

## **Nephrotoxicity of Two Food Additives (Aspartame and Monosodium Glutamate) in Adult Albino Rats: Biochemical and Histopathological Effects**

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**Abstract:** Both aspartame (ASP) and monosodium glutamate (MSG) are added to many food stuffs, hence any meal may contain both of them, so their harmful effects should be studied together. Evidences of ASP nephrotoxic effect have increased; however, little information is available about nephrotoxicity of MSG. This study assessed the nephrotoxic effects of ASP & MSG administered either individually or in-combination. Forty eight male adult albino rats were divided into eight equal groups: negative control; positive control; ASP-treated; MSG-treated; "ASP&MSG"-treated; ASP-recovery; MSG-recovery and "ASP&MSG"-recovery. A single daily dose of ASP (250mg/kg) and MSG (4g/kg) were given orally according to the study regimen. The present study documented nephrotoxic effects of ASP and MSG evidenced by a significant increase in serum urea, creatinine and tissue malondialdehyde levels; a significant decrease in tissue reduced glutathione level; and renal structural damages detected in all treated groups. The worst findings were detected in "ASP+MSG"-treated group. Partial improvement in the renal histopathological and biochemical results was detected in all recovery groups.

**Keywords:** Nephrotoxicity, Food Additives, Aspartame, Monosodium Glutamate, Recovery.

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### **I. Introduction**

Artificial sweeteners are food additives that give sweet taste without increasing caloric intake [1]. Aspartame (L-aspartyl-L-phenylalanine methyl ester) is one of the main artificial substances that replace glucose/fructose sugar [2].

Aspartame (ASP) is rapidly metabolized after ingestion producing three components, namely phenylalanine, methanol and aspartic acid. The major metabolite responsible for the harmful effects of ASP is methanol which is converted into formaldehyde and then to formic acid [3].

Evidences of ASP nephrotoxic effect have increased. Several studies demonstrated that consumption of ASP had led to a dose-dependent increased production of free radicals in renal tissues as well as kidney injury; nevertheless it is difficult to make a definitive conclusion regarding nephrotoxic effect of ASP [1].

Monosodium glutamate (MSG) is a food additive that present in packaged foods and may be hidden on the label [4]. MSG is the sodium salt of glutamic acid, it intensifies the savory relish in foods [5].

Monosodium glutamate is a toxic substance to human and experimental animals. Its ingestion can lead to asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort [4].

Various studies stated that MSG exerts its harmful effects by the induction of oxidative stress in different tissues of experimental animals after chronic administration. However, little information is available about the harmful effects of MSG on kidney functions [6].

Indeed both ASP and MSG are added to many food stuffs all over the world by the food industries to enhance palatability of food or many other reasons, so any food meal may contain both ASP and MSG, hence the harmful effects of the consumption of these food additives should be studied together.

### **II. Aim Of The Work**

The present work was performed to assess the nephrotoxic effects of two food additives [Aspartame (ASP) and monosodium glutamate (MSG)] administered either individually or in combination in adult Wistar albino rats.

### III. Material And Methods

#### 3.1. Chemicals:

Aspartame powder of purity  $\geq 99\%$  was purchased from Sigma-Aldrich, Inc. (3050 Spruce Street, Saint Louis, MO 63103, USA), CAS Number: 22839-47-0. Monosodium glutamate (MSG) powder of purity  $\geq 99\%$  was purchased from Sigma-Aldrich, Inc. (3050 Spruce Street, Saint Louis, MO 63103, USA), CAS Number: 142-47-2. Distilled water was purchased from Misr Chemical Industries Co, Cairo, Egypt. All other used chemical reagents were of analytical grades and were freshly prepared before each experiment.

#### 3.2. Animals:

Forty eight male adult Wistar albino rats brought from Experimental Animal Breeding Farm, Helwan-Cairo, weighing between 180-200 g, at the beginning of the study were acclimatized for one week (6/cage) in a fully ventilated room at Department of Pharmacology, Faculty of Medicine, Benha University. Rats were allowed free access to balanced diet and distilled water. All experimental protocols were approved by the Local Ethical Committee of Faculty of Medicine, Benha University.

#### 3.3. Dosage regimen and vehicle:

Aspartame powder was dissolved in distilled water resulting in a suspension of 50mg/ml, and it was given as a single dose (250mg/kg body weight/day), orally via appropriate gastric gavage, according to [7].

Monosodium glutamate powder was dissolved in distilled water resulting in a solution of 0.8g/ml, and it was given as a single dose (4g/kg body weight/day), orally via appropriate gastric gavage, according to [8].

#### 3.4. Experimental design:

Rats were divided into eight equal groups (six each):

**Group I (negative control):** left without intervention to measure the basic parameters; they were allowed to free access to balanced diet and distilled water for the end of the experiment.

**Group II (positive control):** treated with 1ml of distilled water (solvent) as a single dose, by gastric gavage for 2 months.

**Group III (ASP-treated):** treated with aspartame (250 mg/kg/day) dissolved in distilled water as a single dose, by gastric gavage for 2 months [7].

**Group IV (MSG-treated):** treated with monosodium glutamate (4 g/kg/day) dissolved in in distilled water as a single dose, by gastric gavage for 2 months [8].

**Group V ("ASP & MSG"-treated):** treated with both ASP and MSG with the same doses, routes and period as mentioned before in groups "II & III".

**Group VI (ASP-recovery):** treated with ASP as in group II, but they were left for one month after stopping treatment to recover before they were sacrificed.

**Group VII (MSG-recovery):** treated with MSG as in group III, but they were left for one month after stopping treatment to recover before they were sacrificed.

**Group VIII ("ASP & MSG"-recovery):** treated with both ASP and MSG as in group IV, but they were left for one month after stopping treatment to recover before they were sacrificed.

#### 3.5. Sample preparation:

At the end of the experiment, rats were scarified under ether anesthesia by decapitation after being fasted; blood samples were collected from abdominal aorta in clean and dry centrifuge tubes, which were left for 15 minutes to clot, and then centrifuged at 2500 rpm for 20 min using centrifuge 5418R (Eppendorf, Ontario, Canada) to separate the serum, then it was stored at  $-20\text{ C}^{\circ}$  for biochemical analysis.

