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Original Article	**The Effects of Chronic Aspartame Administration on the Kidney of Albino Rat**

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ABSTRACT

Background: Aspartame is a widely employed synthetic sweetener used in diet control and by diabetic patients. Its safety based on the findings of the previous studies showed controversy.

Aim of the Work: The aim of this study was to demonstrate the chronic effect of aspartame on the structure of the kidney in the newborn, adult and old albino rats.

Materials and Methods: In this work, 60 albino rats were used, 40 of which were three month old, while the remaining 20 rats were twelve month old at the beginning of the study. They were divided into 3 groups: A, B, C and every group was subdivided into 2 subgroups; control and treated. Group A included 20 female albino rats aged three months. The treated subgroup A received aspartame in a dose of 20 mg/kg/day dissolved in tap water through an intragastric tube for three months pregestational and during the gestational period. The offspring numbers, body and kidney weights were estimated and statistically analyzed and their kidneys were examined histopathologically. Group B included 20 adult albino rats aged three months. Group C included 20 albino rats aged twelve months. The treated animals of groups B and C were given aspartame in the same dose and by the same route as in group A for six months. The kidney specimens from all groups were processed for light microscopic examination using Haematoxylin and Eosin stain. Toluidine blue stain was used for the semithin sections of the adult rat's kidney specimens. Electron microscopic study of the proximal convoluted tubules of the adult kidney was done.

Results: The results of this study revealed a delayed development of the kidney of the newborn rat with the maternal aspartame administration in addition to degenerative changes in the renal corpuscles and tubules. The statistical analysis of the newborns' body and kidney weights showed significant reduction. The kidneys of aspartame-treated adult rats showed degenerative changes affecting the renal corpuscles and tubules. The renal corpuscles had shrunken glomerular capillary tuft and widened Bowman`s space. Some of which revealed irregularity of Bowman`s capsule. The renal tubules showed dilatation of the tubular lumen, dense nuclei and vacuolated cytoplasm with sloughed epithelial lining cells. Congested and dilated blood vessels were also observed. The ultrastructural study of the proximal convoluted tubular lining cells revealed an extensive damage of the cytoplasmic organelles and the brush border.

Aspartame-treated aged rat's kidney showed massive degenerative changes in comparison to the other treated groups. All the renal tubules showed thinning of their lining epithelium with dilated lumen. Some of which had destructed or thickened basement membrane. Others showed accumulation of dense acidophilic casts inside the lumen. Dilated and congested blood vessels with vacuolated cytoplasm were noticed.

Conclusion: It was concluded from this study that aspartame had nephrotoxic effects on the newborn, adult and aged rats.

Key Words: Aspartame, kidney, rat, offspring, aged.

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INTRODUCTION

Aspartame is the methylester of N-alpha –aspartyl –L-phenylalanine. It is a polypeptide, widely employed intense synthetic sweetener, having none or insignificant caloric values and a sweetening power higher than sucrose *(European Food Safety Authority, 2002)*. Aspartame took 17 years to be approved for consumption. This period was marked by controversy about its innocuousness that led to requests for toxicological research that still persists at the present time *(Aspartame Information Center, 2005)*.

It received marketing approval in 1973, but only 3 months later aspartame was withdrawn because of allegations based on improperly designed experimental studies dealing with its carcinogenic effects on the rodent brain *(Jankovic, 2003)*.

The reports on the toxic effects of aspartame are numerous and various issues related to the possible adverse effects have been raised as regard its metabolic components phenylalanine, aspartic acid, diketopiperazine and methanol as well as the compound itself *(Trocho et at., 1998)*. However, a conclusive evidence was not found *(Yost, 1989; Stegink et al., 1990; Butchko & Kotsonis ,1991; Butchko et al., 2002)*.

The developing fetuses may be particularly at risk due to the effects of aspartame where the placenta can concentrate amino acids in fetal plasma. However, there are few studies on the use of aspartame in gestation and little is known about its effect on the kidney with maternal administration *(Ranney et al., 1976)*.

Although the renal excretion is the major process for the removal of toxic substances, the renal participation in the assimilation of many substances including aspartame has not been considered. Bearing in mind their indiscriminate utilization, the understanding of how certain peptides are metabolized by the kidney, may fill the existing gaps in the understanding of their morphometric effects on this organ *(Cardelloefaz et al., 2001; Martins & Azoubel, 2007)*. So, the aim of the present work was to clarify the chronic effect of aspartame on the structure of the fetal, adult and old rat's kidney.

MATERIALS AND METHODS

Experimental animals:

In this study, 60 albino rats were used; 40 of which were three month old, while the remaining 20 rats were twelve month old at the beginning of the research. They were obtained from the Animal House of Assiut University and maintained under normal conditions with free access to food and water in the normal daily light and darkness cycle.

In the present work, albino rats were used as an animal model as the data from rats concerning aspartame were closely parallel to human data. The experimental model of long term administration for six months was chosen to mimic the actual practical use of the sweeteners *(Ranney et al., 1976)*.

Treatment regimen: The experimental animals were divided into 3 groups:

- **Group A:** Twenty female albino rats aged three months were subdivided into 2 subgroups: control and treated.

Control subgroup: Consisted of 10 animals that were given tap water through an intragastric tube for three months pregestational and during the gestational period.

Treated subgroup: Consisted of 10 animals that were given aspartame in a dose of 20 mg/kg/day, which was within the limit of the accepted daily intake according to the World Health Organization food additive series, 16 2000 *(Xili et al., 1992; Wasuntarawat et al., 1998; Koyama et al., 2003; Soffritti et al., 2005)*. It was dissolved in tap water and given through an intragastric tube for three months pregestational and during the gestational period.

After the first three months of treatment, mating was allowed. Every morning the female rats were examined for the presence of vaginal plugs. Vaginal smears were tested to detect the occurrence of pregnancy according to *Montes and Luque (1988)*. Pregnancy was diagnosed by the appearance of the vaginal plug and the presence of cornified non- nucleated cells, leucocytes and a large quantity of mucus in the vaginal smear.

The offspring were collected and their body weights were estimated and sacrificed to weigh and investigate their kidneys.

- **Group B:** Twenty adult albino rats aged three months were subdivided into two subgroups: control and treated.

Control subgroup: Consisted of 10 animals that were given tap water through an intragastric tube, then sacrificed after six months and their kidneys were collected.

Treated subgroup: Consisted of 10 animals that were given aspartame in the same dose and by the same route as in group A, then sacrificed after six months and the kidneys were collected.

- **Group C:** Twenty albino rats aged twelve months were subdivided into two subgroups: Control and treated.

Control subgroup: Consisted of 10 animals that were given tap water through an intragastric tube, then sacrificed after six months and their kidneys were collected.

Treated subgroup: Consisted of 10 animals that were given aspartame in the same dose and by the same route as in groups A and B, then sacrificed after six months and the kidneys were collected.

Kidney specimens representing all groups were processed for light microscopic examination using Haematoxylin and Eosin stain *(Drury & Wallington, 1980)*. Also, semithin sections (1micron) were prepared from half of group B kidney specimens and were stained with toluidine blue to be examined under the light microscope.

Ultra-thin sections (0.1micron) were prepared for transmission electron microscopic examination using uranyl-acetate and lead citrate *(Bozzola & Russel, 1992)*.

Statistical analysis:

This was done for the numbers of rats' offspring per pregnant female, their body and kidney weights in both the control and treated subgroups of group A. The variables were represented by M±SE (Mean±Standard error).

Student t-test was used for comparing the means of the variables between the control and treated subgroups of group A.

RESULTS

Histological Results

A. Newborn rats:

The kidney of control animals:

The light microscopic examination of the cortex of the kidney showed the presence of many aberrant well developed renal corpuscles with definitive glomerulus and normal Bowman`s capsule. The renal tubules and collecting tubules had normal epithelial lining cells and normal tubular lumen (Fig. 1).

The kidney of treated animals:

The light microscopic examination of the kidney showed a relative decrease in the cortical thickness with the presence of aberrant less differentiated renal corpuscles as compared with the control (Fig. 2). Some of the renal tubules showed dilated lumen with exfoliation of their epithelial lining cells (Figs. 2, 4, 6). Some tubules showed dense nuclei and vacuolated cytoplasm (Figs. 4, 6). Congested and dilated blood vessels were also observed (Fig. 5). The distal convoluted tubules showed an abnormal v-shaped arrangement (Fig.3). Disorganized corpuscles, loss of Bowman`s capsule and congested glomerulus with dense nuclei were observed (Fig. 4). Some renal corpuscles showed shrunken vacuolated glomerular capillary tuft and dilated Bowman`s capsule (Figs. 4, 5, 6).Completely obliterated proximal convoluted tubules were demonstrated (Fig. 2).

