ORIGINAL ARTICLE

Histological Effects of Continuous Prolonged Use of Monosodium Glutamate on Kidneys of Adult Wistar Rats

[1]FARHANA JAFRI, [2]GODFREY PAUL WILLIAM, [3]FOUZIA FARZANA
[1]Assistant Professor Department of Anatomy Allama Iqbal Medical College; [2]Associate Professor Department of Anatomy FMH College of Medicine and Dentistry; and[3]Professor Department of Anatomy Postgraduate Medical Institute.
Corresponding author: Dr. Farhana Jafri, Department of Anatomy Allama Iqbal Medical College, Lahore. Email: jafrifarhana@gmail.com

ABSTRACT
Background: The generous use of MSG in commercial and domestic areas in Pakistan forms the basis of this study. The hypothesis resides on the fact that kidneys are excretory route of nitrogenous waste products; therefore endure most of the harmful effects of MSG.
Methods: The study was done on 45 adult Wister rats randomly divided in three groups. The experimental groups A was given 6 mg/kg body weight of MSG and group B was given 18mg/kg body weight of MSG once daily for 12 weeks duration whereas group C was kept as control. After 12 weeks, kidneys were removed and processed for routine histological examination, integrity of basement membrane was observed by PAS staining technique. The variables recorded include changes in organ weight, relative tissue weight index and changes in structure of uriniferous tubules.
Results: The microscopic examination of experimental groups A and B showed changes in renal corpuscles, vacuolization in cells of tubules, sloughing off tubular cells as compared to control groups C as a result of continuous use of MSG. Changes were more marked in Experimental Group B as compared to Group A.
Conclusion: Continuous use of MSG in moderate to high doses has a deleterious effect on the nephrons of adult Wister rats. Since, similar changes are expected in human being, a cautious use is advisable. These findings could direct a path for future research in human beings and exploration of preventive measures to minimize the side effects.

Keywords: Monosodium glutamate, Renal corpuscle, Bowman's space, Uriniferoustubules

INTRODUCTION
Monosodium glutamate (MSG) is a sodium salt of glutamic acid, one of the most common amino acid found in nature. MSG is the name allocated by the Food and Drug Administration (FDA) of USA to a substance that contains approximately 78% free glutamic acid[(1)]. Commercially, MSG is produced by fermentation of starch, sugar beets, sugar cane or molasses[(2)]. MSG was discovered as a flavour enhancer food additive and was patented in 1909 by Ajinomoto Corporation of Japan[(3)].

In April 1968, the first report of an adverse reaction to MSG was published in the New England Journal of Medicine reporting that the substance was neurotoxin[(4)]. Ever since a series of animal and human studies have been published reporting adverse effects of MSG on almost all vital organs of the body including nervous system[(5,6)]gastrointestinal tract[(7-9)]liver (causing obesity) and pancreas (resulting in diabetes)[(10,11)] and gonads[(12-14)] and kidneys[(15,16)].

Glutamate receptors are present centrally as well as peripherally, located in bones, heart, pancreas gonads, gastrointestinal tract and kidneys. In kidneys they are located in glomeruli, mesangium, podocytes and proximal convoluted tubules[(17,18)].

In Pakistan, MSG is generously used in both commercial and domestic areas as a flavouring agent in cooked food, without considering the quality and quantity of the substance. This experimental study is based on the hypothesis that excessive use of MSG is potentially harmful to kidneys, being the main excretory path of nitrogenous waste products; therefore endure most of the harmful effects of MSG.

MATERIALS AND METHODS
In this controlled experimental study forty-five adult Wistar albino rats of either sex, weighing approximately 180-200 grams were obtained after thorough examination from the animal house of

National Institute of Health (NIH), Islamabad. The rats were kept according to guidelines of NACLAR under optimum conditions of temperature 24°C±2°C, humidity 50%±10% and in 12 hours light and dark cycles[19].

After initial acclimatization[20], the rats were randomly divided into three groups (A, B and C) fifteen rats in each group (N=15). Group A and B were experimental groups that were given MSG in doses of 6mg/kg of body weight (b.w.) and 18mg/kg b.w. respectively whereas group C was kept as control. The salt was given continuously for a prolonged duration of 12 weeks. All the confounding factors were taken care of for three groups. Dose was calculated by using formula for equivalent dose calculation[21]. Dose calculation and data record was done weekly. MSG selected was a product of Young's Food Products. Daily administration of salt dissolved in distilled water was done by gavage method.