Both kidneys were quickly removed, washed with ice cold physiological saline; part of each kidney was rapidly frozen at  $-80\text{ C}^{\circ}$  until analyzed, then homogenized in 10 ml ice-cold phosphate buffer (50 mM, pH 7.4, 0.1% triton and 0.5 mM EDTA), this homogenate was centrifuged at 3000 g for 15min at  $4^{\circ}\text{C}$  using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany) and the resulting clear supernatant was used for biochemical analysis. The other parts were preserved in 10% formalin for histopathological examination.

#### 3.6. Studied parameters:

##### 3.6.1. Renal functions:

- Serum creatinine was determined by Jaffé. Colorimetric - kinetic method, using the commercial kit of Spinreact Creatinie-J with spinlab (Spinreact Company), Spain [9].
- Serum urea was determined spectrophotometrically using the commercial kit of Spinreact Urea-37 with spinlab (Spinreact Company), Spain, according to [9].

### **3.6.2. Malondialdehyde (MDA) level in renal tissues:**

- Malondialdehyde concentration in renal tissue homogenate was determined spectrophotometrically using the method described by [10].

### **3.6.3. Reduced glutathione (GSH) level in renal tissues:**

- Reduced glutathione level in renal tissue homogenate was measured spectrophotometrically as described by [11].

### **3.6.4. Histopathological evaluation of the kidney:**

- Both kidneys were removed and the upper 1/3 of each kidney was put into a buffered 10% formalin fixation solution and processed with paraffin wax for histopathological examination.
- Sections (5µm) were cut, and stained with conventional hematoxylin and eosin (H&E) stain [12].

### **3.6.5. Statistical analysis:**

The collected data were analyzed using SPSS version 21 software (Spss Inc, ILL Company, Chicago, USA). Continuous data were expressed as mean ± standard deviation. Differences between the studied groups were tested by using ANOVA (F) test and Kruskal-Wallis (KW) test. The accepted level of significance in this work was stated at 0.05 [13].

## **IV. Results**

### **4.1. Biochemical results:**

#### **4.1.1. Renal functions:**

The present study showed a highly significant ( $p < 0.001$ ) increase in serum urea & creatinine levels recorded in ASP-treated, MSG-treated & “ASP + MSG”-treated groups as compared to control groups; the highest values of urea & creatinine were recorded in “ASP + MSG”-treated group. Furthermore, the values of urea & creatinine were improved in ASP-recovery & MSG-recovery groups with an insignificant statistical difference against control groups, but the difference between the results of “ASP + MSG”-recovery group and control groups was statistically significant, as illustrated in table 1 and fig. 1 & 2.

#### **4.1.2. Oxidative stress indices:**

The present study showed a highly significant ( $p < 0.001$ ) increase in renal tissue malondialdehyde (MDA) levels recorded in ASP-treated, MSG-treated & “ASP + MSG”-treated groups as compared to control groups, the highest value of MDA was recorded in “ASP + MSG”-treated group. Furthermore, the values of MDA were improved in ASP-recovery & MSG-recovery groups with an insignificant statistical difference against control groups, but the difference between the results of “ASP + MSG”-recovery group and control groups was statistically significant, as showed in table 1 and fig. 3.

The present study showed a highly significant ( $p < 0.001$ ) decrease in renal tissue reduced glutathione (GSH) levels recorded in ASP-treated, MSG-treated & “ASP + MSG”-treated groups as compared to control groups; the lowest value of GSH was recorded in “ASP + MSG”-treated group. Furthermore, the values of GSH were improved in ASP-recovery & MSG-recovery groups with an insignificant statistical difference against control groups, but the difference between the results of “ASP + MSG”-recovery group and control groups was statistically significant, as illustrated in table 1 and fig. 4.

### **4.2. Histopathological results:**

In control groups, the normal histological picture of renal corpuscles and tubules were recorded: renal corpuscles contained normal glomerular tuft & normal urinary space, with normal proximal and distal convoluted tubules, as showed in fig. 5.

In ASP-treated group, the renal glomeruli were disfigured and the renal tubules showed vacuolar degeneration, while in ASP-recovery group, the glomeruli retained normal lobulation with improvement of vacuolar degeneration of renal tubular epithelium, some tubules showed only some cloudy swelling in their lining, as illustrated in fig. 6 (A & B).

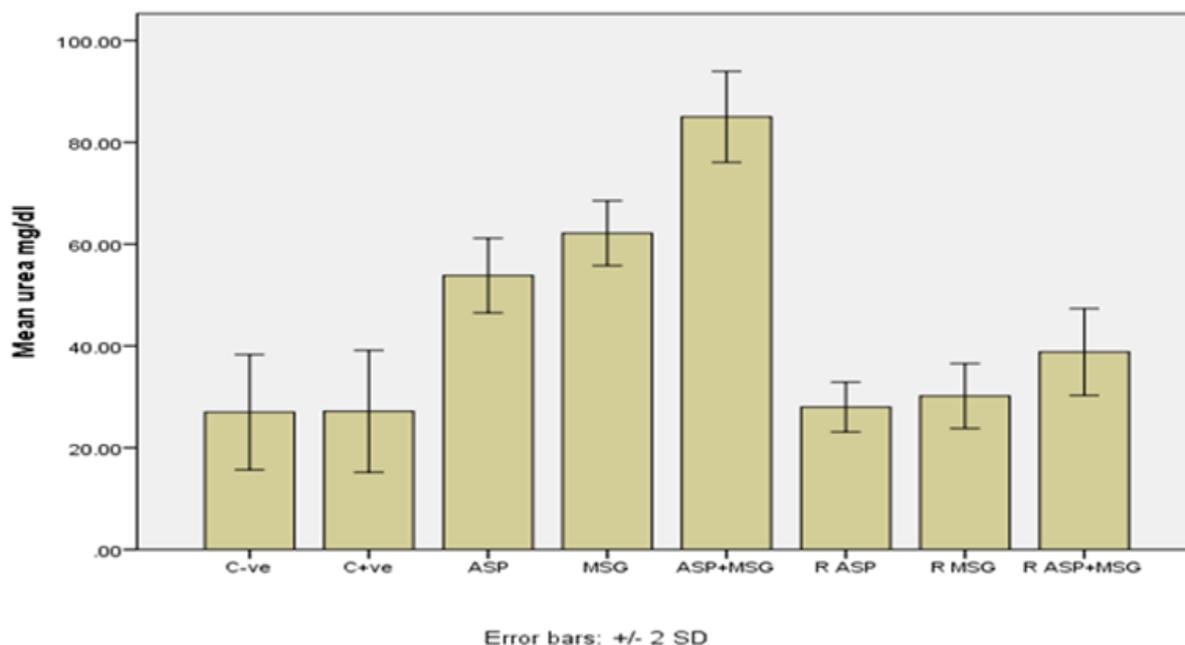
In MSG-treated group, glomerular degeneration in the form of increased number of mesangial cells with renal tubular atrophy, tubular casts and mononuclear cell infiltrate of the kidney stroma were detected, while in MSG-recovery group, the renal glomeruli retained their normal lobulation but the lining epithelium of few renal tubules showed vacuolar degeneration, as showed in fig. 7 (A, B & C).