B. Adult rats

The kidney of control animals:

The light microscopic examination of the kidney demonstrated normal histological picture. The proximal convoluted tubular lining cells were cuboidal to columnar and had a large rounded nucleus. The distal convoluted tubular lining cells were cuboidal with a central rounded nucleus (Fig. 7).

The examination of the semi-thin sections of the kidney at this age revealed that the lining cells of the proximal convoluted tubules had normal nuclei and cytoplasm with typical brush border (Fig. 15).

The ultrastructure of the proximal convoluted tubular lining cells revealed the typical appearance of the microvilli forming the brush border. The cell had a normal euchromatic nucleus limited by a regular nuclear membrane with one nucleolus. The cytoplasm was rich in healthy lightly stained oval or rounded mitochondria with well defined cristae and rough endoplasmic reticula (Figs. 18,19).

The kidney of treated animals:

The light microscopic examination of the kidney revealed many degenerative changes. The renal corpuscles had shrunken glomerular capillary tuft and dilated Bowman`s capsule (Figs. 8, 11). Some renal corpuscles showed irregularity of Bowman`s capsule as well as congestion and destruction of the glomerular capillary tuft (Figs. 9, 11).

The renal tubules showed dilatation of the tubular lumen with dense nuclei and vacuolated cytoplasm (Figs. 11,12, 14). Some tubules showed sloughed epithelial lining cells with exfoliated nuclei and accumulation of dense acidophilic casts inside the lumen (Figs. 10, 14). Some tubules were destructed and fused with each other (Fig.13). Cellular infiltrations among the tubules (Figs. 9, 13) with congested and dilated blood vessels were observed (Figs. 8, 12, 13). Extravasated RBCs were seen between the tubules (Figs. 11, 12).

The examination of the semi-thin sections of the kidney at this age revealed that the lining cells of the proximal convoluted tubules had small condensed pyknotic nuclei, some of which showed loss of the nuclear membrane and other nuclei had irregular outline. Also, fragmentations of some nuclei with indistinct nuclear boundaries were manifested. The cytoplasm of the lining epithelium showed vacuolization (Fig. 16). The lining cells of the proximal convoluted tubules showed leakage of the cellular materials with formation of cellular debris (Fig.17). Damage of the microvilli forming the brush border was observed (Figs. 16, 17).

The ultrastructure of the proximal convoluted tubular lining cells revealed an extensive damage of the microvilli forming the brush border. The cells became shrunken. The cytoplasm appeared rarified with scanty haphazard cell organelles. There were decreased numbers of mitochondria with destructed cristae, lysosomes and vesicles (Figs. 20, 21). Some nuclei became shrunken and revealed a peripheral condensation of chromatin (Fig.20). Other nuclei were more or less normal (Fig. 21).

C. Old rats

The kidney of control animals:

The light microscopic examination of the kidneys of the old rats showed the typical histological picture of the normal adult rat's kidney. However, the majority of the renal corpuscles had enlarged glomerular capillary tuft, narrow or sometimes obliterated Bowman`s space (Fig. 22).

The kidney of treated animals:

The treatment with aspartame for six months led to obvious histological changes in the kidney. The light microscopic examination of the renal tubules showed thinning of their lining epithelium with dilated lumen (Fig. 23). Most of these revealed thickened basement membrane. Some tubules showed destructed or detached basement membrane (Figs. 24, 27, 29). Exfoliation of the lining epithelium and fusion of the tubules were observed (Figs. 24, 26, 28, 29). Also, completely obliterated tubules were demonstrated (Figs. 28, 29). Most of the proximal and distal convoluted tubular lining cells had dense nuclei associated with vacuolated cytoplasm (Figs. 25, 28, 29). Some tubules showed accumulation of dense acidophilic casts inside the lumen (Fig. 25). Most of the renal corpuscles had disrupted histological structure in the form of lost, irregular or detached Bowman`s capsule, obliterated Bowman`s space and congested or vacuolated glomerular capillary tuft with dense nuclei (Figs. 24, 25, 26, 27, 29). Dilated blood vessels with vacuolated cytoplasm were noticed (Figs. 24, 27).

Statistical Results

All rats' offspring of group (A) control and treated subgroups appeared externally normal and viable. The means of their numbers per pregnant female were similar in both the control and treated subgroups. The mean±standard error (M±SE) of the numbers of the control subgroup was (9.73±0.38) and of the treated subgroup was (9.55±0.45) with no significant difference (Table 1; Histogram 1).

The mean ± standard errors (M±SE) of their body weights were (8.50±0.25 gm) in the control subgroup and (4.87±0.06 gm) in the treated subgroup which showed an apparent decrease in body weight in the treated subgroup offspring as compared with the control subgroup offspring. This decrease was found to be very highly significant (Table 2; Histogram 2).

The mean ± standard errors (M±SE) of their kidney weights were (0.17±0.01 gm) in the control subgroup and (0.03±0.00 gm) in the treated subgroup which showed an apparent decrease in kidney weight in the treated subgroup offspring as compared with the control subgroup offspring. This decrease was found to be very highly significant (Table 3; Histogram 3).

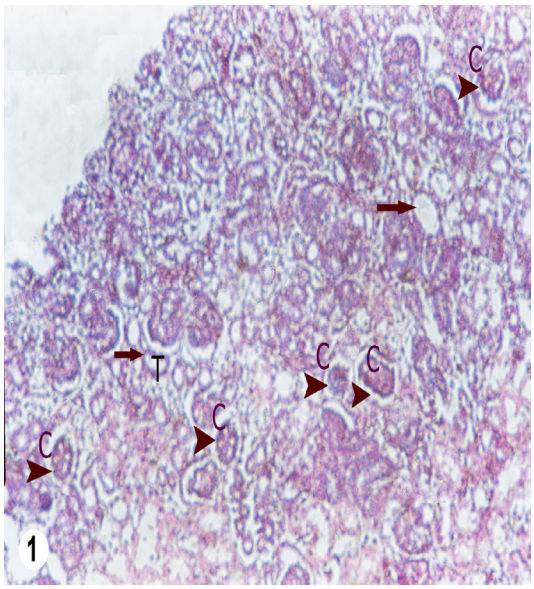


Fig. 1: A photomicrograph of a section in the kidney of a control newborn rat showing many aberrant well developed renal corpuscles (C) with definitive glomerulus and normal Bowman`s capsule with its parietal and visceral layers (arrowheads). Notice the renal tubules (T) with normal epithelial lining cells (arrows) and normal tubular lumen. Hx. & E.; X 250

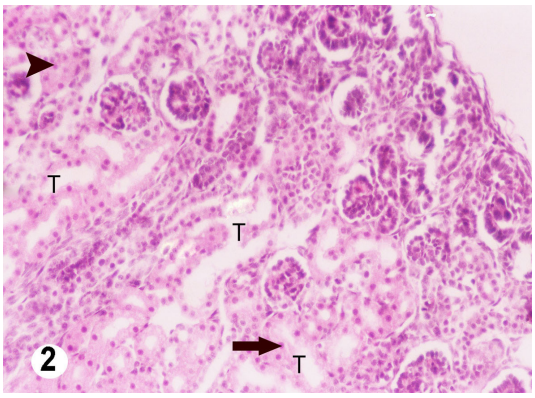


Fig. 2: A photomicrograph of a section in the kidney of aspartame-treated newborn rat. It shows a decreased cortical thickness and aberrant less differentiated renal corpuscles as compared with the control. Notice the dilated renal tubules (T) with dense nuclei (arrow) and the completely obliterated tubules (arrowhead). Hx. & E.; X 250

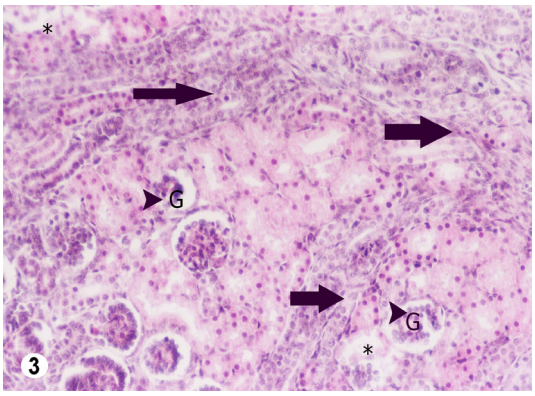


Fig. 3: A photomicrograph of a section in the kidney of aspartame-treated newborn rat. It shows disorganized corpuscles (G) with dense nuclei (arrowheads). Notice the v- shaped arrangement and dilatation of the distal convoluted tubules (arrows). Some tubules show exfoliation of the lining epithelium (star). Hx. & E.; X 250