After allocated time of 12 weeks of daily salt administration rats were weighed and data recorded for calculation of mean body weight (Table 1). Then Kidneys were dissected out from group A, B and C using chloroform anaesthesia[22,23]. The weights of kidneys were measured to calculate paired kidney weight (Table 1). Then they were fixed in 10ml of 10% neutral buffered formalin (NBF) (Merck Pharma®) for 48 hours in small plastic containers clearly labelled. Then kidneys were processed and prepared for routine histological slides. 3-5 µm thick sections were obtained and routine histological staining with heamotoxylin and eosin was done. To observe integrity of basement membrane and brush border slides were stained with PAS (periodic acid- Schiff reaction) and counter stained by heamotoxylin. The basement membrane appeared magenta and nuclei were stained blue[24]. Stained slides were studied under light microscope with magnifications of X10, X40 and X100 (oil immersion) to see the histological architecture of the kidney. Changes in the Bowman's corpuscle and cellular architecture of PCT and DCT were studied in comparison with control group. The quantitative parameters include paired kidney weight (PKW), relative tissue weight index (RTWI) and glomerular diameter. Qualitative parameters include changes in renal tubules.

Data was analysed by SPSS (version 22.0). For quantitative parameters ANOVA was applied, if significant then Post-hoc analysis (Tukey's test) was applied to find the association between groups. Pearson's Chi square test was applied for association between qualitative data. P-value ≤ 0.05 was taken as statistically significant association.

RESULTS

Dissected kidneys were pale brown and smooth, without any gross abnormality. Relative tissue weight index (RTWI) was calculated by using mean weight of rats and mean paired kidney weights (Table 1). The longitudinal section of each kidney had single cone shaped medullary pyramid; external to it lies cortex covered by a thin fibrous capsule as seen under light microscope. The reddish-brown cortex mainly consisted of renal corpuscles (RC), proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). The structures that showed changes after a continuous ingestion of monosodium glutamate include renal corpuscle, PCTs and DCTs.

Renal corpuscles of group C (control animals) look like spherical structures measuring approximately 110µm in diameter (Table 2). It consisted of an inner tuft of capillaries, the glomerulus, that was covered by bowman's capsule; a cup formed by epithelium with inner indistinguishable visceral layer and outer parietal layer of simple squamous epithelium resting on a basement membrane. The two layers of Bowman's capsule were separated from each other by Bowman's space or urinary space (Figure 1). Each RC was surrounded by a large number of PCTs and a few sections of DCTs. The basement membrane and brush border high in glycogen content were visible in PAS stained slides (Figure 2).

When renal corpuscles of groups A and B were compared with the control groups C, occulometery showed statically non-significant change in sizes of renal corpuscles (Table 2). Microscopic study revealed focal areas of reduced urinary space to such an extent that renal corpuscles were not identifiable from their surroundings. No increase in number of any type of cells (endothelial, mesangial, podocytes or mononuclear cells) was found in these renal corpuscles as counted by using the counting grid. These changes were more marked in group B as compared to A (Figure 3 & 4). When basement membrane was observed by PAS staining, it was found intact in all experimental groups (Figure 5& 6).

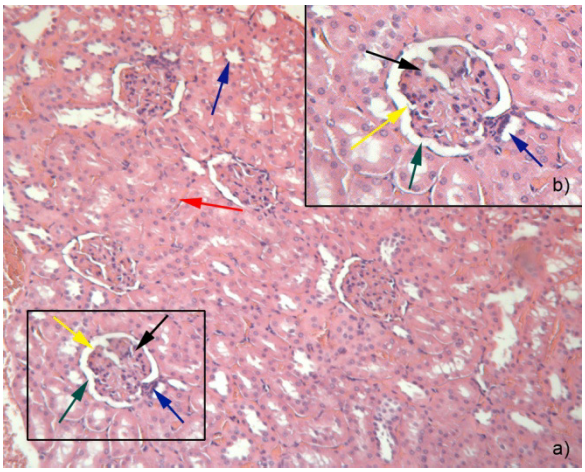

Figure 1: (a) Photomicrograph showing section of renal cortex of control animal. H & E Stain X100. Showing renal corpuscles composed of glomerulus (black arrow), parietal layer of Bowman's capsule (dark green arrow), urinary or bowman's space (yellow arrow), PCTs (red arrow), DCTs (blue arrow) are visible. (b) Enlarged view of renal corpuscle. H & E stain. X200. Showing Multi-lobed glomerulus (black arrow), Parietal layer (dark green arrow) is clearly visible, Bowman's space (yellow arrow), A section of DCT with macula densa (blue arrow) is also visible.