In “ASP + MSG”- treated group, the worst histopathological results were detected in the form of renal tubular epithelial atrophy, disfigured glomeruli, with mesangial proliferation, while in “ASP + MSG”-recovery group, The glomeruli retained their normal lobulation but renal tubular epithelium showed cloudy swelling, as illustrated in fig. 8 (A & B).

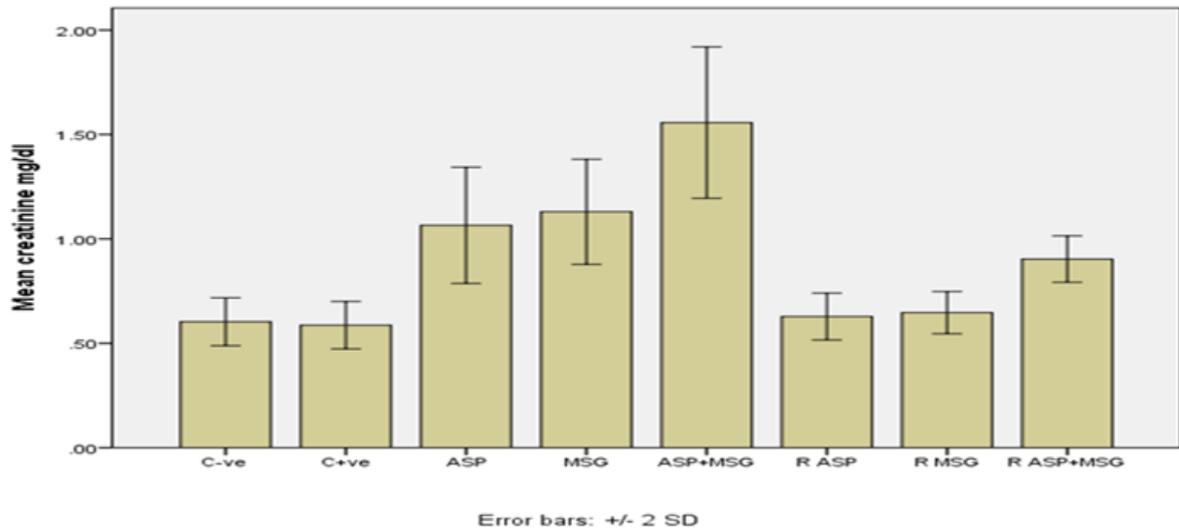
**Table (1):** Distribution of the study groups according to the mean values of urea, creatinine, malondialdehyde (MDA) and reduced glutathione (GSH):

Group Parameter	C -VE	C +VE	ASP	MSG	ASP + MSG	R ASP	R MSG	R ASP+ MSG	Test	P value
<b>Urea</b>			**	**	**	^	^	*		
Mean	27.0	27.17	53.83	62.17	85.0	28.0	30.17	38.83	KW=38.25	<0.001 **
±SD	±5.66	±5.98	±3.65	±3.19	±4.47	±2.45	±3.19	±4.26		
Range	20-35	20-35	50-60	58-65	80-90	25-30	25-35	28-36		
<b>Creatinine</b>			**	**	**	^	^	*		
Mean	0.60	0.59	1.07	1.13	1.56	0.63	0.65	0.75	KW=39.75	<0.001 **
±SD	±0.06	±0.05	±0.14	±0.13	±0.18	±0.06	±0.05	±0.09		
Range	0.54-0.70	0.52-0.67	0.89-1.2	0.98-1.3	1.3-1.8	0.58-0.70	0.57-0.70	0.65-0.88		
<b>MDA</b>			**	**	**	^	^	*		
Mean	51.5	51.0	68.33	74.0	93.83	51.83	53.0	57.83	F test=65.06	<0.001 **
±SD	±4.85	±4.0	±2.88	±3.1	±5.31	±5.34	±2.28	±7.22		
Range	45-59	45-56	65-72	68-77	88-100	43-58	50-56	50-70		
<b>GSH</b>			**	**	**	*	**	*		
Mean	45.17	44.83	35.67	31.67	19.5	42.67	42.0	43.17	F test=189.27	<0.001 **
±SD	±0.75	±0.75	±1.75	±1.63	±1.38	±1.37	±1.41	±2.48		
Range	44-46	44-46	33-38	30-34	18-21	41-45	40-44	39-46		

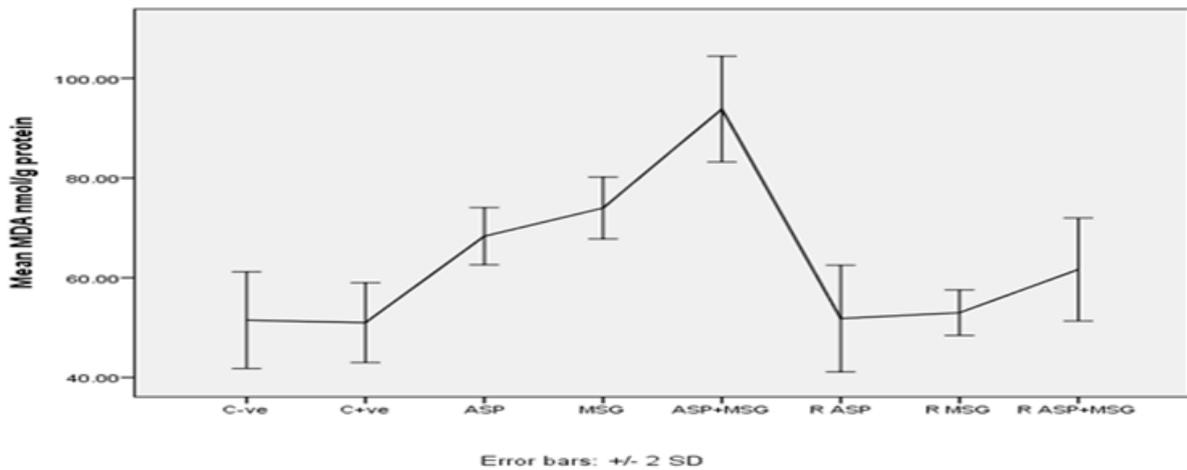
\*\* : highly significant; \* : significant; ^ : non-significant (as compared to control groups); ±SD : standard deviation; KW : Kruskal-Wallis test; F test : ANOVA; C -VE : negative control group; C +VE : positive control group; ASP : aspartame-treated group; MSG : monosodium glutamate-treated group; ASP + MSG : “aspartame + monosodium glutamate”-treated group; R ASP : aspartame-recovery group; R MSG : monosodium glutamate-recovery group; R ASP + MSG : “aspartame + monosodium glutamate”- recovery group.