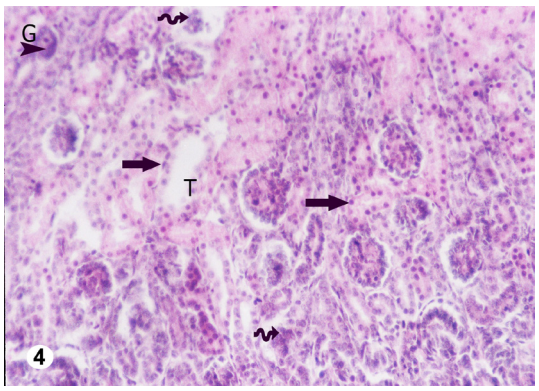


Fig. 4: A photomicrograph of a section in the kidney of aspartame-treated newborn rat. It shows a congested glomerulus (G) with dense nuclei (arrowhead) and loss of Bowman's capsule. Some renal corpuscles show shrunken glomerular capillary tuft (curved arrows). The renal tubules (T) show dense nuclei and cytoplasmic vacuolization(arrows). Hx. & E.; X 250

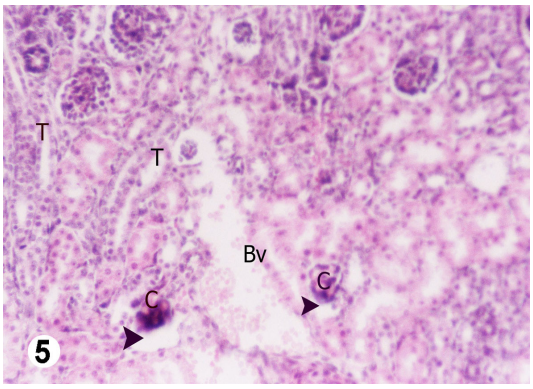


Fig. 5: A magnified photomicrograph of a section in the kidney of aspartame-treated newborn rat showing a dilated congested blood vessel (Bv). Some renal corpuscles (C) have congested glomerulus and dilated Bowman`s space (arrowhead). The tubules (T) show dilated tubular lumen and dense nuclei. Hx. & E.; X 400

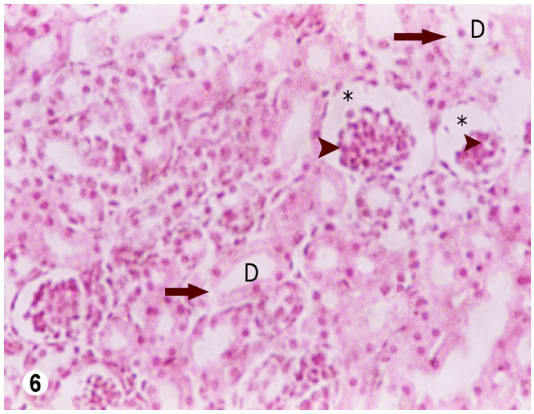


Fig. 6: A magnified photomicrograph of a section the kidney of aspartame-treated newborn rat. The renal corpuscles shows shrunken vacuolated glomerular tuft with dense nuclei (arrowhead) and widened Bowman's space (stars). The distal (D) convoluted tubules show destructed epithelium (arrows) with dense nuclei and vaculoted cytoplasm. Hx. & E.; X 400

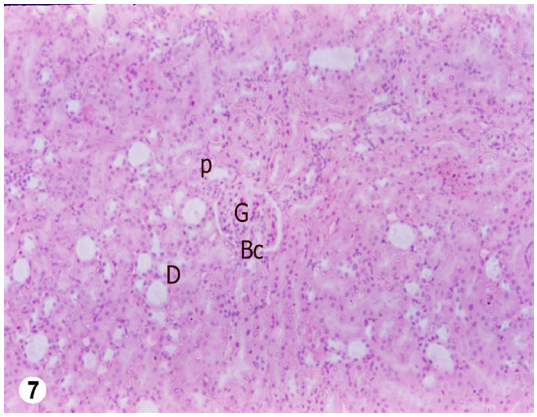


Fig. 7: A photomicrograph of a section in the kidney of a control adult rat. The renal corpuscles have normal capillary tuft (G) and Bowman's capsule (Bc). The proximal (p) and distal (D) convoluted tubules have normal epithelial lining cells and normal tubular lumen. Hx. & E.; X 250

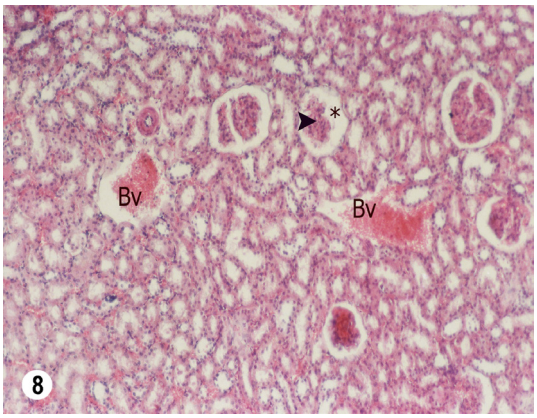


Fig. 8: A photomicrograph of a section in the kidney of aspartame-treated adult rat. It shows congested and dilated blood vessels (Bv). Notice the shrunken glomerular tuft (arrowhead) with widened Bowman's space (star). Hx. & E.; X 100

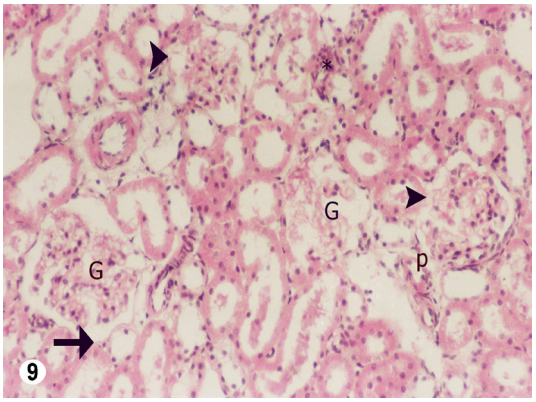


Fig. 9: A photomicrograph of a section in the kidney of aspartame-treated adult rat showing irregularity in Bowman`s capsule (arrow), distortion of the renal corpuscle and destruction of the glomerular capillaries (G). The proximal convoluted tubules (p) have dense nuclei. Notice the destructed Bowman`s capsule (arrowhead) and cellular infiltration (star). Hx. & E.; X 250

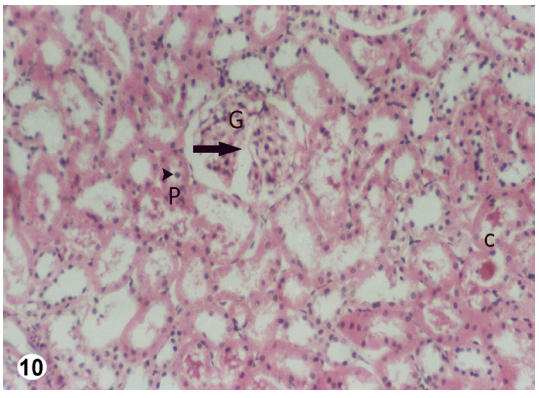


Fig. 10: A photomicrograph of a section in the kidney of aspartame-treated adult rat. The proximal convoluted tubules (p) show dense exfoliated nuclei (arrowhead). Some tubules show dense acidophilic casts in the lumen (C). Notice the glomerulus (G) with lobulated capillary tuft (arrow). Hx. & E.; X 250

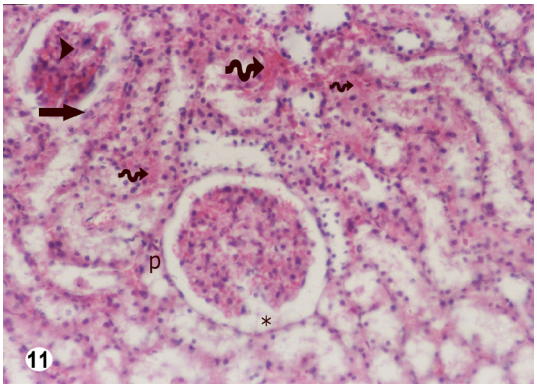


Fig. 11: A magnified photomicrograph of a section in the kidney of aspartame-treated adult rat. The proximal convoluted tubules (p) show dense nuclei and vacuolated cytoplasm with dilated lumen.The renal corpuscles reveal distortion in Bowman`s capsuale (arrow), dilatation of Bowman`s space (star) and congestion of the glomerular tuft (arrowhead). Notice the extravasated RBCs between the tubules (curved arrows). Hx. & E.; X 400