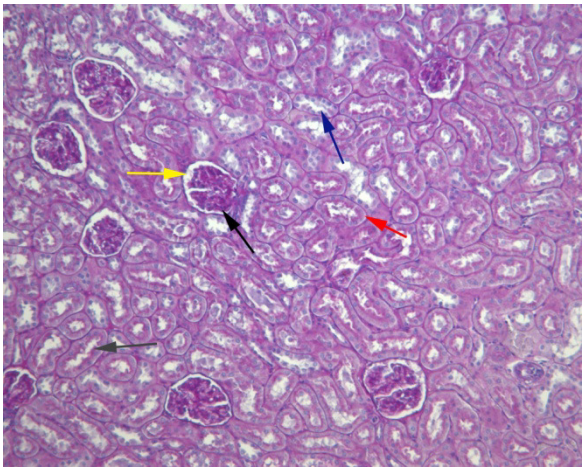

Figure 2: Photomicrograph showing section of renal cortex of control group. PAS stain. X100. Showing Complete basement membrane of renal corpuscles (black arrow) Bowman's space (yellow arrow), PCTs with complete basement membrane (red arrow) Brush border is also visible as dark magenta inside lumen of PCTs (grey arrow), DCTs also having complete basement membrane (blue arrow) are visible.

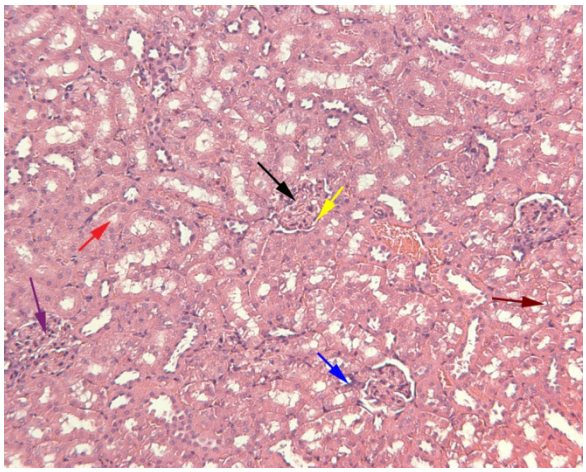

Figure 3: Photomicrograph showing section of renal cortex of experimental group A (6mg/kg MSG once daily for 12 weeks). H & E Stain X100. Showing Renal corpuscles (purple arrow), Glomerulus (black arrows), Surrounded by merely visible bowman's space (yellow arrows), PCTs (red arrows), Cellular vacuolization (maroon arrows), DCT (blue arrows) can be seen.

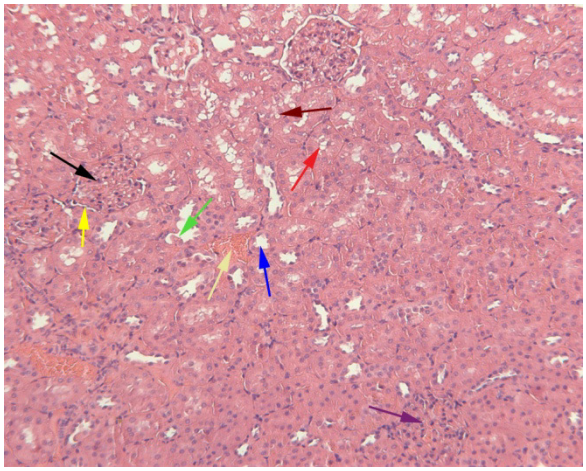

Figure 4: Photomicrograph showing section of renal cortical tissue experimental group B(18mg/kg MSG for 12 weeks). H & E Stain X100. Showing Renal corpuscle (purple arrow) difficult to differentiate from surrounding, Glomerulus (black arrow), Decreased urinary space (yellow arrow), PCTs (red arrow) show no lumen near RC without bowman's space but prominent lumen in upper part of photograph, DCTs have prominent lumen (blue arrow), Tubules show sloughed off cells (light green arrow), Interstitial hemorrhage is visible (off-white arrow).