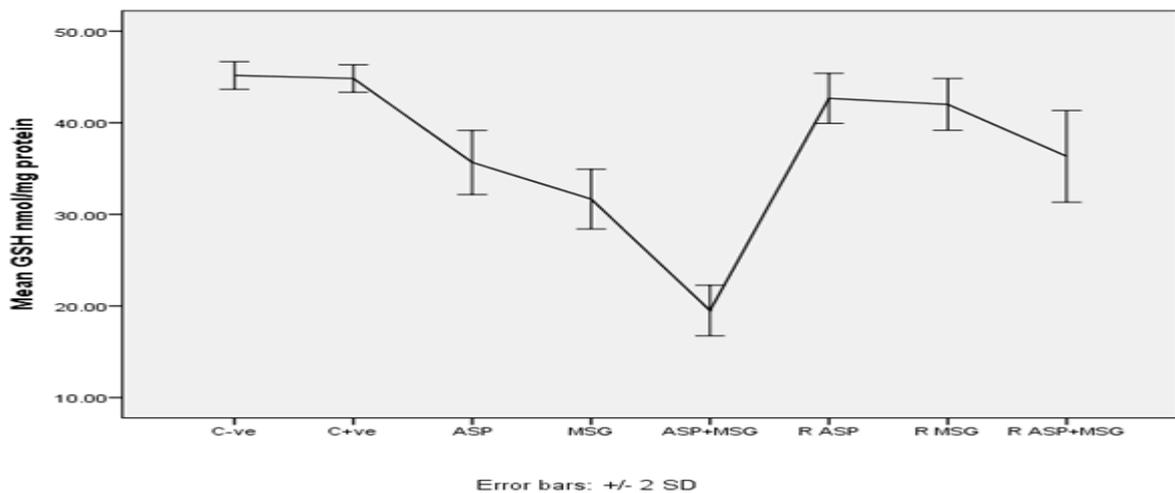
**Fig. (1):** Bar chart showing comparison between the studied groups regarding the mean of blood urea levels.



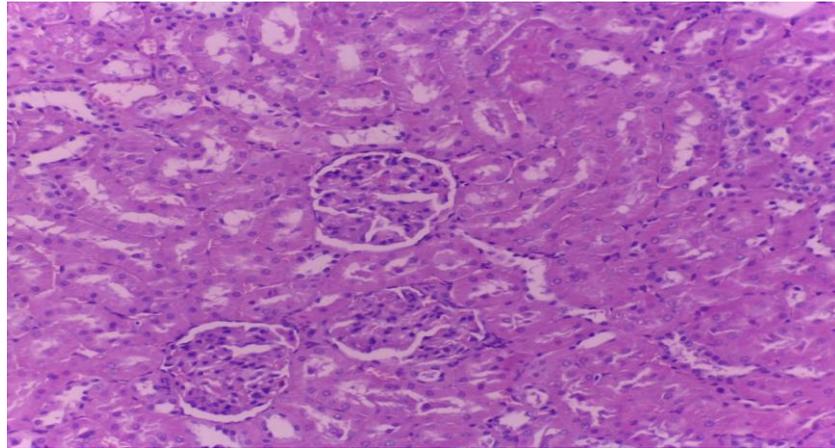
**Fig. (2):** Bar chart showing comparison between the studied groups regarding the mean of serum creatinine levels.



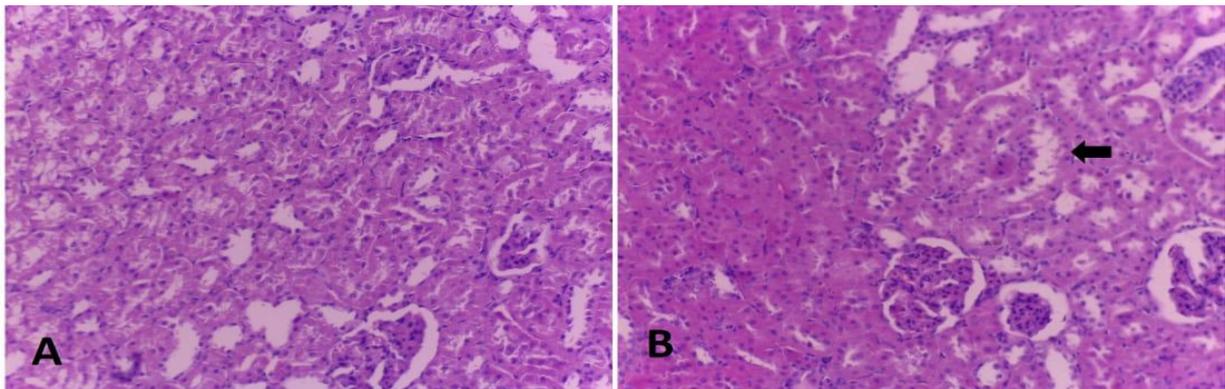
**Fig. (3):** Line chart showing comparison between the studied groups regarding the mean of malondialdehyde (MDA) levels in renal tissue.



**Fig. (4):** Line chart showing comparison between the studied groups regarding the mean of reduced glutathione (GSH) levels in renal tissue.