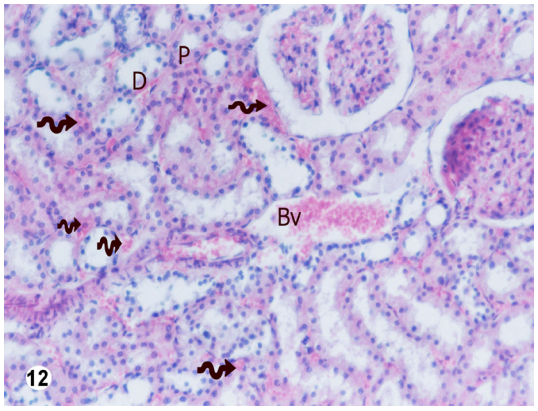


Fig. 12: A magnified photomicrograph of a section in the kidney of aspartame-treated adult rat. It shows congested and dilated blood vessels (Bv). The proximal (p) and distal (D) convoluted tubules have dense nuclei and vacuolated cytoplasm with dilated lumen. Notice the extravasated RBCs between the tubules (curved arrows). Hx. & E.; X 400

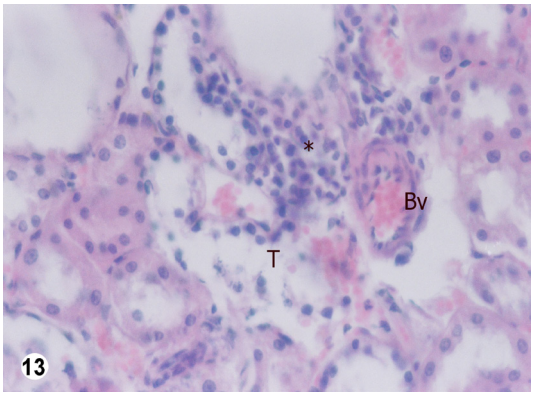


Fig. 13: A magnified photomicrograph of a section in the kidney of aspartame-treated adult rat showing cellular infiltration among the tubules (star) ,congested and dilated blood vessels (Bv), fusion and destruction of the walls of some tubules (T). Hx. & E.; X 400

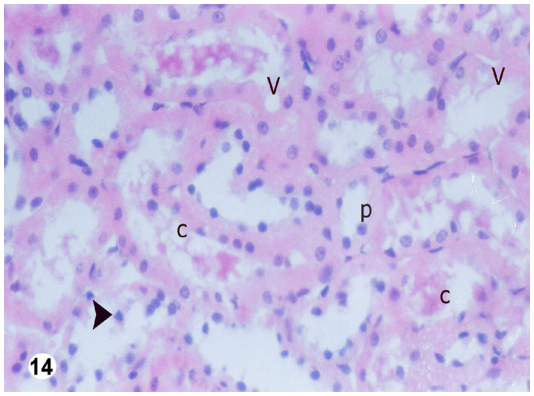


Fig. 14: A magnified photomicrograph of a section in the kidney of aspartame- treated adult rat. It shows accumulation of casts inside the renal tubules (c). The proximal convoluted tubules (p) show dense nuclei and dilated lumen. The distal convoluted tubules show exfoliated nuclei (arrowhead) and vacuolated cytoplasm (v) with dilated lumen. Hx. & E.; X 400

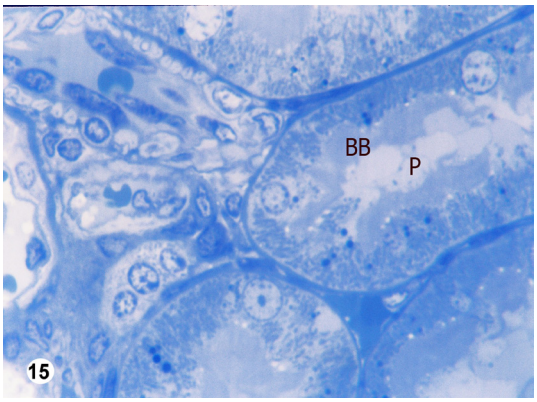


Fig. 15: A photomicrograph of a semithin section in the kidney of a control adult rat showing that the proximal convoluted tubule (P) has normal epithelial lining cells with the presense of the brush border (BB). Toluidine blue; X 1000

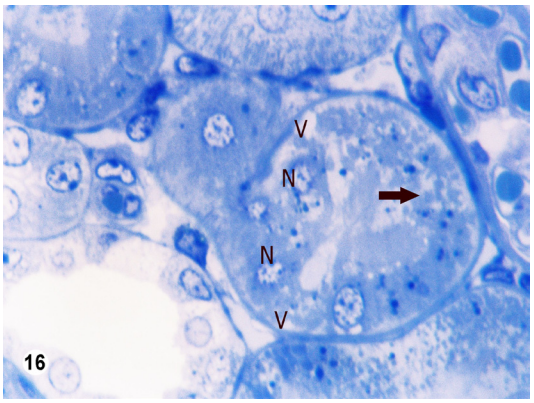


Fig. 16: A photomicrograph of a semithin section in the kidney of aspartame- treated adult rat showing that the proximal convoluted tubule has vacuolated cytoplasm (v) and a destructed brush border (arrow). The nuclei show apoptotic figure, fragmentation, indistinct nuclear boundaries and irregular outline (N). Toluidine blue; X 1000

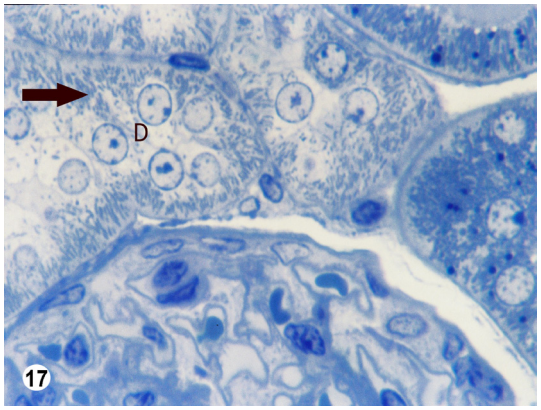


Fig. 17: A photomicrograph of a semithin section in the kidney of aspartame-treated adult rat. The proximal convoluted tubules show destructed brush border (arrow) and presense of cellular debris inside the lumen (D). Toluidine blue; X 1000

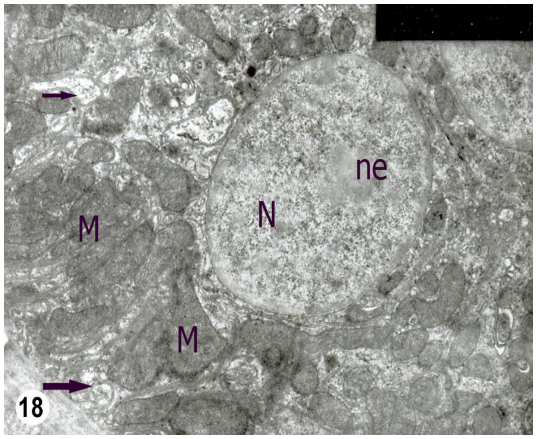


Fig. 18: An electron micrograph of a proximal convoluted tubular lining cell in the kidney of a control adult rat. It shows an euchromatic nucleus (N) with a regular nuclear membrane and a prominent nucleolus (ne). The cytoplasm is rich in healthy appearant mitochondria (M) and rough endoplasmic reticula (arrows). X 5,000

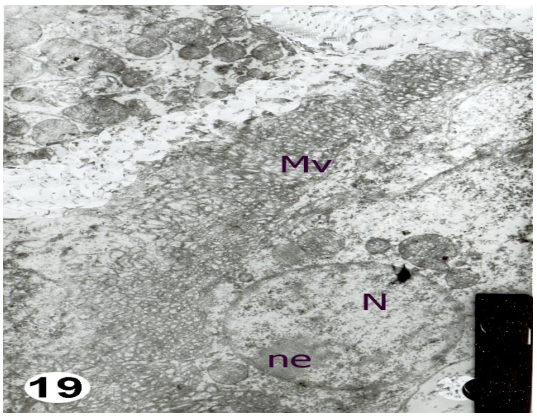


Fig. 19: An electron micrograph of a proximal convoluted tubular lining cell in the kidney of a control adult rat. It shows the typical appearance of the microvilli (Mv) forming the brush border and an euchromatic nucleus (N) with a regular nuclear membrane and a prominent nucleolus (ne). X 5,000

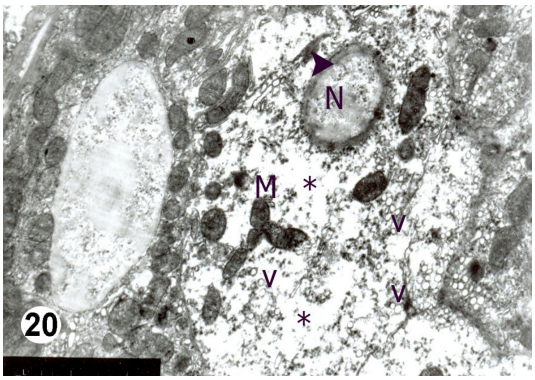


Fig. 20: An electron micrograph of a proximal convoluted tubular lining cell in the kidney of aspartame-treated adult rat. The cytoplasm shows a decreased number of mitochondria with destructed cristae (M) and vesicles (V). The nucleus (N) is shrunken with peripheral condensation of chromatine (arrowhead). Notice the rarified cytoplasm (stars) with scanty haphazard cell organelles. X 5,000

