

# Protective Effect of Curcumin Against Monosodium Glutamate-Induced Oxidative Renal Damage: biochemical and histopathological study

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## Abstract

**Introduction:** Monosodium glutamate (MSG) is frequently used as a flavoring agent and taste enhancer in processed modern foods. **Aim of study:** to assess the effects of MSG on the renal cortex and renal indices of rats and to investigate the possible protective effect of curcumin (biochemically, histologically and immunohistochemically). **Material and methodology:** for this study, 40 adult male albino rats (10 in each group) were used, first group (control) was given 2 ml / kg olive oil, second group received 100 mg/kg curcumin (CUR) dissolved in 2 mL/kg olive oil once daily, third group orally received 4 g/kg of MSG once daily for 14 days dissolved in distilled water and the fourth one orally received the same doses of CUR and MSG mentioned above once daily for 14 days. Blood and renal samples were collected from each group then analyzed. **Results:** MSG caused significant increase in urea, creatinine and renal malondialdehyde while, superoxide dismutase and catalase activities were decreased. Additionally, the histopathological deterioration matched with the biochemical analysis. Curcumin caused improvement of the alterations induced by MSG. **Conclusion:** MSG caused impairment of renal function by inducing oxidative damage, however, curcumin protected against MSG-induced nephrotoxicity by its antioxidative properties.

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## Key words

Curcumin- Monosodium glutamate- Nephrotoxicity

## Introduction

Monosodium glutamate (MSG) is used worldwide as a food flavor in variable kinds of processed foods (food additive number E621) (Owoeye and Salami, 2017). However, its safety is a controversial issue. It is a white, odorless, and crystalline powder which contains 78% glutamic acid, 22% sodium salt and water (Hajjhasani et al., 2020). The European Food Safety Authority proposed acceptable daily intake (ADI) of 30 mg/kg per day (Cynober et al., 2018).

Although there will be no issue with small amounts of MSG, this may be of a great problem at high doses and with frequent intake of regular small amounts. Moreover, MSG may be under other titles, causing difficulty in determination its amount in dietary supplies (Ragab, 2018).

The kidney is a vital organ with a central role in preservation the volume and composition of the total body fluid and acid-base balance (Ibrahim et al., 2019). It is more vulnerable to toxicity as it is exposed directly to the blood via open fenestrae in the glomerular capillaries. Additionally, the toxin is concentrated several times more than in other organs (Abass and Abd El-Haleem, 2011). The kidney has a vital role in elimination of MSG (Osman et al., 2012).

Curcumin is a yellow colored phenolic pigment widely used as a spice and a coloring agent in several

foods. Pharmacologically, curcumin has anti-inflammatory and antioxidant activities (Bayomy et al., 2011). The antioxidant effect happened by different mechanisms; it scavenges free radicals. Also, it inhibits reactive oxygen species-generating enzymes including lipoxygenase, cyclooxygenase, and xanthine oxidase (Puneeth and Sharada, 2015).

Curcumin was previously investigated against MSG-induced neurotoxicity in rats. The study of Khalil and Khedr, (2016) demonstrated its neuroprotective role. Curcumin also protected against monosodium glutamate induced rat thymocytes toxicity by reduction of apoptosis and reactive oxygen species formation (Vucic et al., 2018). Additionally, the beneficial action of curcumin in testicular toxicity was recorded in the study of Sakr and Badawy, (2013). It succeeded in restoration of testicular weight and the sperm count.

Therefore, the present work aimed to evaluate the nephrotoxicity caused by MSG, its effects on the oxidative state and the possible protective action of curcumin.

## Patients and Methods

### Animals:

Forty adult male albino rats were purchased from the experimental animal unit of Menoufia Faculty of medicine. Their weight ranged from 200-250 g. They

were housed in polypropylene cages and had free access to laboratory food and water during the experimental period. Rats were allowed to acclimatize for two weeks then were randomly divided into 4 groups.

Group I (control): Rats were orally given vehicle oil (2 ml / kg body weight olive oil) by stomach tube once daily for 14 consecutive days.