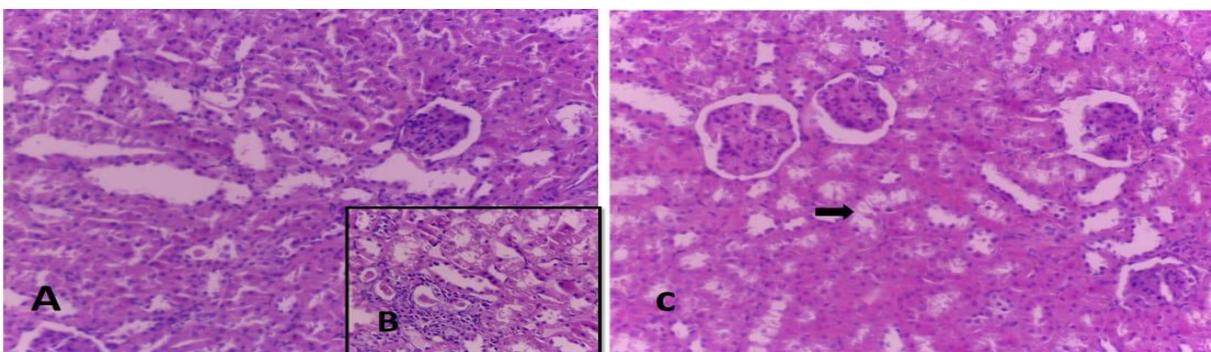


**Fig. (5):** A photomicrograph of kidney section of a rat from a control group showing normal histological structure: renal corpuscles contain normal glomerular tuft & normal urinary space, proximal convoluted tubules, and, distal convoluted tubules (H&E, x200).



**Fig. (6A):** A photomicrograph of kidney section of a rat from ASP-treated group: The glomeruli are disfigured and the tubules show vacuolar degeneration (H&E, x200).

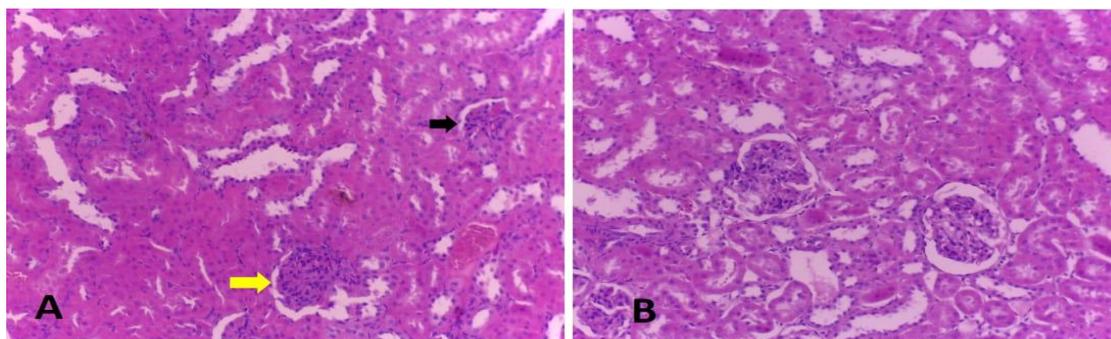
**Fig. (6B):** A photomicrograph of kidney section of a rat from ASP-recovery group: The glomeruli retained normal lobulation with improvement of vacuolar degeneration of renal tubular epithelium; some tubules show only some cloudy swelling in their lining (black arrow) (H&E, x200).



**Fig. (7A):** A photomicrograph of kidney section of a rat from MSG-treated group showing renal tubular atrophy and glomerular degeneration in the form of increased number of mesangial cells (H&E, x200).

**Fig. (7B):** A magnification of the previous photomicrograph showing renal tubular atrophy, tubular casts and mononuclear cell infiltrate of the stroma (H&E, x400).

**Fig. (7C):** A photomicrograph of kidney section of a rat from MSG-recovery group: The glomeruli retained their normal lobulation while the lining epithelium of few renal tubules still shows vacuolar degeneration (black arrow) (H&E, x200).



**Fig. (8A):** A photomicrograph of kidney section of a rat from (ASP+MSG)-treated group showing renal tubular epithelial atrophy, some glomeruli are disfigured (black arrow), and others show mesangial proliferation (yellow arrow) (H&E, x200).

**Fig. (8B):** A photomicrograph of kidney section of a rat from (ASP+MSG)-recovery group: The glomeruli retained their normal lobulation while renal tubular epithelium show cloudy swelling (H&E, x200).

## V. Discussion

Aspartame (ASP) - a low-calorie artificial sweetener - found in about 6000 products, such as carbonated soft drinks, hot chocolate, chewing gums, candies and many other dietary products can result in toxic effects in animal models [14].

Monosodium glutamate (MSG) -a food flavor enhancer- is used worldwide in the food industry; its utilization has been linked with many toxic metabolic effects in many tissues such as the liver, kidney, and brain [15].

The present study showed a highly significant increase in urea and creatinine serum levels recorded in ASP-treated, MSG-treated and “ASP+MSG”-treated groups as compared to control groups, with the highest values of serum urea and creatinine were recorded in “ASP+MSG”-treated group.

Several previous studies have linked ASP to deleterious nephrotoxic effects; [16] revealed that ASP administered orally (0.25 g/L, in drinking water, daily for 60 days) in adult albino rats resulted in significantly increased serum urea and creatinine levels compared to controls. [17] Found that ASP given orally to a rat model (500 mg/kg body weight for 42 days), significantly increased serum urea and creatinine levels, as compared to control group. Also [18] concluded that female rats treated daily with ASP at a dose of either 50 or 150 mg/rat/day, orally for six months resulted in a significant increase in the levels of serum urea and creatinine as compared to the control group.

However, [19] stated that ASP may prevent the nephrotoxicity induced by ochratoxin A “a mycotoxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum*” in experimental animals. The mechanism of this protection is due to the fact that ASP is a structural analogue of ochratoxin A.

On the other hand, [1] stated that ASP-induced renal adverse effects in human have not been evaluated. There are only some conflicting studies that relate between artificially sweetened soda and the occurrence of chronic renal disorders. Previously, [20] concluded that the safety of ASP has been controversial, as ASP administration at doses of 30 to 77 mg/kg/day over 13 weeks in human showed no significant impact on renal or hepatic function.

[21] Concluded that MSG given orally at a dose of 4 g/kg to adult female Wistar rats for six months, resulted in a significant increase in renal function markers (urea, uric acid, and creatinine), indicated that MSG produced functional disorders in the kidney. [22] Found that MSG resulted in toxic renal biochemical effects (significant increased urea and creatinine levels) at a low dose (5mg/kg) given orally to adult albino rats for 28 days.

[23] Reported that serum urea and serum creatinine were significantly increased in adult albino rats treated with MSG at doses of 0.6 and 1.6 mg/g body weight for 14 days, as a result of MSG-induced oxidative stress in renal tissues.