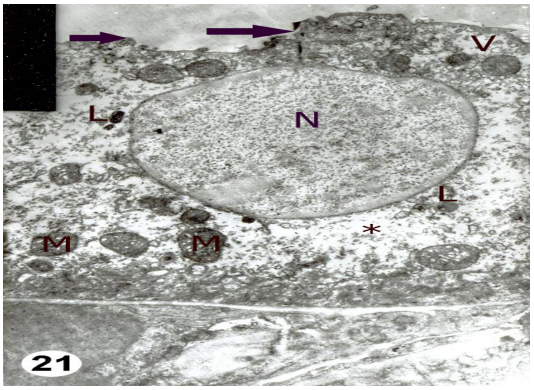


Fig. 21: An electron micrograph of a proximal convoluted tubular lining cell in the kidney of aspartame-treated adult rat. It shows damage in the microvilli forming the brush border (arrows). The cytoplasm shows a less number of mitochondria with destructed cristae (M), some lysosomes (L) and vesicles (V). The nucleus (N) is more or less normal. Notice the rarified cytoplasm (star) with scanty haphazard cell organelles and the shrinkage of the cell. X 5000

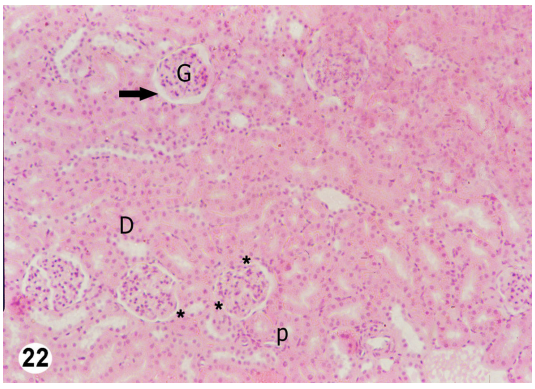


Fig. 22: A photomicrograph of a section in the kidney of a control old rat. The renal corpuscles have enlarged capillary tuft (G) and narrow or sometimes obliterated Bowman's space (star) with regular Bowman`s capscule (arrow). The proximal (P) and distal (D) convoluted tubules have normal epithelial lining cells and normal tubular lumen. Hx. & E.; X 250

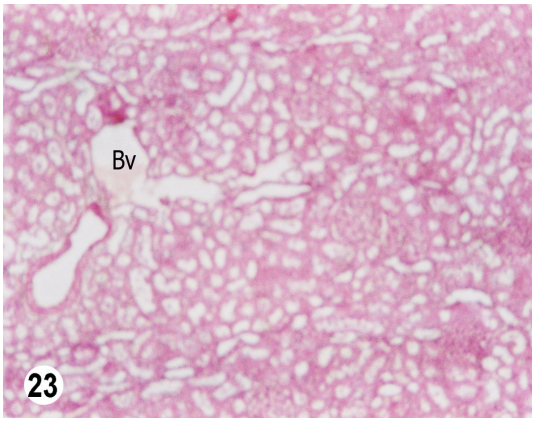


Fig. 23: A photomicrograph of a section in the kidney of aspartame-treated old rat. All renal tubules show thinning of the lining epithelium with diluted lumen. Notice the dilated blood vessel (Bv). Hx. & E.; X 100

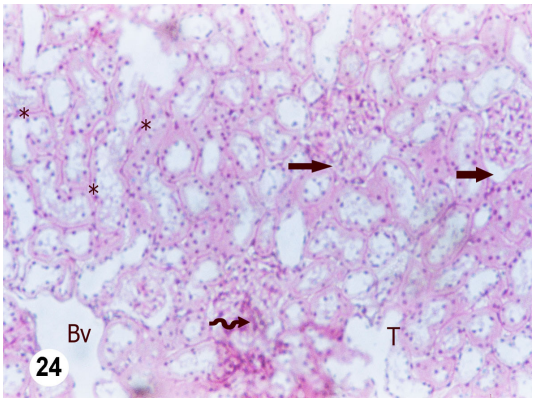


Fig. 24: A photomicrograph of a section in the kidney of aspartame-treated old rat. The renal corpuscles show congested capillary tuft (curved arrow). Some corpuscles show irregular Bowman`s capscule (arrows). The renal tubules show thickened basement membrane (stars). There are destructed and fused tubules (T). Notice the dilated blood vessels (Bv). Hx. & E.; X 250

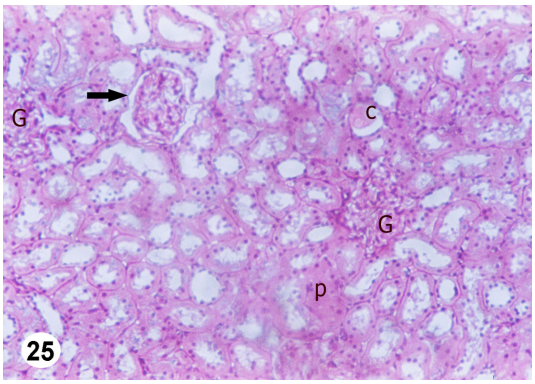


Fig. 25: A photomicrograph of a section in the kidney of aspartame-treated old rat showing disorganized renal corpuscles (G) with obliterated Bowman`s space. Other renal corpuscles reveal irregular Bowman`s capuscle (arrow). The proximal convoluted tubules (p) show dense nuclei and obliterated lumen. Notice the accumulation of acidiophilic casts (c) inside the tubules. Hx. & E.; X 250

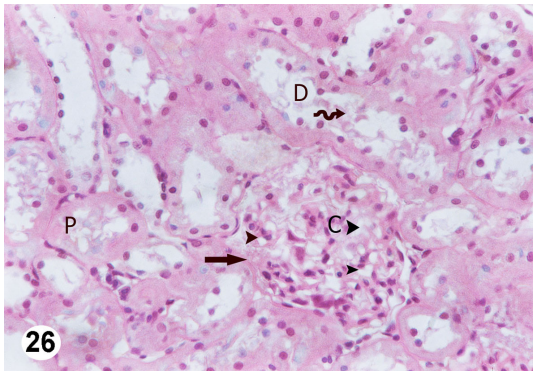


Fig. 26: A magnified photomicrograph of a section in the kidney of aspartame- treated old rat showing a disfigured renal corpuscle (C) with an absent Bowman`s space. Notice the vacuolated glomerular tuft (arrowhead) and irregular Bowman`s capuscle (arrow). The distal convoluted tubules (D) show exfoliated epithelium and dilated lumen (curved arrow).The proximal convoluted tubules (p) reveal destructed epithelial lining and vacuolated cytoplasm. Hx. & E.; X 400

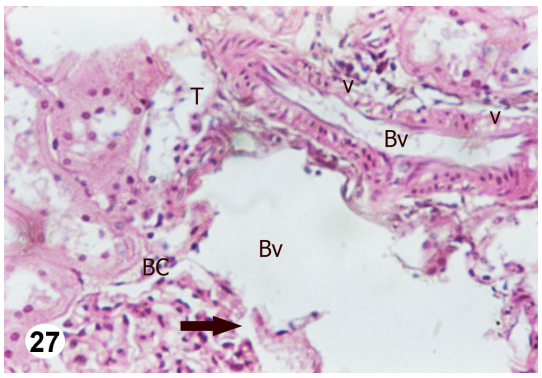


Fig. 27: A magnified photomicrograph of a section in the kidney of aspartame- treated old rat showing dilated fused renal tubules with disturbed lining epithelium and dense nuclei (T). Bowman`s capuscle (BC) shows detachment and irregularity (arrow). Notice the dilated blood vessels (Bv) with vacuolated cytoplasm (v). Hx. & E.; X 400

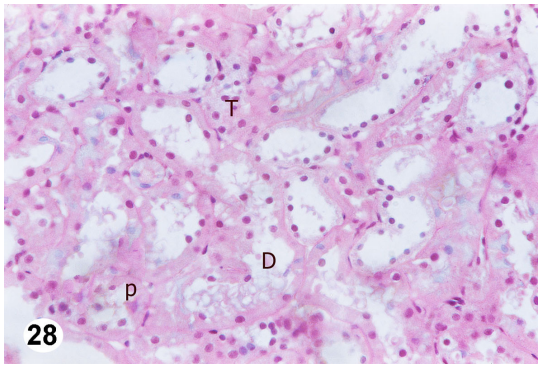


Fig. 28: A magnified photomicrograph of a section in the kidney of aspartame- treated old rat. The distal convoluted tubules (D) show exfoliated nuclei, vacuolated cytoplasm, dilated lumen and destructed epithelial lining. The proximal convoluted tubules (p) have dense exfoliated nuclei and vacuolated cytoplasm. Notice the compeletly obliterated tubules (T). Hx. & E.; X 400