Group II (CUR): Experimental animals were orally given 100 mg/kg curcumin (CUR) dissolved in 2 mL/kg olive oil which increases its absorption once daily for 14 consecutive days by stomach tube (Damiano et al., 2020).

Group III (MSG): Rats were orally given 4 g/kg of MSG dissolved in distilled water once daily for 14 days by stomach tube (Owoeye and Salami, 2017).

Group IV (CUR + MSG): Rats orally received the same doses of CUR and MSG mentioned above separated by one hour by stomach tube once daily for 14 days.

The study was approved by the Institutional Ethical Committee (code: 9/2022FORE2).

#### Chemicals

- MSG and curcumin were purchased from Sigma-Aldrich Chemicals Co. (St.Louis, Mo, USA).
- Kits for urea, creatinine and oxidative stress markers were obtained from Biodiagnostic Co., Egypt.
- Monoclonal antibody against Bax was obtained from (Dako, Carpinteria California, USA).

Blood samples: 24 hours after the last dose of MSG, blood sample was obtained from all rats by puncturing their retro-orbital plexus using microcapillary tubes according to Joslin, (2009) then the rats were sacrificed. Serum was obtained by centrifugation for determination of urea and creatinine levels. Serum levels of urea were estimated according to Rock et al., (1987). Determination of serum creatinine was done as described by Bjurosson, (1979). Results were expressed as mg/dl.

Rats were sacrificed by cervical dislocation after being anaesthetized by ether. The kidneys were dissected out, weighted then divided into two parts. The first part was homogenized as 1g of tissue in 10 ml of phosphate buffer using a homogenizer then centrifuged. The obtained supernatants were stored at -20 °C until tissue assay. The second part was fixed and processed for paraffin sections.

Determination of oxidative stress markers in kidney homogenate

- The malondialdehyde concentration was measured spectrophotometrically (T60 UV VIS Spectrophotometer, UK) according to Draper and Hadley, (1990) using the thiobarbituric acid reaction and expressed as nmoles /g of tissue.
- The activities of superoxide dismutase (SOD) and catalase (CAT) were measured by the methods of Beauchamp and Fridovich (1971) and Aebi, (1984) respectively and the results were expressed as units/ mg of protein.

#### Histopathological examination:

Kidneys were fixed in 10% formalin solution. Paraffin wax blocks were prepared, sectioned then were processed for staining with hematoxylin and eosin (H&E) according to Bancroft and Layton, (2012). Sections from all groups were examined by light microscope.

Immunohistochemical staining for proapoptotic protein Bax

Sections were incubated with a monoclonal antibody against Bax in a dilution of 1:200. After washing, biotinylated secondary antibodies were added, followed by avidin-biotin peroxidase complex. Next, Visualization of the immune reaction was done with diaminobenzidine. The sections were counterstained with hematoxylin. Positivity appears as brown precipitation in the cells.

#### Statistical analysis:

The data obtained were analyzed by the SPSS for windows, version 26 and were demonstrated as mean  $\pm$  standard deviation. ANOVA test was applied to compare experimental groups followed by Tukey's post hoc test. Differences were regarded as significant when P values were less than 0.05

#### Results

None of the rats died during the study period. Rats in group (II) showed nearly the same results as control group indicating safety of curcumin dose with no histological difference observed by light microscope.

#### Laboratory analysis

There was no significant difference between the mean values of the control and curcumin groups in all measured parameters. MSG caused significant increase in urea, creatinine, MDA levels and renal weight and a significant reduction in SOD and CAT activities than the control and curcumin groups. Co-administration of curcumin with MSG decreased urea, creatinine, MDA and kidney weight and increased SOD and CAT significantly than MSG group. The correction of creatinine, kidney weight and CAT were insignificant with the control.

#### Serum levels of urea and creatinine

The mean value of serum urea was significantly higher in MSG exposed rats ( $53.5 \pm 7.4$  mg/dl) in comparison with the control value ( $32.4 \pm 4.5$  mg/dl) (p-value <0.001). Administration of curcumin with MSG corrected these changes significantly in relation to MSG group (p-value 0.007), but they were still significantly higher than the controls (p-value <0.001) (table 1).

