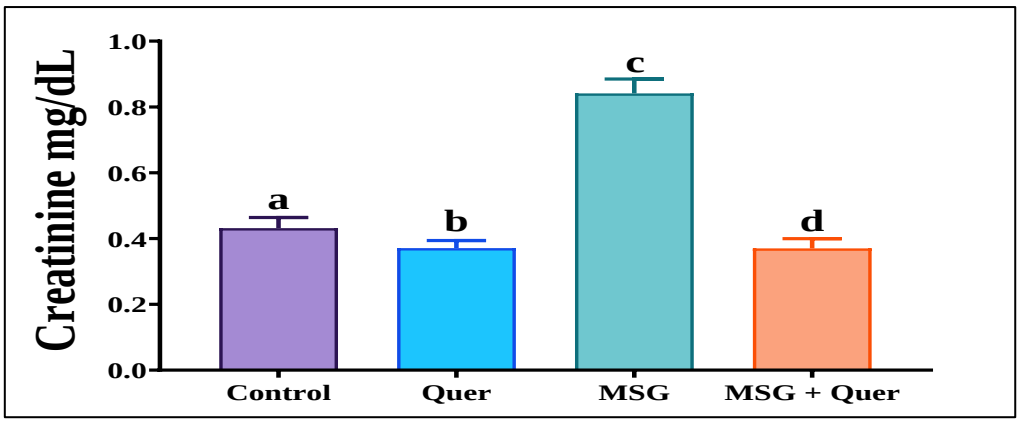

Fig 2. The effect of quercetin on the level of creatinine in e rats treated with monosodium glutamate.

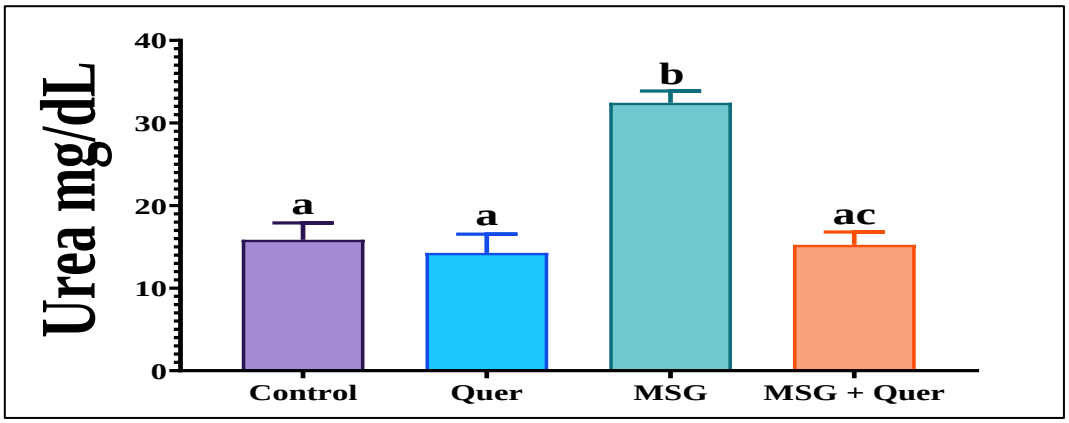

Fig.3 The effect of quercetin on the level of urea in male rats treated with monosodium glutamate. -Values are expressed as means ±SD; n=5 for each treatment group; means in each column with different superscript letters are significantly different ($p<0.05$).

The results of this investigation showed that when rats were given MSG at a dose of 15 mg/kg, the average levels of total antioxidants in their bodies considerably decreased ($P\leq0.05$), while the average level of MDA increased (Table 1). This conclusion was consistent with recent studies that showed that giving rats MSG caused oxidative stress by markedly increasing MDA levels and decreasing antioxidant levels **[11].** However, studies revealed that rats treated with MSG regularly suffered from oxidative stress and increased levels of free radicals, which damaged their liver and kidneys **[12].**

Elevated MDA indicates lipid peroxidation, a critical indicator of oxidative damage caused by reactive oxygen species (ROS). This suggests that the body produces more free radicals when MSG is consumed. These free radicals oxidise polyunsaturated fatty acids to lipid peroxidation, which produces MDA. By rupturing cell membranes and disrupting the balance between oxidants and antioxidants, this results in oxidative stress and can damage several organs **[13].** MSG facilitates this process by increasing intracellular Ca2+ levels, which either promote phospholipid degeneration or lipid peroxidation, according to one research **[14].** One study found that an increase in monosodium glutamate diet reduces total antioxidant capacity (T-AOC), hence increasing the population's risk of cardiovascular disease. The results of our investigation, which showed a significant decrease in antioxidants, indicated that this risk could be connected to increased intake of meat and meat-based processed foods **[15].**