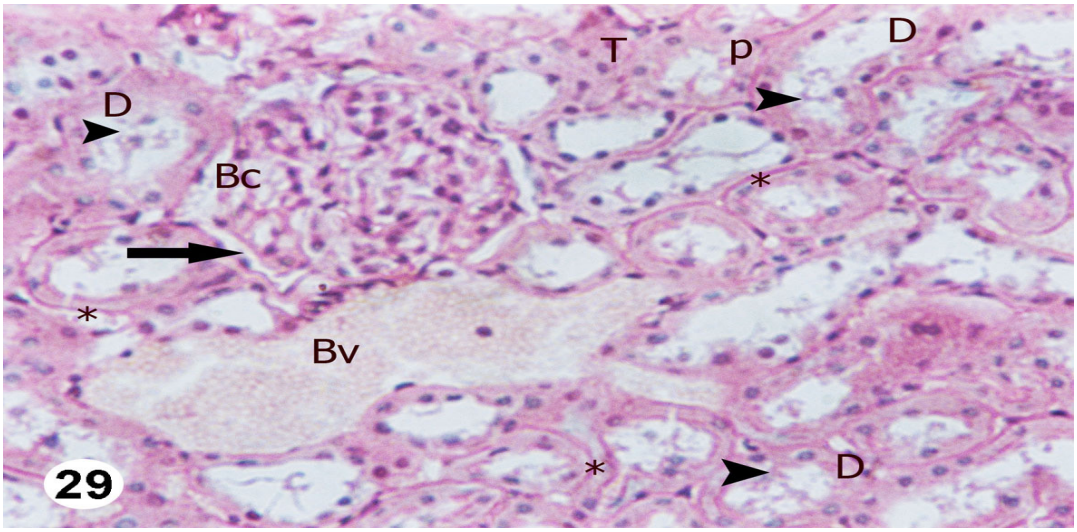
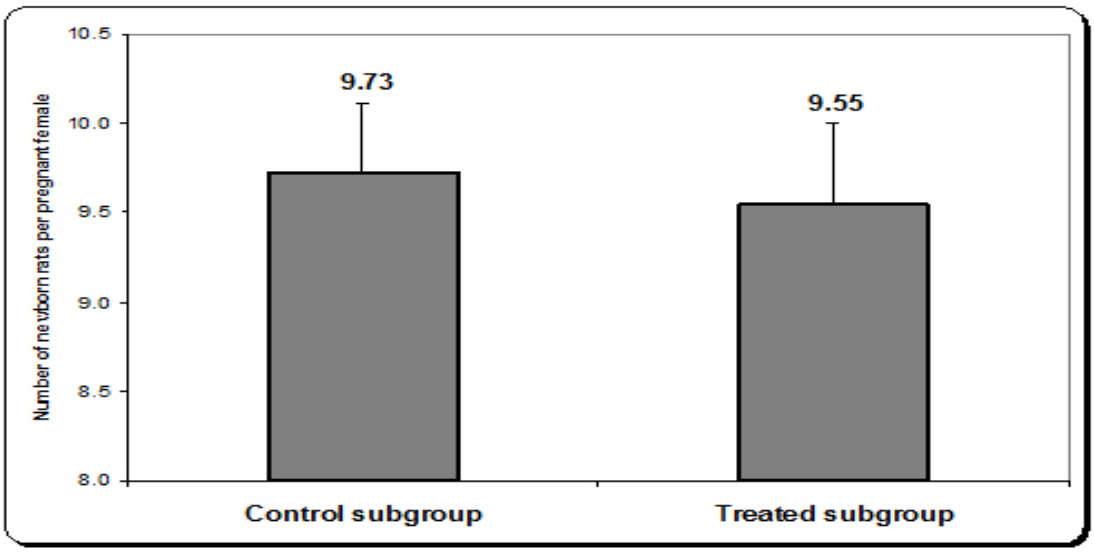


Fig. 29: A magnified photomicrograph of a section in the kidney of aspartame-treated old rat. The distal convoluted tubules (D) show exfoliated nuclei (arrowhead).The renal corpuscle shows an irregular Bowman`s capuscle (arrow). The proximal convoluted tubules (p) have dense nuclei and vacuolated cytoplasm .All renal tubules reveal thickened basement membrane (star). Notice the obliterated tubule (T). Hx. & E.; X 400

Table 1: The numbers of newborn rats per pregnant female of the control and treated subgroups.

	Mean	Standard error	P value
Control subgroup	9.73	0.38	0.76 (NS)
Treated subgroup	9.55	0.45	

P> 0.05 (NS) → Non-significant difference.

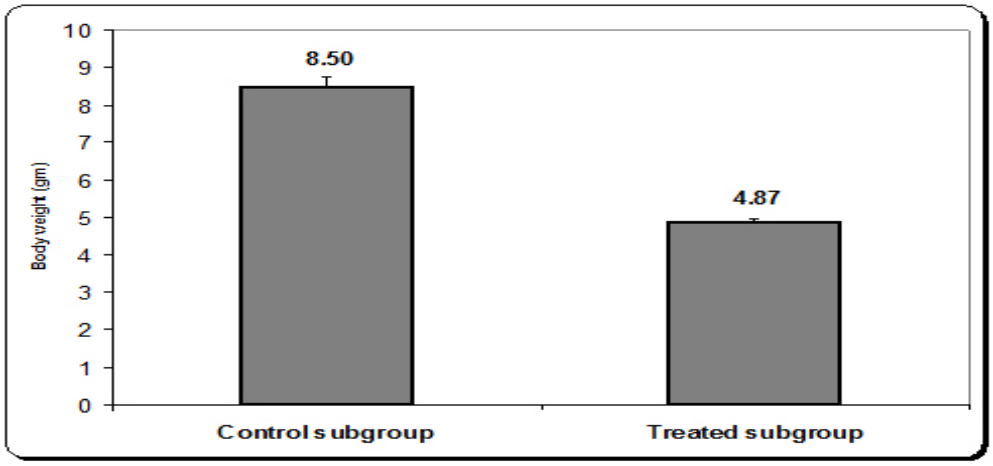


Histogram 1: A graphic representation showing the mean numbers of newborn rats per pregnant female of the control and treated subgroups

Table 2: The body weights (gm) of the control and treated rats' offspring.

	Mean	Standard error	P value
Control subgroup	8.50 gm	0.25 gm	***
Treated subgroup	4.87 gm	0.06 gm	0.000

P< 0.001 (***) → very highly significant difference.

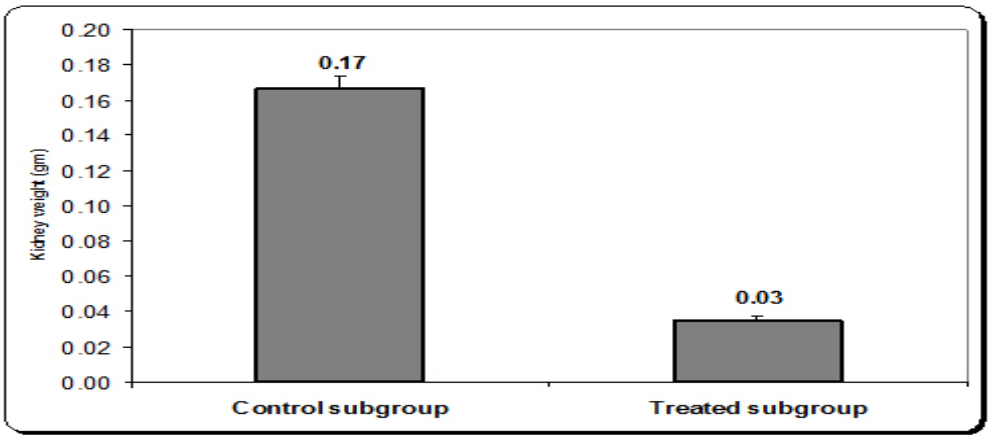


Histogram 2: A graphic representation showing the means of body weights of the control and treated rats' offspring

Table 3: The kidney weights (gm) of the control and treated rats' offspring.

	Mean	Standard error	P value
Control subgroup	0.17 gm	0.01 gm	***
Treated subgroup	0.03 gm	0.00 gm	0.000

P < 0.001 (***) → very highly significant difference.