As regard creatinine, it was significantly higher in MSG intoxicated rats ( $1.3 \pm 0.4$  mg/dl) when compared to the control animals ( $0.7 \pm 0.09$  mg/dl) (p-value <0.001). Addition of curcumin significantly showed significantly lower creatinine level ( $0.9 \pm 0.2$  mg/dl) than in MSG treated group. CUR + MSG treated group was statistically unchanged compared with the control values, table (1).

#### Kidney weight

MSG administration caused significantly higher renal weight ( $1.4 \pm 0.3$  g) compared to the measurements of the controls ( $0.8 \pm 0.04$  g) (p-value

<0.001). The kidney weight of CUR + MSG group (0.9±0.2 g) was significantly (p-value <0.001) less than that of the MSG-given group with insignificant change comparable to the control animals as demonstrated in table (2).

#### Oxidative stress markers in kidney homogenate

MSG group (Group III) revealed a significantly higher MDA level (15.3±2.1 nmoles /g) than the control group (8.7±0.9 nmoles /g). Concomitant administration of curcumin antagonized the oxidative stress caused by MSG with significant reduction of renal MDA level (12.5±1.6 nmoles /g) compared to the MSG group (p-value =0.001), but these reduced values still differed significantly from the control rats (p-value <0.001).

MSG-intoxicated rats showed significant decrease (p<0.001) in SOD activity (12.8±1.6 units/mg protein) as compared with the controls (18.7±0.9 units/mg protein). Concomitant treatment of rats with curcumin significantly increased SOD activity in the renal tissue (14.7±1.6 units/mg protein) (p -value = 0.022) in relation to group III as shown in table (2).

Table (2) also revealed similar changes related to CAT activity. The activity was decreased significantly (p-value <0.001) in MSG group (55.7±9.8 units/mg protein) compared to the control group (83.2±5.8 units/mg protein). Curcumin addition in group IV caused significantly higher activity (74.7±6.8 units/mg protein) compared to that in MSG group (p-value <0.001) with no significant change in relation to the control value.

#### Histopathological examination

*Sections stained with hematoxylin and eosin:*

The sections of the renal cortex of the control and curcumin groups were nearly similar, showing normal appearance of the renal corpuscle containing the glomerulus, proximal tubules and distal tubules (Fig. 1).

MSG group showed disorganized renal structure; shrunken glomerular tufts with dilatation of the capsular space, vacuolated cytoplasm of tubular cells with separation from basement membrane, tubular dilatation and interstitial hemorrhage (Fig. 2A and 2B).

Renal cortex of combined curcumin and monosodium glutamate group (Group IV) showed improvement of renal corpuscles and tubules except for the presence of congested intertubular capillaries (Fig.3).

*Sections immunostained of BAX antigen:*

In both the control and curcumin-treated rats, the renal tissue showed negative immunostaining for Bax (Fig.4).

In MSG intoxicated rats (Group III); the sections showed strong positive immunoreaction for BAX appeared as dark brown granules throughout the cytoplasm of most of the cells (Fig.5).

Kidneys of combined curcumin and monosodium glutamate group (Group IV) revealed that some cells appeared negative staining and others were weakly positive (Fig.6).

**Table 1: Statistical comparison between the mean values of serum level of urea and creatinine in the studied groups using ANOVA test.**

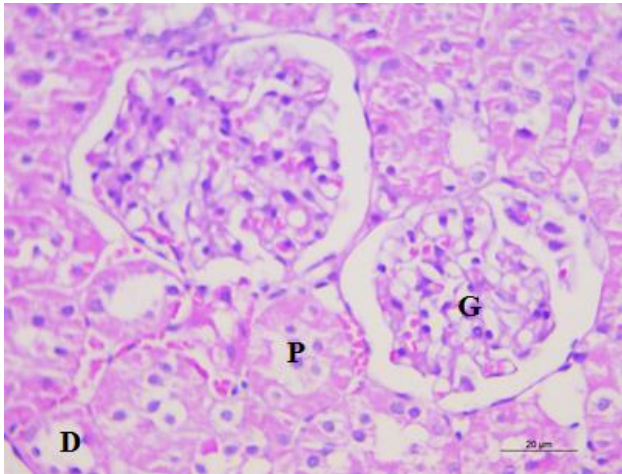
	Group I Control	Group II CUR	Group III MSG	Group IV CUR + MSG
Urea (mg/dl)	32.4±4.5	31.4±4.8	53.5±7.4*	45.1±4.0* <sup>@</sup>
Creatinine (mg/dl)	0.7±0.09	0.7±0.06	1.3±0.4*	0.9±0.2 <sup>@</sup>