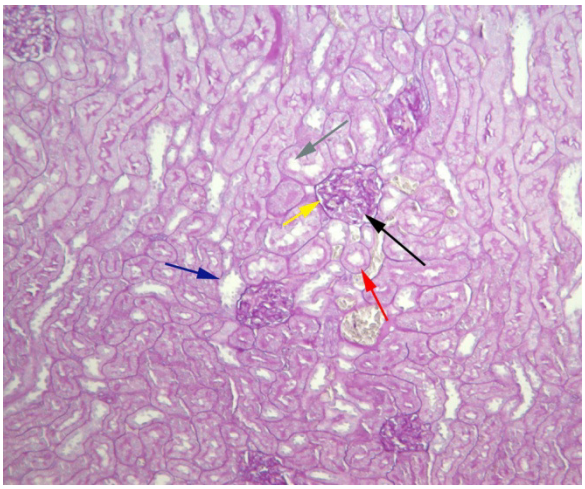

Figure 5: Photomicrograph showing section of cortical tissue of experimental group A (6mg/kg MSG once daily for 12 weeks). PAS stain. X100. Showing Renal corpuscles with obliterated bowman's space (black arrow), Complete basement membrane and reduced bowman's space (yellow arrow), PCTs with intact basement membrane (red arrow), Patchy disintegration of brush border (grey arrow), DCTs are visible without brush border but intact basement membrane (blue arrow).

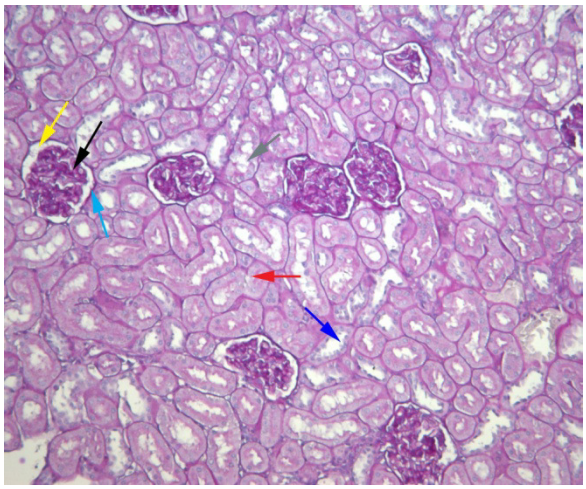

Figure 6: Photomicrograph showing section of renal cortex of experimental group B2 (18mg/kg b.w. MSG for 12 weeks). PAS stain. X100. Showing Renal corpuscles (RC) showing intact basement membrane (light blue arrow), glomerulus (black arrow) Bowman's space (yellow arrow), Complete basement membrane of PCTs (red arrow) DCTs (blue arrow), Patchy destruction of brush border (gray arrow).

The comparison of Bowman's space showed a significant association with 73.33% animals of group A and 86.67% animals in group B displayed a reduced space as compared to controls. The comparison of decreased bowman's space in groups A, B with C was done by applying chi square test. It was found statistically significant with a p-value of < 0.001 (Table 3).The large cuboidal cells of PCT had lightly stained basally located basophilic nucleus with indistinct lateral boundaries (Figure 1). The cells had prominent brush border stained magenta with PAS staining technique, and were resting on a basement membrane well marked by PAS stain (Figure 2). In experimental groups A and B the lumen of PCTs was barely visible in same focal areas where urinary space was reduced and appeared normal in other areas. Tubules showed degenerative changes with vacuolization of cytoplasm and sloughing of cells, light microscopic study also revealed presence of pyknosis of nuclei is at places as compared to Group C (Figure 3 & 4). Patchy destruction of brush border was seen with PAS staining technique for groups A and B, respectively (Figure 5 & 6).

All these changes were dose related and were more marked in Group B.

DCT of study groups were lined by simple cuboidal epithelium and were differentiated from PCT by a larger more clearly defined lumen; cells were smaller than those of PCT having rounded dark staining nucleus with scanty brush border as shown by PAS staining technique (Figure 2). The tubules showed vacuolization of cytoplasm in focal areas in Groups A and B as compared to group C (Figure 3 & 4), Apical protrusions of cells, separation from neighboring cells and sloughing of cells was more marked in groups B than group A as compared to control groups C (Figure 5 & 6).

The architectural comparison of histo-pathological changes of cells of PCTs and DCTs was statistically significant as shown by ANOVA (Table 4). Cell surface projections distorting cuboidal shape were seen in approximately 60% of animals in group A whereas approximately 67% of animals in group B showed similar changes as compared to group C. The degenerative changes in nuclear shape and position were also observed (Table 4).