Histogram 3: A graphic representation showing the means of kidney weights of the control and treated rats' offspring

DISCUSSION

The renal cortex of the control rat's offspring in the present work showed that nephrogenesis proceeded from the deep to the outer cortex. This result is in agreement with those of *Friis (1980), Poules et al. (1996) and Belles et al. (1999)* who further suggested that nephrogenesis was directed by a second, entirely different developmental process. The formation of nephrons continued up to about 3 weeks of age, after which the morphological development was a differentiation of the nephrons already presented.

In the present work, the kidney of aspartame-treated newly born rats showed a massive retardation in renal development as well as destructive histological changes. These findings are in the same line as the results of *Marielza et al. (2007)* who suggested that the administration of aspartame (APM) on the 9^{th}, 10^{th} and 11^{th} days of pregnancy led to alterations in the development of renal structures and a delayed fetal growth as expressed by cell damage during this period. Also, the present results are supported by the study of *McConnell (1973)* on neonate rats that had been exposed to APM in utero and showed massive changes in the kidney in the form of minimal to slight hypertrophy and vacuolization of the tubular cells nuclei in the inner cortex. *Soffritti et al. (2007)* demonstrated that when life-span exposure to APM began during fetal life, its carcinogenic effects increased.

Simintzi et al. (2007) stated that people on weight reduction programs and especially pregnant women might take care avoiding aspartame consumption because aspartic acid and phenylalanine crossed the placenta in a concentration-dependent manner and possibly crossed the blood–brain barrier entering the CSF of the fetus.

In contrast, *Sturtevant (1985)* stated that there was no evidence of risk to the fetus as aspartate did not cross the placenta and small elevations of blood methanol following abuse doses of aspartame were observed. Also, fetal blood phenylalanine levels did not rise to the level associated with the development of mental retardation in the offspring. Adverse effects of phenylalanine on fetuses were observed only when blood phenylalanine levels remained high as opposed to spiking occasionally *(London, 1988)*.

As regard the histological structure of the control adult rat's kidney, the current study revealed that the renal corpuscles were oval to round structures. Each corpuscle consisted of a glomerular tuft of capillaries surrounded by Bowman's capsule. The Bowman's capsule had two layers: parietal and visceral with Bowman's space in between. The same findings were obtained by *Junqueira and Carneiro (1998), Young and Heath (2000) and Van De Graaff (2002)*.

The proximal convoluted tubules were lined with a simple cuboidal epithelium with a well-developed brush border. The basal parts of the cells showed basal acidophilic striations due to the presence of basal infoldings of the cell membrane; which entrapped a layer of longitudinally oriented large elongated mitochondria. The distal convoluted tubules were lined with cuboidal cells which had less acidophilic cytoplasm and rounded apical nuclei. These findings are in agreement with those of *Kessel (1998) and Young and Heath (2000)*.

In the present work, the kidney of aspartame-treated adult rat revealed destructive histological changes on light microscopic examination. These changes coincide with the results of *Zararsiz et al. (2007).*Who found that in rats treated with formaldehyde as one of aspartame`s metabolites, there were degenerated glomerulei and vacuolization and dilation of the distal tubules. The authors explained these changes by the significant decrease in the renal tissue activities of superoxide dismutase and glutathione peroxidase.

The same results were obtained by *Tada et al. (2008)* who reported that the subchronic oral use of aspartic acid for 90 days led to decreased blood urea nitrogen, creatinine and uric acid levels associated with raised urinary ketone and protein. On histopathological examination, degenerated renal tubules and tubular dilation were observed with an increased kidney weight.

Belpoggi et al. (1995) and *Soffritti et al. (2005, 2006)* stated that aspartame was a multipotential carcinogenic agent. In their studies, aspartame was given to Sprague-Dawley rats from 8 weeks of age until natural death. The authors detected significant dose-related increased incidence of transitional cell carcinomas, dysplasias and calcification of the renal pelvis and ureter. Microscopically, the carcinomas were invading with various levels of extension and mitotic figures.

Ranney et al. (1976) demonstrated that the increased incidence of lymphomas in APM-treated rats could be related to its metabolite methanol which was in turn metabolized to formaldehyde in both humans and rats. Also, *Soffritti et al. (2002)* reported that methanol administered in drinking water at doses ranging from 20,000 to 500 ppm induced a statistically significant increase in the incidences of lymphomas and leukemias in female rats.

Kitahori et al. (1996) observed a dose-related hyperplasia of the renal pelvis when monosodium aspartate (MSA) was administered with drinking water to groups of male and female Fischer rats for 100 weeks. The same effect was found by the same group of investigators in another study in which MSA was administered to evaluate its promoting activity of carcinogenesis of the transitional epithelium of the renal pelvis *(Kitamura et al., 1996)*. In both studies, a clear evidence was provided of a relationship between MSA treatment and the transitional cell hyperplasia. The authors indicated that calcification could have an important role in inducing simple and papillary hyperplasia of the renal pelvis transitional cell epithelium and consequently in the induction of transitional cell tumors. These findings are in agreement with *Soffritti et al. (2006)* who reported that calcification might be the mechanism responsible for this effect.

The destructive effects of aspartame observed in the current work are in contrast with the results obtained with the long-term carcinogenicity bioassays performed on Sprague-Dawley rats, which concluded that aspartame did not have any carcinogenic effects on the kidney *(Food and Drug Administration, 1981; Ishii, 1981; Ishii et al., 1981)*.

Soffritti et al. (2005) stated that the carcinogenic changes in the renal pelvis, ureter, peripheral nerves and proliferative changes in the olfactory epithelium were not observed with long-term administration of methanol and formaldehyde. To investigate if the other two metabolites of APM were responsible for inducing these lesions, it was of a paramount importance to perform adequate life-span carcinogenicity studies on aspartic acid or phenylalanine.

The examination of the semi-thin sections of the kidney of adult rat confirmed the destructive changes demonstrated by light microscopic examination. Also, electron microscopic examination of the lining cells of the proximal convoluted tubules revealed an extensive damage.

Karikas et al. (1998) measured the direct molecular interaction of aspartame and its metabolites with DNA as an indicator of a possible carcinogenic potential in an in vitro model. They observed a measurable dose-related molecular interaction with DNA by aspartame, as well as its amino acid components (aspartic acid and phenylalanine) that might explain the nuclear changes observed in the current study.

On the other hand, *Jeffrey and Williams (2000)* tested the DNA-damaging activity of several sweeteners in a rat hepatocyte/ DNA repair assay. They stated that none of the sweeteners, including aspartame, showed an increase in NNG (net nuclear grain) counts and consequently aspartame had no effect on DNA.

Gombos et al. (2007) stated that after one week of administration of various doses of aspartame to female mice, the p53, c-myc and Ha-ras gene expression increased especially in organs with high proliferation rates such as lymphoreticular organs, bone-marrow and kidney. The authors concluded that aspartame had a biological effect even at the recommended daily maximum dose.

In the present study, mitochondrial changes were observed in the cytoplasm of proximal tubular cells of adult rat kidney in agreement with the study of *Teng et al. (2001)* who detected a marked decrease in the mitochondrial membrane potential and an inhibition of the mitochondrial respiration with aspartame. *Gombos et al. (2007)* stated that the mitochondria and their DNA (MtDNA) were subjected to damage by aspartame. As MtDNA lacked the repair mechanisms of nuclear DNA, the damage was highly cumulative and even passed on to the fetus by the mother.

World Health Organization (1981) reported that with aspartame administration in human, the kidneys showed a significant weight increase and high levels of red and white blood cells were observed in the urine .

Gurney et al. (1997) stated that individuals with renal disease were an especially important population as they could have altered amino acid profiles. Many of them were diabetics and would also be likely to consume aspartame, which pro-

vided two amino acids; aspartic acid and phenylalanine that increased the load on their kidneys. The authors studied 23 diabetic patients with renal failure who were on maintenance hemodialysis to evaluate the effect of aspartame and the postprandial condition on plasma amino acid profiles. The plasma phenylalanine concentrations at 1 and 2 hs after aspartame administration were significantly higher than after placebo but were within the normal postprandial range in these subjects. The plasma concentrations of tyrosine were also higher after aspartame vs placebo but were below the normal postprandial range in these subjects. So the authors concluded that aspartame was safe for diabetic subjects with chronic renal failure.

Older women drinking over 2 aspartame beverages weekly had 30% decline in the kidney function in 11 years *(Lin & Curhan, 2009)*. Also, these authors examined the effects of sodium and artificial sweeteners on the kidney function among more than 3,000 women in the Nurses Health Study. A higher dietary sodium intake was found to be associated with a greater kidney function decline in women with well-preserved kidneys, while the odds for kidney decline doubled for women consuming two or more daily servings of artificially sweetened soda.

On light microscopic examination of the aged control rat's kidney, the renal corpuscles revealed aging changes. Most of them had enlarged glomerular capillary tuft and narrow or sometimes obliterated Bowman`s space.