*The results are expressed as Mean ± SD. \* Significant compared to control group, @ significant compared to MSG group*

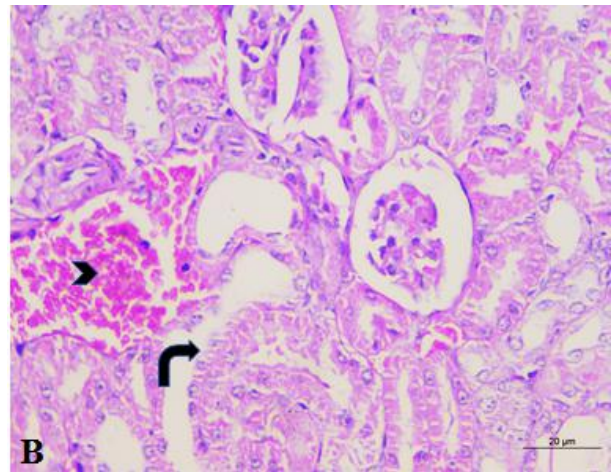
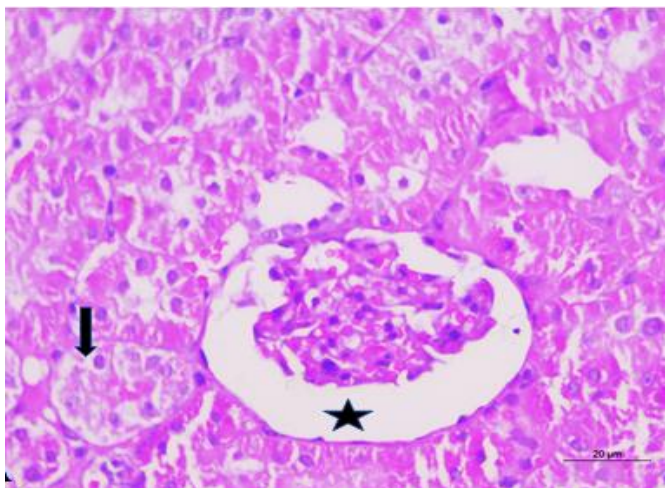
**Table 2: Statistical comparison between the mean values of Kidney weight and oxidative stress markers measured in the renal tissues of studied groups using ANOVA test.**

	Group I Control	Group II CUR	Group III MSG	Group IV CUR + MSG
Kidney weight (g)	0.8±0.04	0.8±0.04	1.4±0.3*	0.9±0.2 <sup>@</sup>
MDA (nmoles /g)	8.7±0.9	8.2±1.0	15.3±2.1*	12.5±1.6* <sup>@</sup>
SOD (units/mg protein)	18.7±0.9	18.5±1.1	12.8±1.6*	14.7±1.6* <sup>@</sup>
CAT	83.2±5.8	84.3±8.5	55.7±9.8*	74.7±6.8 <sup>@</sup>

*The results are expressed as Mean ± SD, CAT: Catalase, SOD: Superoxide dismutase. \* Significant compared to control group, @ significant compared to MSG group*

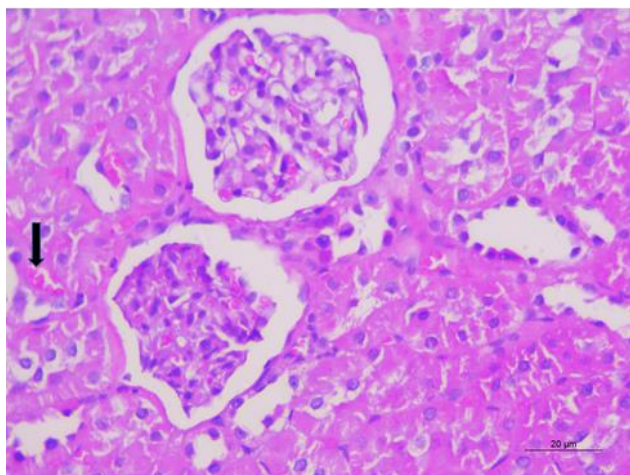


**Figure 1:** Photomicrograph of a transverse section of adult male albino rat renal cortex of control group showing normal appearance of the renal corpuscle containing the glomerulus (G), proximal tubules (P) and distal tubules (D) (Hx & E x400).