The results of our investigation showed that the total antioxidant capacity of the second group was significantly more than that of the control group, and that of the fourth group was greater than that of the third. Total antioxidant capacity (T-AOC) increased in the quercetin-treated animal group, according to **[16]**. These outcomes are in line with our research, showing that long-term quercetin intake supports memory performance in older male rats and shields neurons from the harmful effects of oxidative stress brought on by ageing. This might be because quercetin strengthens the body's antioxidant defenses, which enhance organ function as a whole. The injection of quercetin, a potent antioxidant, is responsible for the rise in antioxidant levels in MSG treatments, either alone or in combination **[17].**

By reducing MDA levels because of higher antioxidant levels, the pycristine extract was found to help decrease lipid peroxidation levels in the groups that received it. By scavenging free radicals and blocking lipid peroxides in membranes that induce cell necrosis, these antioxidants help to maintain a balance between oxidants and cells. It also helped to rearrange the architecture of cells. Pycristine is an antioxidant that may scavenge free radicals.

According to our present study's findings, the animals in the third group that received MSG treatment had significantly higher levels of tumour necrosis factor (TNF-α). This could be because the endogenous development of cells produces more ROS. Numerous studies show that pathologic levels of endogenous TNF-α promote the development and spread of tumours. The cytokine TNF-α, which is generated by stromal cells and cancer cells in the tumour microenvironment, is linked to increased expression in cancer **[18].** Acute inflammation brought on by proinflammatory cytokines enhances the activity of tumour necrosis factor alpha (TNF-α), an active proinflammatory cytokine. Additionally, it plays a crucial role in mediating a number of physiological processes, such as insulin resistance, apoptosis, cell

proliferation, and the activities of blood vessel lining cells **[19].**

The results of our current study (as shown in Figure 1) verified that the animals in the third group that received MSG treatment had a markedly higher level of tumour necrosis factor TNF-α. This is in line with several studies that demonstrate a direct or indirect relationship between MSG consumption and an elevation in TNF-α [20, 21]**.**

The creation of free radicals, which react under specific circumstances to generate nitroso compounds-which are inflammatory substances may be the cause. Since cancer is linked to high expression of the cytokine (TNF-α), which is produced in the tumour microenvironment by stromal cells and cancer cells, there is ample evidence that pathological physiological concentrations of endogenous TNF-α promote tumour formation and growth **[22].** When MSG is consumed continuously, the liver of infected animals undergoes further alterations because of increased oxidative stress brought on by the buildup of free radicals, which causes the liver cells to collapse and lipid peroxides to accumulate in the cell or mitochondrial membranes. This causes sinusitis to worsen, inflammatory regions to develop, and poor venous flow at the level of the hepatic vein, or inferior vena cava **[5].**

The kidneys typically remove metabolic wastes such urea, uric acid, and creatinine from the body and acidify urine to maintain the ideal chemical composition of bodily fluids. The blood concentrations of these compounds rise with renal disease. Thus, elevated creatinine and urea levels in the impacted mice in our investigation suggest renal impairment. Our study's physiological and histological investigation results supported and demonstrated this **(Figures 2-3)**. The current study's findings demonstrated that mice given monosodium glutamate, which causes renal failure, had significantly higher blood levels of creatinine and urea. Elevated urea levels are caused by a low rate of urea elimination into urine. The findings of a study that found rats fed with MSG had higher blood urea levels support this **[11].**

Monosodium glutamate may be the cause of elevated urea and creatinine levels, which can be a sign of kidney dysfunction because of the increased production of free radicals that lead to oxidative stress from the buildup of nephrotoxic agents, which in turn causes necrosis or apoptosis from lipid oxidation in cell membranes. As a result of the oxidation of proteins and amino acids, the concentration of serum urea rises. Moreover, oxidative stress results in chronic complications from elevated free radicals, such as renal impairment, which raises urea and creatinine levels, as well as functional disruption of the cells of the inner layer of the kidney's glomerular capillaries, which raises blood creatinine concentration and lowers urine creatinine **[23].**

The oxidative stress linked to lower levels of antioxidants and antioxidant enzymes and increased production of free radicals that can peroxidase lipids in the kidney may be the cause of the raised creatinine levels and alterations in renal tissue. Our findings are in line with a prior study that found acute MSG poisoning in mice resulted in elevated blood serum levels of creatinine, urea, and calcium **[24].**

The anti-inflammatory and antioxidant properties of Christin are responsible for the reduction in the combined parameters of creatinine and urea levels in the fourth group when compared to the third group and the reduction in creatinine and urea levels in the fourth group when compared to the control group. Phenolic phytochemicals have a strong ability to scavenge several kinds of reactive oxygen species (ROS), which is one of their primary antioxidant activity mechanisms. The findings of our investigation align with the findings of the study by **[25],** which showed improvement in kidney histological alterations and a decrease in creatinine and urea levels. This might be explained by the protective function of Christin, which considerably lowers tubular necrosis and mitigates nephrotoxicity. In a prior study, **[26]** showed that rats given quercetin showed a smaller difference in basic renal parameters than the control group. This is what defines quercetin's successful application in our present investigation. Because quercetin is a natural antioxidant that acts on carbonates and amino acids from the body's urea production, it may help eliminate or repair cell damage from oxidative stress without raising glomerular filtration rate, which could explain the drop in urea and creatinine concentrations. Given the current study's results, it may not be feasible to draw the practical conclusion that quercetin, which lessened the physiological effects of MSG in the experimental group, may be a viable substitute for a number of pharmaceuticals that are made chemically.