After 16 weeks of MSG treatment at a dose of 4.25 g/kg orally to adult albino rats [24] recorded a decrease in urine excretion of creatinine as a result of renal pathological effects and induced oxidative stress in renal tissues.

[25] Found that treatment of adult mice with both MSG and ASP resulted in a significant neuronal damage due to the synergistic effects of MSG and ASP with the influence and accumulative actions of their individual use.

The present study showed a highly significant increase in renal tissue malondialdehyde (MDA) levels, and a highly significant decrease in renal tissue reduced glutathione (GSH) levels recorded in ASP-treated,

MSG-treated and ASP+MSG-treated groups as compared to control groups, with the highest values of MDA and the lowest value of GSH were recorded in ASP+MSG-treated group.

[26] Found that rats treated with ASP at a dose of 50 mg/kg for 30 and 60 days, showed a significant increase in MDA level associated with a significant reduction in GSH levels in renal tissues, indicating that that ASP intake induced oxidative stress and affected the antioxidant defense system in renal tissues.

[27] Showed that daily intake of ASP at a dose of 40 mg/kg body weight for six weeks resulted in oxidative stress in the kidney tissue with a significant increase in hydrogen peroxide, lipid hydroperoxides and carbonyl protein levels, as well as, a significant decrease in GSH and glutathione peroxidase activities as compared to control rats.

Also [10] concluded that ASP given orally to albino rats at a dose of 40 mg/kg body weight for six weeks resulted in an oxidative stress in the liver and kidney [a significant increase in MDA levels and a significant decrease in GSH levels].

Aspartame is metabolized into 50% phenylalanine, 40% aspartic acid and 10% methanol. Most of phenylalanine is integrated into the amino acids pool for protein biosynthesis. Aspartic acid is further metabolized to carbon dioxide and excreted through the lungs. Meanwhile methanol is oxidized to formaldehyde then to formic acid with liberation of hydrogen peroxide and superoxide anion (oxidative stress) leading to degradation of cell membranes (lipid peroxidation) and cell injury [26] and [28].

Lipid peroxidation (LPO) is an auto catalytic process resulted in oxidative destruction of cellular membrane and reduction of membrane fluidity which is essential for cell functions. It is started by the removal of a hydrogen atom from the polyunsaturated fatty acids in the cell membrane, with its degradation to form a wide variety of byproducts particularly MDA [28] and [29].

The decreased level of GSH is due to the increased LPO [superoxide and hydrogen peroxide free radicals] following ASP intake. Considering that GSH and GSH-dependent enzymes (glutathione peroxidase & glutathione reductase) have vital roles in detoxification of electrophilic xenobiotics and in maintaining the antioxidant status [30] and [31].

[32] Concluded that MSG-induced nephrotoxic biochemical effects may be attributed to the formation of reactive oxygen species (ROS) in renal tissues, including the increased lipid peroxidation byproducts such as MDA and decreased activities of superoxide dismutase and GSH.

[33] Found that MSG given to adult albino rats orally for 30 days at a dose of either 6, 17.5 or 60 mg/kg body weight resulted in an increase in lipid peroxidation (MDA levels) as well as a significant decrease in antioxidants (Glutathione peroxidase) in testis tissues.

[34] In their study on adult male Wistar rats treated with MSG orally at a dose of 4g/kg body weight for ten days showed a significant increase in oxidative stress indices, as a result of increase renal uptake of glutamate (a gluconeogenic precursor that increases glucose concentration). This may be explained by the fact that hyperglycemia results in oxidative stress and free radicals formation in the kidney [35].

[36]; [37]; [38] Confirmed that glutamate -a byproduct from MSG metabolism- can lead to oxidative stress state (production of superoxide and hydrogen peroxide) in renal or other body tissues through (1) increased activity of  $\alpha$ -Ketoglutarate dehydrogenase ( $\alpha$ - KGDH) which increases ROS formation, (2) glutamate itself can enter the Krebs cycle and modifies the redox state of the cell, (3) additionally, glutamate can increase the intracellular calcium levels through glutamate receptors resulted in lipid peroxidation and free radicals formation.

[39] Observed a synergistic effect in mice group treated with both MSG and ASP as evidenced by a significant oxidative stress indices (increased lipid peroxides and decreased GSH levels), as compared to the other studied groups.

As regard the histopathological findings; the renal glomeruli were disfigured and the renal tubules showed vacuolar degeneration in ASP-treated group; glomerular degeneration, tubular casts and mononuclear cell infiltrate of the renal stroma were detected in MSG-treated group, while in "ASP+MSG"-treated group, the worst histopathological results were detected in the form of renal tubular epithelial atrophy, disfigured glomeruli, with mesangial proliferation. There was some improvement in renal histopathology in all recovery groups especially in ASP-recovery and MSG-recovery groups.

[18] Illustrated structural changes in the renal tubules and the Malpighian corpuscles of ASP-treated rats; tubules were disrupted, their walls were fragmented and the epithelium was degenerated with scattered tubular necrosis. These degenerative changes could be related to the toxic effects of ASP metabolites on the cellular proteins.

[40] In their study on ASP-treated rats showed severe glomerular damages, including loss of normal architecture and decrease in normal sizes. They attributed these histopathological changes to the metabolic sequence of methanol-formaldehyde-formic acid, with synergistic damage by free radicals.

[7] In their study on ASP-treated rats found structural damage in both renal glomeruli and renal tubules with loss of the brush border of proximal convoluted tubular epithelium, and widening of their lumens.

[24] and [41] found similar morphological alterations in MSG-treated rats in both renal glomeruli [focal degeneration and infiltration of inflammatory cells] and renal tubules [focal atrophy with loss of the brush border and cellular desquamation], as a consequence of oxidative stress and inflammatory reactivity.

[42] in their study on adult Wistar male rats treated with MSG showed hydropic degenerative changes and necrosis in the kidney, either in renal glomeruli or in the convoluted tubules, which were parallel with the lipid peroxidation products levels.

The present work found that the values of serum urea and creatinine and the values of MDA and GSH, as well as the histopathological findings were improved to some extent in recovery groups, as an insignificant statistical difference was found in ASP-recovery and MSG-recovery groups against control groups, but the difference between the results of "ASP+MSG"-recovery group and control groups was statistically significant [as regard the studied biochemical & the oxidative stress indices].

[43] In a study on adult albino rats treated with ASP orally at a dose of 250mg/kg daily for three months, followed by one month of stoppage, found that ASP resulted in toxic effects on the structure of sciatic nerve and that one month of ASP cessation was not sufficient to obtain complete recovery (only partial amelioration was detected in the recovery group).