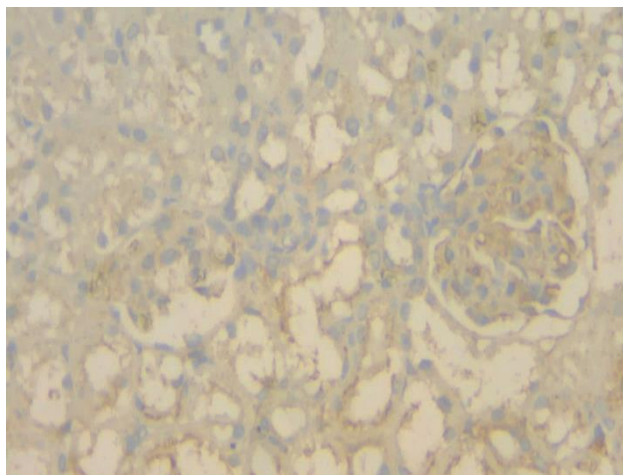


**Figure 2 A, B:** Photomicrographs of a transverse section of adult male albino rat renal cortex of monosodium glutamate group showing shrunken glomeruli with widening of the capsular space (star), vacuolated cytoplasm of tubular cells with separation from basement membrane (arrow), tubular dilatation (bended arrow) and interstitial hemorrhage (arrowhead) (Hx & E x400).

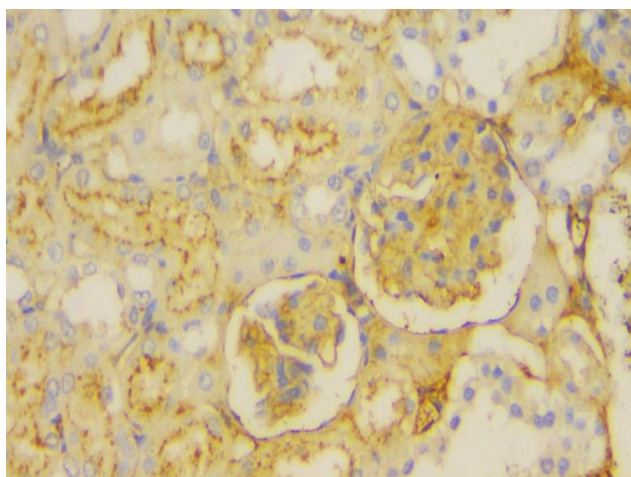




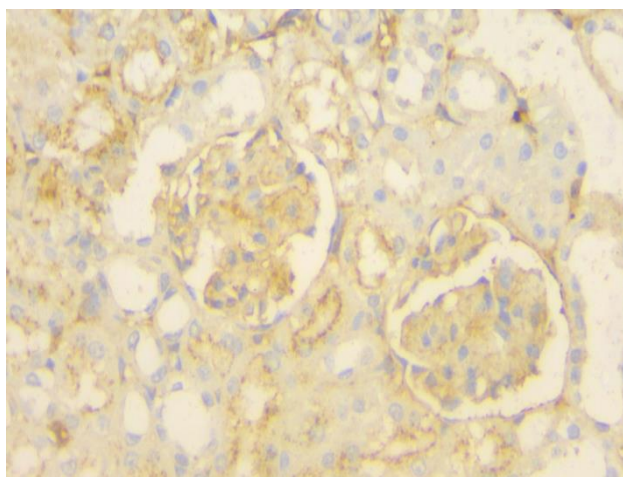
**Figure 3:** Photomicrograph of a transverse section of adult male albino rat renal cortex of curcumin and monosodium glutamate group showing improvement of renal corpuscles and tubules except for the presence of congested intertubular capillaries (arrow) (Hx & E x400).