Table 1: Comparison of mean values in study groups (A, B and C) for mean Paired Kidney Weight (PKW) and mean Relative Tissue Weight Index (RTWI) after 12 weeks

Groups	PKW	RTWI	Tukey HSD		
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	Groups	PKW	RTWI
C (control)	2.036±0.135	0.919±0.056	C – A	0.969	0.017
A (6mg/kg)	2.005±0.157	0.798±0.157	C – B	0.081	0.002
B (18mg/kg)	1.763±0.109	0.763±0.109	A – B	0.132	0.687
ANOVA (p-value)*	0.064	0.002			

*p≤0.05 was taken as statistically significant

Table 2: Comparison of Mean Renal Corpuscular Diameter in study groups (A, B, and C) after 12 weeks

Groups	Duration	N	Renal Corpuscle Diameter	ANOVA p-value*
			$\bar{x}$ ± SD	
C (control)	12 weeks	15	110.52 ± 6.10	0.737
A (6mg/kg b.w.)		15	111.83 ± 3.51	
B (18mg/kg b.w.)		15	111.81 ± 5.82	

*p≤0.05 was taken as statistically significant

Table 3: Comparison of Bowman's space of study groups A, B and C for the presence of histopathlogical changes after 12 weeks

Groups	Sacrifice Timing	Bowman's Space	df*	p-value**
C (control)	12 weeks	00(00.00)	2	0.000
A (6mg/kg b.w. MSG)		11(73.33)		
B (18mg/kg b.w. MSG)		13(86.67)		

*df: degrees of freedom

**p≤0.05 was taken as statistically significant

Table 4: Comparison of structural changes in renal tubules of Study Groups A, B and C for the presence of histopathlogical changes after 12 weeks; assessed by chi square test.

Groups	Timings	N	Cell shape	Nucleus shape	Nucleus position
C (control)	12 weeks	15	02 (13.33)	02 (13.33)	01 (06.67)
A (6mg/kg)		15	9 (60.00)	7 (46.67)	8 (53.33)
B (18mg/kg)		15	10 (67.00)	8 (53.33)	10 (67.00)
df*		2			
p-value**			0.000	0.000	0.000

*df: degrees of freedom

**p≤0.05 was taken as statistically significant

DISCUSSION

Monosodium glutamate (chemical compound) and an increase in serum levels produce unwanted effects on almost all cells and tissues of body. This study showed similar changes. Keeping in view of available literature, this research was done with a daily administration of small doses of MSG in distilled water, to study effects of continuous use of MSG for 12 weeks on weight of kidneys and histological architecture of kidneys. These changes were compared with control animals.

The mean value of paired kidney weights (PKW) of experimental group A (2.005±0.16 grams) showed a decline as compared to control group C (2.036±0.14 grams); whereas a marked reduction in mean values of group B (1.763±0.11 grams) was found. This significant decrease in mean values of group A and B might be due to destruction of renal parenchyma or a decline in growth of organ or both processes were going on at the same time. The microscopic study showed degenerative changes that can account for this decrease. When group A and B were compared with each other as well as with control group C changes were more marked in group B; that means whatever process was producing this decrease that was more marked in high dose group. RTWI calculation showed a borderline value of ANOVA that means cellular degeneration might be responsible for mean kidney weight changes after twelve weeks, these findings are in agreement with previous research[15].

Renal corpuscles showed degenerative changes that include obliterated urinary space in focal areas with non-significant increase in size of renal corpuscles (Figure 3 & 5); these findings were in accordance with the previous work[16,25]. No hyper-cellularity or congestion was present on histological examination. Therefore, the increase in size might be due to the swelling of glomerular cells including endothelial cells and podocytes. This swelling can be explained by presence of glutamate receptors in glomerulus as reported earlier[18,26].Degenerative changes observed in proximal and distal convoluted tubules were in agreement with observations reported earlier[16,26],and were explained by presence of glutamate receptors in renal glomeruli and PCTs that causes the influx of Ca^{2+} and Na^{2+} with passive influx of water resulting in appearance of vacuoles[25].

Most harmful effects caused by MSG might be explained by its mode of action. It acts on glutamate receptors and activation results in influx of Ca^{2+} and Na^{2+} resulting in commencement of number of events that include activation of several enzyme pathways and signalling chain of reactions resulting in cellular injury by activation of phospholipases, protein kinase C, proteases, protein phosphatases, nitric acid synthases and generation of free radicals. The destabilization of Ca^{2+} homeostasis results in membrane destabilization adding to Ca^{2+} mediated toxicity[25, 27].It is known that ionotropic GluR act as mediators of inflammation and cellular injury[27].

CONCLUSION

Continuous use of MSG in moderate to high doses has a deleterious effect on the nephrons of adult Wister rats. Since, similar changes are expected in human being, a cautious use is advisable. These findings could direct a path for future research in human beings and exploration of preventive measures to minimize the side effects.

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