## VI. Conclusion:

The present study documented nephrotoxic effects of ASP and MSG administered either individually or in combination in adult Wistar albino rats, with the worst effects were detected in "ASP+MSG"-treated group, and these effects were improved to some extent in recovery groups, as evidenced by:

1. A highly significant increase in urea and creatinine serum levels in all treated groups as compared to control groups, with the highest values were recorded in "ASP+MSG"-treated group.
2. A highly significant increase in renal tissue MDA levels, and a highly significant decrease in renal tissue GSH levels recorded in all treated groups as compared to control groups, with the highest values of MDA as well as the lowest value of GSH were recorded in "ASP+MSG"-treated group.
3. The renal structural damages detected in all treated groups, with the worst histopathological findings were detected in "ASP+MSG"-treated group.
4. Partial improvement in the renal histopathological and biochemical results in all recovery groups.

## VII. Recommendations:

- 1- Performing several clinical trials on nephrotoxic effects of ASP & MSG on humans, as clinical data is lacking.
- 2- In general, food consumers should put in mind the potential nephrotoxic effects of ASP & MSG; thereby they should consume only minimal amounts of them as possible as they can.

## Acknowledgment

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## References

- [1]. M.R. Ardalan, H. Tabibi, V.E. Attari, A.M. Mahdavi, Nephrotoxic Effect of Aspartame as an Artificial Sweetener. Iran. J. Kid. Dis., 11(5), 2017, 339 – 343.
- [2]. M.A. Lebda, H.G. Tohamy, Y.S. El-Sayed, Long-term soft drink and aspartame intake induces hepatic damage via dysregulation of adipocytokines and alteration of the lipid profile and antioxidant status. Nutr. Research, 41, 2017, 47 – 55.
- [3]. A. Iyaswamy, D. Wankhar, S. Loganathan, S. Shanmugam, R. Rajan, S. Rathinasamy, Disruption of redox homeostasis in liver function and activation of apoptosis on consumption of aspartame in folate deficient rat model. J. Nutr. Intermed. Metabol., 8, 2017, 41-50.
- [4]. A.D. Alalwani, Monosodium glutamate induced testicular lesions in rats (histological study). J. Mid. East Fertil. Soc., 19, 2014, 274–280.
- [5]. C.O. Ibegbulem, P. C. Chikezie, A.I. Ukoha, C. N. Opara, Effects of diet containing monosodium glutamate on organ weights, acute blood steroidal sex hormone levels, lipid profile and erythrocyte antioxidant enzymes activities of rats. J. Acute Dis., 5(5), 2016, 402–407.
- [6]. S. Mahieu, M. Klug, N. Millen, A. Fabro, A. Benmelej, M.C. Contini, Monosodium glutamate intake affect the function of the kidney through NMDA receptor. Life Sci., 149, 2016, 114–119.
- [7]. N.G.A. El Haliem, D.S., Mohamed, The effect of aspartame on the histological structure of the liver and renal cortex of adult male albino rat and the possible protective effect of Pimpinella anisum oil. Egy. J. Histol., 34, 2011, 715-726.
- [8]. O.O. Onyema, C.S. Alisi, A.P. Ihetuge, Monosodium glutamate induces oxidative stress and affects glucose metabolism in the kidney of rats. Int. J. Biochem. Res. Rev., 2 (1), 2012, 1–11.
- [9]. M.R. Pincus, M.S. Lifshitz, Evaluation of Renal Function, Water, Electrolytes and Acid–Base Balance. In: Henry's Clinical Diagnosis and Management by Laboratory Methods, (Saunders Company, Philadelphia, U.S.A., 2007). By: McPherson, R.A. and Pincus, M.R. (eds.), 21<sup>st</sup> ed., Chapter (14), pp: 360-376.
- [10]. I.M. Mourad, Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. Afri. J. Pharm. Pharmacol., 5 (6), 2011, 678-682. ISSN 1996-0816
- [11]. A. Shalaby, H. Abdel Malek, Renoprotective effect of sitagliptin (dipeptidyl peptidase- 4 inhibitor) against cisplatin induced nephrotoxicity in mice. Brit. J. Pharm. Research, 4 (9), 2014, 1116-1129.
- [12]. J.D. Bancroft, M. Gamble, (eds.) Fixatives. In: Theory and Practice of Histology Techniques, (Churchill Livingstone, Elsevier, China, 2008), 8<sup>th</sup> ed., chapter (10), pp: 72-75.