**Figure 4:** Photomicrograph of a transverse section of adult male albino rat renal cortex of control group showing negative immunostaining reaction for BAX (x400).



**Figure 5:** Photomicrograph of a transverse section of adult male albino rat renal cortex of MSG group showing a strong positive immunoreaction for BAX (x400).



**Figure 6:** Photomicrograph of a transverse section of adult male albino rat renal cortex of curcumin and monosodium glutamate group showing some cells appear negative staining and others are weakly positive for BAX (x400).

## Discussion

MSG is a slow poison that negatively affects the human body especially with chronic administration. With the increasing consumption of MSG, daily intake may be exceeding the recommended dose and the toxicity will be finally resulted (Osman et al., 2012).

MSG increased the kidney weight significantly. This can be explained by increased food consumption by increasing its palatability and also by the inflammatory activity with resultant tissue edema (Badawi, 2019). The study of Tawfik and Al-Badr (2012) reported significant elevation of the relative liver and kidney weights of rats administered MSG. Similar results were observed in albino mice by Muslim, (2020).

These results were in line with the previously published data from other researches that demonstrated MSG induced obesity (Hermanussen et al., 2006; Seiva

et al., 2012). In a similar way, He et al. (2011) recorded that its intake is associated with overweight in Chinese adults.

MSG-treated group developed variable histopathological changes in the renal cortex with distortion of renal cytoarchitecture in the form of shrunken glomerular tufts with dilatation of the capsular space, vacuolated cytoplasm of tubular cells with separation from basement membrane, tubular dilatation and interstitial hemorrhage. Similar observations were made by Dixit et al., (2014).

Regarding serum biochemical analysis, the current work demonstrated a significant elevation in the levels of the kidney indices measured in MSG-intoxicated rats indicating renal dysfunction. This was previously recorded by Sandharbh et al. (2015) and Elbassuoni et al., (2018).

In agreement with the results of the present study, Paul et al. (2012) recorded reduced renal activities of antioxidant enzymes and increased MDA level (the final result of lipid peroxidation) after MSG administration. The plenty of long-chain polyunsaturated fatty acids in the kidney lipids makes it more prone to oxidative stress by reactive oxygen species (Sharma, 2015). Superoxide dismutase and catalase activities decrease resulted in renal cellular injury and dysfunction.

The changes related to the activity of the antioxidant enzymes due to MSG were similarly recorded in the cardiac tissue by Singh and Ahluwalia (2012) and in the brain tissue by El-Shobaki et al., (2016) and Hazzaa et al., (2020). The same was reported also in MSG induced hepatotoxicity and testicular toxicity by Khayal et al. (2018). These results confirmed that the toxicity of MSG on different body tissues is through oxidative stress.

MSG caused upregulation of Bax protein (apoptotic inducer) in renal tissue. BAX overexpression leads to cell death as it increases mitochondrial permeability (Dejean et al., 2006; Sanz et al., 2008). Similar observations were made by Abass and Abd El-Haleem (2011).

Marked improvement was observed in renal tissue of rats of group IV with milder histopathological changes. These results agreed with the study of Manikandan et al., (2011) who demonstrated the nephroprotective action of curcumin against toxicity induced by gentamicin in rats.

Curcumin which is a natural product successfully improved the renal dysfunction evident by the significantly lower renal dysfunction biomarkers. This effect may be related to its antioxidant activities as it was found that reactive oxygen species (ROS) may be concerned with the reduction of glomerular filtration rate (Bayomy et al., 2011). In accordance with the present findings, previous researches concluded that curcumin lowered the elevation of these serum parameters in gentamicin-induced nephrotoxicity (Al-kuraishy et al., 2019). Moreover, the study of Al-Amoudi, (2013) showed that curcumin improved kidney dysfunction induced by Chlorpyrifos (Organophosphate insecticide).