These results are in agreement with those of *Goyal (1982)* who found that the sizes of Malpighian corpuscles and glomerular tufts increased significantly in the senile kidney. On the contrary, *Baylis et al. (1995)* stated that the lumina of a few tubules contained small amounts of a colloid like material and that there was a moderate accumulation of lymphocytes and plasma cells in the adventitia and surrounding connective tissue of the arteries. Within the aging process, both the afferent and efferent arterioles tended to atrophy in a parallel manner resulting in bloodless glomeruli.

Graune and Stratton (1971) stated that the kidneys of senile rats differed from those of the young and middle-aged animals as the tubules and arteries were affected more than the glomeruli. A high proportion of the tubules had large masses of colloid and aberrant epithelial cells which were usually much larger than the neighboring cells. They also found a marked infiltration of the adventitia of many arteries and of the adjacent connective tissue by masses of lymphocytes and plasma cells. Many arteries presented vacuoles among the muscle cells of the media. The glomeruli appeared in various stages of fibrosis that indicated a functional obliteration of the capillary bed of the glomerulus. But in the present work, the vacuoles in the blood vessels were detected only in aspartame-treated aged animals. They also demonstrated that in the senile human kidney, four main types of histological change were listed as glomerular changes ranging from congestion to fibrosis or hyaline transformation, tubular distension and atrophy, vascular changes and interstitial changes especially sclerosis of the connective tissue in the cortex.

In the present work, the treatment with aspartame for six months led to a marked degeneration of the kidney of old rats. This might be due to the association between aging and the destructive effects of aspartame.

The present observation of obliterated lumen of some tubules was explained by *Mayam et al. (2009)* who suggested that this obliteration might be the consequence of a swelling of the tubular lining cells accompanied by an alteration in the nuclei and an initiation of necrosis.

The increased thickness of Bowman`s capsule in the treated adult group and the increased thickness of the renal tubular basement membrane in the treated aged group observed in this study, could be explained according to *Nilsson et al. (1998)* and *Soffritti et al. (2005)* who suggested the effect of formaldehyde –one of aspartame metabolites- on fibroblast. Fibroblast tended to accumulate the highest intracellular level of formalin than any other cells.

The present research revealed that the treatment with aspartame in the dosage of 20mg/kg/day administered in the gestational period did not induce abortions, gross anatomical malformations or meaningful differences in the numbers of the newborn rats as compared to the control group. However, their body and kidney weights decreased. These results are in accordance with *Portela et al. (2007)* who suggested that aspartame had a damaging effect on the rat fetal liver, associated with reduction in the placental and ma-

ternal–fetal weights. On the other hand, *Yirmiya et al. (1989)* reported that the perinatal exposure to aspartame did not cause difference in the body weight of rat pups.

The present work did not show a significant difference between the numbers of rat offspring per pregnant female of the control and treated female rats. This is consistent with *Lennon et al. (1980)* who stated that the intragastric administration of approximately 300 mg/kg/day of APM to the female rats for seven days and to the female hamsters for five days after mating did not affect the postcoital fertility as measured by the number of implantation sites and the normally appearing fetuses.

Schroeder et al. (1973) demonstrated that aspartame had no effect on the survival rate, mating performance, fertility or body weight gain.

Schroeder and McConnell (1970), Bantle et al. (1990) and Kotsonis and Hjelle (1996) reported that the continuous dietary administration of aspartame to the primigravid rat employing dosages up to 4g/kg/day, exerted neither embryotoxic nor teratogenic effects in the developing foetus.

Holder (1989), McAnulty et al. (1989) and Yirmiya et al. (1989) found no effect on the maternal body weight, visual placing response, food consumption, length of gestation and reproduction indices. The perinatal exposure to aspartame did not affect the reflex development, morphological development or spatial memory in the rat pups.

Lennon et al. (1980) stated that the higher levels of 7.5 and 9 g/kg/day APM caused reduced food consumption due to the diet palatability and resulted in a body weight loss in dams and retarded growth rates in the young.

The degenerative affection of the kidney observed in the different age groups examined in this work suggested nephrotoxic effects of aspartame. The histopathological study of the newborn kidney provoked questions regarding the maturation and degree of renal damage with the use of aspartame during gestation, necessitating future investigations and caustion or even avoidance of consumption of aspartame containing products during pregnancy.

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تأثير التناول المزمن للأسبرتام على كلية الفأر الأبيض

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ملخص البحث

الأسبرتام مادة تحلية صناعية تستخدم على نطاق واسع فى الحمية الغذائية ولدى مرضى السكر. الهدف من هذه الدراسة هو إيضاح التأثير المزمن للأسبرتام على تركيب الكلية في الفأر الأبيض حديث الولادة، بالغا، ومتقدم العمر.

لقد استخدم فى هذه الدراسة ستون فأراً أبيضاً؛ أربعون منهم كانوا يبلغون ثلاثة أشهر من العمر، بينما العشرون فأراً الباقون فكانوا يبلغون إثنى عشر شهراً من العمر عند بداية هذه الدراسة. ولقد تم تقسيمهم إلى ثلاث مجموعات أ، ب، ج. و قسمت كل مجموعة إلى مجموعتين فرعيتين؛ ضابطة و معالجة بالأسبرتام.

اشتملت المجموعة أ على عشرين أنثى فأراً أبيضاً بلغت أعمارهن ثلاثة أشهر. تم إعطاء مجموعتها الفرعية المعالجة بالأسبرتام جرعة قدرها ٢٠ ملجرام / كجم / اليوم مذابة فى ماء الصنبور من خلال أنبوبة معدية لمدة ثلاثة أشهر قبل الحمل وأثنائه. ثم تم تقدير أعداد المواليد وأوزانهم و أوزان الكلى وتحليل ذلك إحصائيا.كما تم فحص كلى المواليد مجهرياً. أما المجموعة ب فقد شملت عشرين فأراً أبيضاً بالغاً بلغت أعمارهم ثلاثة شهور. وضمت المجموعة ج عشرين فأراً أبيضاً بلغت أعمارهم إثنى عشر شهراً. وتم إعطاء الأسبرتام للحيوانات المعالجة في المجموعتين ب، ج بنفس الجرعة والطريقة المستخدمة في المجموعة أ لمدة ستة أشهر. وقد تم إعداد عينات الكلى من كل المجموعات للفحص بالمجهرالضوئى باستخدام صبغه الهيماتوكسيلين و الإيوسين. وأستخدمت صبغة التولويدين الأزرق لصبغ القطاعات شبه الرقيقة لعينات كلى الفئران البالغة. كما تمت دراسة التركيب الدقيق النبيبات الملتفة القريبة لكلية الفأر البالغ باستخدام الميكروسكوب الإلكتروني.

وقد أظهرت نتائج هذه الدراسة تأخر نمو كلية الفئران حديثة الولادة الذين تناولت أمهاتهم الأسبرتام، بالإضافة إلى وجود تغيرات تنكسية فى الكبيبات والنبيبات الكلوية. وأوضح التحليل الإحصائى وجود تراجع ملحوظ فى أوزان أجسام وكلى الفئران حديثى الولادة مقارنة بالمجموعة الضابطَة.

أما كلية الفئران البالغة المعالجة بالأسبرتام فقد أظهرت وجود تغيرات تنكسية فى الكبيبات والنبيبات الكلوية. فبالنسبة للكبيبات الكلوية فقد لوحظ انكماش الخصلة الدموية الخاصة بها وانتظام و اتساع فضاء بومان . كما أظهر بعضها عدم انتظام حافظة بومان. أما النبيبات الكلوية فقد أظهرت اتساعاً فى تجاويفها، وبدت الأنوية داكنة اللون، مع وجود فجوات في السيتوبلازم وسقوط للخلايا المبطنة لها. ولوحظ أيضاً وجود أوعية دموية محتقنة ومتسعة.

وقد أوضحت دراسة التركيب الدقيق للخلايا المبطنة للنبيبات الملتفة القريبة تدميراً واسع المدى لعضيات الخلية و الحافة الفرشية.

لقد عانت كلية الفئران المتقدمة العمر المعالجة بالأسبرتام من تغيرات تنكسية كبيرة مقارنة بالمجموعات المعالجة الأخرى. حيث أظهرت كل النبيبات الكلوية رقة فى النسيج المبطن لها مع اتساع تجاويفها. كما أظهر بعضها تهدم و زيادة سمك الغشاء القاعدى وتجمعت ترسيبات فى تجاويف بعضها. و لوحظ وجود أوعية دموية محتقنة ومتسعة ذات فجوات في السيتوبلازم.

ولقد استنتج من هذه الدراسة أن الأسبرتام له تأثيرات سمية على كلية الفأر جنيناً وبالغاً ومتقدماً فى العمر.