Conclusion

The present investigation found that male albino rats exposed to monosodium glutamate

(MSG) for an extended period had reduced antioxidant capacity (AOCT) and increased oxidative stress (MDA), inflammatory markers (TNF-α), and the kidney enzymes (creatinine and urea). Compared to the MSG-only group, quercetin treatment considerably improved these parameters, resulting in a drop in enzymes and indicators of oxidative and inflammatory damage as well as an increase in antioxidant capacity.

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Declaration of interests

The authors declare that they have no competing interests

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Publication consent

All authors have read and approved the final version of the manuscript and consent to its publication.

Data and material availability:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author contribution:

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by the authors. The first draft of the manuscript was written by the first author, and all authors reviewed, revised, and approved the final version of the manuscript.

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الدور الوقائي لـ Quercetin في التخفيف من السمية الكلوية الناجمة عن MSG و TNF-α في ذكور الفئران البيضاء

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الملخص

الخلفية والهدف:
هدفت الدراسة الحالية إلى معرفة التأثير العلاجي للكيرسيتين Quercetinفي ذكور الجرذان البيض المعرضة للتسمم بكلوتاميت أحادي الصوديوم Mono sodium Glutamate (MSG).

المواد وطرائق العمل:
أجريت هذه الدراسة في البيت الحيواني التابع لقسم علوم الحياة - كلية التربية للعلوم الصرفة / جامعة الأنبار لمدة (30 يوم). اشتملت الدراسة على (20) ذكر من الجرذان البيض Males Albino Rates من نوع *Sprague Dawley* تراوحت أعمارهم بين (3 - 4 أشهر) بوزن (200gm – 250) ، قُسمت الحيوانات وفق أوزان متقاربة إلى أربع مجاميع، كل مجموعة تضم (5 جرذ). المجموعة الاولى (Control) جُرعت(5 mL/kg) ماء مقطر Distilled Water، المجموعة الثانية (Quercetin) جُرعت فموياً (100 mg/kg) بالكيرسيتين وبجرعة (2ml)، المجموعة الثالثة (MSG) جرعت فموياً بمحلول كلوتاميت أحادي الصوديوم تركيز (15 mg/kg) ، المجموعة الرابعة(MSG+ Quer) جرعت فموياً بـ (15mg/kg) بـ محلول كلوتاميت أحادي الصوديوم ، ثم تركت لمدة 48 ساعة بعدها جُرعت بـ (100 mg/kg) بـ الكيرسيتين يومياً لمدة شهر واحد، وبنفس الجرعة للمجموعتين الثانية والثالثة ، بعد انتهاء مدة التجربة (3 أشهر) تم التضحية بجميع الحيوانات.

النتائج:
أدى تجريع ذكور الجرذان بـMSG في المجمعة الثالثة إلى زيادة معنوية عند مستوى الاحتمالية وفي مؤشرات وظائف الكلى (Urea) و(Creatinine)، مع زيادة في تركيز الـ Malondialdehyde (MDA) ومستويات عامل نخر الورمي Tumor necrosis factor alpha (TNF-α)، وانخفاض معنوي في فعالية سعة مضادات الأكسدة الكلية (T-AOC) مقارنة بمجموعة السيطرة. من جهة أخرى لوحظ في المجموعة الرابعة ذات المعاملة المشتركة (كلوتاميت أحادي الصوديوم مع الكيرسيتين) انخفاض في المعنوية عند مستوى الاحتمالية ($P\leq0.05$) في فعالية وظائف الكلى (Urea) و(Creatinine)، وفي الـ (MDA)، وفي عامل نخر الورمي (TNF-α) ، ولكن زيادة في سعة مضادات الاكسدة الكلية (T-AOC) مقارنةً مع المجموعة الثالثة .

الاستنتاج:
تشير هذه النتائج إلى أن الكيرسيتين قد يساعد في تخفيف الآثار الفسيولوجية السلبية لاستهلاك MSG. علاوة على ذلك، وبفضل خصائصه المضادة للأكسدة، يمكنه أن يقلل بشكل فعال من سمية الكلى كعامل مساعد، مما يجعله بديلاً طبيعيًا محتملًا للعديد من أدوية العلاج الكيميائي المستخدمة لعلاج أمراض الكلى أو سميتها.

الكلمات المفتاحية: ***الكيرسيتين؛ الغلوتامات أحادية الصوديوم (MSG)؛ الإجهاد التأكسدي، الالتهاب؛ السمية الكلوية.***