Curcumin showed strong antioxidant activities and free radicals scavenging properties as it reduced the elevated MDA levels, and significantly increased the SOD and CAT activities. Curcumin protected against oxidative stress damage resulted from over formation of free radicals than their detoxification by antioxidants (Al-kuraishy et al., 2019). Similarly, administration of curcumin attenuated the nephrotoxic effect of paracetamol by its ability to decrease MDA level and increase its antioxidant enzymes activity (Ibrahim et al., 2019).

Curcumin renoprotective effect occurs through different mechanisms. The reduction of oxidative stress by prevention of ROS formation and promoting transcription of genes of antioxidant enzymes. Curcumin can also reduce the inflammatory process through decreasing the inflammatory mediators.

Additionally, it reduces the cytokines which lead to renal fibrosis (Trujillo et al., 2013).

## Conclusion

Regular intake of MSG can lead to renal damage; hence it should be avoided in renal disorders. Curcumin had a protective effect against its nephrotoxicity. It caused improvement of renal function parameters and correction of oxidative stress as well as histopathological changes. More public awareness towards the toxic effects of MSG should be considered. The amount of MSG should be listed by food manufacturers on their packaging to know its daily consumption. Minute amount of curcumin can be added to food which contains MSG to overcome its toxicity.

## Conflict of interest

None declared

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## التأثير الوقائي للكرمين ضد الضرر التأكسدي بالكلية المحدث بالجلوتامات أحادية الصوديوم: دراسة بيوكيميائية و هستولوجية

شيرين رجب سليمة<sup>١</sup> و رشا رجب<sup>٢</sup>

### الملخص العربي

#### ملخص البحث:

**المقدمة:** تستخدم الجلوتامات أحادية الصوديوم كمحسنة للنكهة. أظهرت العديد من الدراسات أن لها تأثيرات سامة على مختلف الأعضاء. يعتبر الكركمين من مضادات الأكسدة القوية. **الهدف:** تقييم السمية الكلوية الناتجة عن تناول جلوتامات أحادية الصوديوم في ذكور الجرذان البيضاء والتأثير الوقائي المحتمل للكرمين. **المواد والطرق المستخدمة:** اشتملت هذه الدراسة على ٤٠ من ذكور الجرذان البيضاء لمدة ١٤ يوماً. تم تقسيم الجرذان إلى أربع مجموعات كل مجموعة تكونت من ١٠ جرذاً :

المجموعة الأولى (مجموعة الضابطة) : كل جرذ تم إعطاؤه زيت الزيتون ٢ مل/كجم مرة واحدة يومياً عن طريق الفم.

المجموعة الثانية (مجموعة الكركمين) : كل جرذ تم إعطاؤه ١٠٠ مجم / كجم من الكركمين مذاب في ٢ مل/كجم زيت زيتون مرة واحدة يومياً عن طريق الفم. المجموعة الثالثة (مجموعة الجلوتامات أحادية الصوديوم) : كل جرذ تم إعطاؤه ٤ جم / كجم من الجلوتامات أحادية الصوديوم مذاب في ماء مقطر مرة واحدة يومياً عن طريق الفم. المجموعة الرابعة (مجموعة الجلوتامات أحادية الصوديوم و الكركمين) : كل جرذ تم إعطاؤه الكركمين مع الجلوتامات أحادية الصوديوم بنفس الجرعات والطريقة السابقة. تم أخذ عينات دم من الجرذان لقياس مستويات اليوريا والكرياتينين. ثم تم أخذ عينات من الكلية لعمل دراسة نسيجية ومناعية، وكذلك قياس دلالات الإجهاد التأكسدي. **النتائج:** أظهرت النتائج في مجموعة الجلوتامات أحادية الصوديوم زيادة في مستوي اليوريا والكرياتينين وتسبب تغيرات نسيجية ملحوظة في الكلي وتغيرات في دلالات الإجهاد التأكسدي بالكلية وبإعطاء الكركمين حدث تحسناً ملحوظاً في النتائج السابقة . **الإستنتاج :** أكدت النتائج التأثير السام للجلوتامات أحادية الصوديوم على الكلي من خلال الإجهاد التأكسدي. وأظهرت النتائج أيضاً أن الكركمين له تأثير وقائي من خلال تأثيره المضاد للأكسدة.

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