- [13]. R.S. Greenberg, R.S. Daniels, W.D. Flanders, J.W. Eley, J.R. Boring, (eds.) Diagnostic Testing. In: Medical Epidemiology, (McGraw-Hill, New York, 1996), 3<sup>rd</sup> edition., pp: 77-89.
- [14]. A. Rafati, A. Noorafshan, M. Jahangir, L. Hosseini, S. Karbalay-Doust, Vitamin E can improve behavioral tests impairment, cell loss, and dendrite changes in rats' medial prefrontal cortex induced by acceptable daily dose of aspartame. *Acta. Histochem.*, 120, 2018, 46–55.
- [15]. S.H. Abdel-Aziem, H.A.M. Abd El-Kader, F.N. Ibrahim, H.A. Sharaf, A.I. El makawy, Evaluation of the alleviative role of *Chlorella vulgaris* and *Spirulina platensis* extract against ovarian dysfunctions induced by monosodium glutamate in mice. *J. Genetic Engine. Biotechnol.*, 16 (2), 2018, 653-660.
- [16]. H.I. Bahr, M.S. Zaki, Renal Genomic Instability Induced by Aspartame and the Possible Influence of the Flaxseed Oil and Coenzyme Q10 in Male Rats. *Life Sci. J.*, 11(8), 2014, 301-308.
- [17]. A.A.S. Saleh, Synergistic effect of N-acetylcysteine and folic acid against aspartame- induced nephrotoxicity in rats. *Int. J. Adv. Res.*, 2 (5), 2014, 363-373.
- [18]. A. Waggas, K. Soliman, G. Moubarz, A. Abd Elfatah, M. Taha, Potential protective effects of aqueous extract of Majoram leaves, against aspartame induced renal toxicity in female rats. *Am. J. Toxicol. Sci.*, 7 (4), 2015, 267-278.
- [19]. R.D. Patil, R. Sharma, R.K. Asrani, Mycotoxicosis and its control in poultry: A review. *J. Poul. Sci. Tech.*, 2 (1), 2014, 1-10.
- [20]. C.R. Whitehouse, J. Boullata, L.A. McCauley, The potential toxicity of artificial sweeteners. *J. Am. Assocat. Occup. Health Nurs.*, 56 (6), 2008, 251-259.
- [21]. M.V. Paul, M. Abhilash, M.V. Varghese, M. Alex, R.H. Nair, Protective effects of  $\alpha$ -tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. *Toxicol. Mech. Meth.*, 22 (8), 2012, 625–630.
- [22]. A.C.C. Egbuonu, O. Obidoa, C.A. Ezeokonkwo, P.M. Ejikeme, L.U.S. Ezeanyika, Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats: body weight changes, serum cholesterol, creatinine, and sodium ion concentrations. *Toxicol. Environm. Chem.*, 92 (7), 2010, 1331–1337.
- [23]. M.S. Tawfik, N. Al-Badr, Adverse effects of monosodium glutamate on liver and kidney functions in adult rats and potential protective effect of vitamins C and E. *Food and Nutr. Sci.*, 3 (5), 2007, 651-659.
- [24]. M.C. Contini, A. Fabro, N. Millen, A. Benmelej, S. Mahieu, Adverse effects in kidney function, antioxidant systems and histopathology in rats receiving monosodium glutamate diet. *Experim. Toxicol. Pathol.*, 69, 2017, 547–556.
- [25]. C.H. Park, S.H. Choi, Y. Piao, S. Kim, Y. Lee, H. Kim, S.J. Jeong, J.C. Rah, J.H. Seo, J.H. Lee, K. Chang, Y.J. Jung, Y.H. Suh, Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol. Lett.*, 115(2), 2000, 117–125.
- [26]. S.A. Alwaleedi, Alterations in antioxidant defense system in hepatic and renal tissues of rats following aspartame intake. *J. Appl. Biol. Biotechnol.*, 4 (2), 2016, 46-52.
- [27]. F. Isabelaa, P.M. Amália, P. Tanisea, O. Giovana, S. Etiane, S. Sun, L. Susana, P. Wania, N-acetylcysteine protects the rat kidney against aspartame-induced oxidative stress. *Free Radi. Biol. Med.*, 75 (P27), 2014, S21–S53.
- [28]. J.N. Parthasarathy, S.K. Ramasundaram, M. Sundaramahalingam, S.D. Pathinasamy, Methanol induced oxidative stress in rat lymphoid organs. *J. Occup. Health*, 48, 2006, 20-27.
- [29]. L. Bergendi, L., Benes, Z. Durackova, M. Ferencik, Chemistry, physiology and pathology of free radicals. *Life Sci.*, 65, 1999, 1865- 1874.
- [30]. A.K. Choudhary, L. Sundareswaran, R.S. Devi, Aspartame induced cardiac oxidative stress in Wistar albino rats. *Nutr. Clin. Métabol.*, 30, 2016, 29–37.
- [31]. I. Finamora, S. Péreza, C.A. Bressanb, C.E. Brennerb, S. Rius-Pérez, P.C. Brittesc, G. Cheirand, M.I. Rochad, M. Veigad, J. Sastrea, M.A. Pavanatob, Chronic aspartame intake causes changes in the trans-sulphuration pathway, glutathione depletion and liver damage in mice. *Redox Biol.*, 11, 2017, 701–707.
- [32]. A. Sharma, Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: a mini-review. *J. Biomed. Sci.*, 22, 2015, 93- 98.
- [33]. R. Hamza, M.S. AL-Harbi, Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. *Toxicol. Reports*, 1, 2014, 1037–1045.
- [34]. O.O. Okwudiri, A.C. Sylvanus, I.A. Peace, Monosodium glutamate induces oxidative stress and affects glucose metabolism in the kidney of rats. *Int. J. Biochem. Research & Review*, 2 (1), 2012, 1-11.
- [35]. D. Koya, K. Hayashi, M. Kitada, A. Kashiwagi, R. Kikkawa, M. Handeda, Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. *J. Am. Soc. Nephrol.*, 14 (8), 2003, S250-S253.
- [36]. L. Tretter, V. Adam-Vizi, Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. *Philos. Trans. Royal Soc. Biol. Sci.*, 360 (1464), 2005, 2335–2345.
- [37]. A. Sharma, C. Wongkham, V. Prasongwattana, P. Boonnate, R. Thanan, S. Reungjui, Proteomic analysis of kidney in rats chronically exposed to monosodium glutamate. *PLoS One*, 9 (12), 2014, e116233.
- [38]. O.J. Onaolapo, A.Y. Onaolapo, M.A. Akanmu, O. Gbola, Evidence of alterations in brain structure and antioxidant status following 'low-dose' monosodium glutamate ingestion. *Pathophysiol.*, 23, 2016, 147–156.
- [39]. G.M. Abu-Taweel, M.A. Zyadah, J.S. Ajarem, M. Ahmad, Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. *Neurotoxicol. Teratol.*, 42, 2014, 60–67.
- [40]. J. Ashok, D. Wankhar, R. Sheeladevi, W. Wankhar, Long-term effect of aspartame on the liver antioxidant status and histopathology in Wistar albino rats. *Biomed. Prevent. Nutr.*, 4, 2013, 299–305.
- [41]. S.G. Dixit, P. Rani, A. Anand, K. Khatri, R. Chauhan, V. Bharihoke, To study the effect of monosodium glutamate on histomorphometry of cortex of kidney in adult albino rats. *Ren. Fail.*, 36 (2), 2014, 266–270.
- [42]. G.G. Ortiz, O.K. Bitzer-Quintero, C. Beas Zárate, S. Rodríguez Reynoso, F. Larios-Arceo, I.E. Velázquez-Brizuela, F. Pacheco-Moisés, S.A. Rosales-Corral, Monosodium glutamate-induced damage in liver and kidney: a morphological and biochemical approach. *Biomed. Pharmacotherapy*, 60, 2006, 86–91.
- [43]. E.F. Okasha, Effect of long term-administration of aspartame on the ultrastructure of sciatic nerve. *J. Microscopy Ultrastruct.*, 4, 2016, 175–183.

Abdelmonem G. Madboly. "Nephrotoxicity of Tow Food Additives (Aspartame and Monosodium Glutamate) in Adult Albino Rats: Biochemical and Histopathological Effects." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)* 13:4 (2019): 20-29.