Research Article

Histological study of the protective effect of Selenium against Nephrotoxicity induced by Aspartame in adult male albino rats

Mohammed A. Desouky, Medhat A. Salah, Abdel Hamid S. Abo Bakr, and Heba H. Sedki Tony.
Department of Anatomy and Embryology, Faculty of Medicine, Minia University

Abstract
The objective of the present study is to explore the protective effect of selenium against nephrotoxicity induced by aspartame in adult male albino rat. Forty-five adult male albino rats, weighting 180 were randomly divided into three groups as follows: first group was given distilled water and served as control group; the second group was given aspartame dissolved in distilled water in a dose of 500 mg /kg b. wt.; the third group was given both aspartame and selenium. Selenium was given at daily dose 0.2 mg/kg dissolved in distilled water and given intra peritoneal followed by aspartame with an hour interval for 6 weeks. Serum urea, creatinine were significantly increased in rats that had received aspartame also the body was significantly decreased in rats received aspartame. Combined Treatment with selenium significantly restored kidney functions, modulate weight parameters.
Keywords: selenium, nephrotoxicity, aspartame, Serum urea, creatinine

Introduction
Aspartame is the most widely used artificial sweeteners. It is L-aspartyl L-phenyl alanine methyl ester. It is a synthetic, white, odorless, and a crystalline powder sweetener. Its sweetener effect is 180–200 times higher than sucrose (Baky, 2016). It was discovered in 1965 by James M. Schlatter, a chemist in G.D. Searle &company. He synthesized aspartame in the course of producing an anti-ulcer drug candidate. He discovers its sweet taste when he licked his finger which had accidentally become contaminated with aspartame (Ager et al., 1998).

For more than 30 years, aspartame has widely used as food addictive because of it is very strong and sweet taste. However, there is big controversial about its safety (Aspartame Information Center, 2005& Directorate, 2002). Aspartame received marketing approval in 1973, but was withdrawn because of doubts related to its carcinogenic effect on rodent brain (Ager et al., 1998). Aspartame is used as artificial sweetener for dietetic purpose (DeKoning et al., 2011) due to its sweetening effect. It is used in hygiene products and drugs such as cough therapy (Soffritti et al., 2018). The acceptable daily intake of aspartame is 40 mg/kg of the body weight based on 1980 joint FAQ/WHO committee on food additives (Butchko et al., 2002).

Once ingested, 50 % of aspartame is broken down into phenylalanine, one of nine essential amino acids commonly found in food and a precursor to tyrosine. Excessive levels of phenylalanine in the brain can cause decrease in the level of serotonin in the brain leading to emotional disorders such as depression (Spiers et al., 1998).

An essential mineral trace element and foods are major natural source of it. The richest sources of selenium (Se) are sea food, liver, kidney, other meats, grains and seeds (Burk and Levander, 1999). It is essential for healthy immune function; so it is used in autoimmune thyroiditis (Hashimoto's thyroiditis) as well as in patient with high cholesterol (Duntas and Benvenga, 2015). Selenium appears to play an important role in maintain the viability of sperm cells (Shahidi, 1997). Recent researches reported the role of selenium in cancer prevention, immunity, heart, and renal disease (Hasanvand et al., 2017 & Mix et al., 2006).

The importance of selenium in humans is well established as it has antioxidant properties because of its biological function as a scavenger of reactive oxygen species (Tinggi, 2008 &

Ostadalova et al., 2007) and its deficiency has caused serious health effects in humans, such as congestive cardiomyopathy (Keshan disease) (Toufektsian et al., 2000). There has been an

increased interest in the study of Se and its compounds as proteins (selenoproteins) led to the discovery of at least 30 selenoproteins and enzymes (selenoenzymes) as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD) (Tapiero et al., 2003).

The antioxidant activity of selenium is mainly reported for its role in the formation and function of the selenium dependent glutathione peroxidases. Glutathione peroxidases detoxify hydro peroxidases and prevent oxidative damage to cell membrane (Chen and Toppel, 1995).

Material and methods

Experimental animals:

In this study, 45adult male albino rats were used. They were obtained from the Animal House of Minia University and maintained under normal conditions with free access to food and water in the normal daily light and darkness cycle.

Forty-five adult male albino rats, their weight range about 180gm were used. Animals were housed in standard clean plastic cages and were given regular diet and water under controlled conditions. The experiment was approved by the Ethical Committee for animal handling for research work in Minia University.

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

Group I (control): fifteen rats, each received a daily dose of distilled water comparable to the dose given to the other groups throughout the experiment.

Group II (APM): fifteen rats, each received aspartame, which was given at daily dose 500 mg/kg dissolved in distilled water and given orally to the animals by intra gastric tube for 6 weeks according to (Saleh, 2014). The ASP tablets are used, each one containing 20 mg.

Group III (APM-Se): fifteen rats, each was co-administered with aspartame and selenium. Selenium was given at daily dose 0.2 mg/kg dissolved in distilled water and given intra peritoneal (Hasanvand et al., 2017) followed by aspartame with an hour interval (Sadek et al., 2017) for 6 weeks.

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:

The kidney of control animals:

The light microscopic examination of the kidney demonstrated normal histological picture.It showed normal renal corpuscles with rounded glomeruli and regular intact Bowman's capsule. The Bowman's capsule was consisted of double layer of epithelial cells, the inner visceral layer, the outer parietal layer and the bowman's space in between. The juxtaglomerular apparatus lied in between the glomerulus and distal convoluted tubule which formed of juxtaglomerular cells and Macula densa cells of DCT (Fig.1, 2).

The proximal convoluted tubular lining cells were cuboidal to columnar and had a large rounded nucleus with brush border (+ve) PAS. The distal convoluted tubular lining cells were cuboidal with a central rounded nucleus without brush border (–ve) PAS (Fig. 3).

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. 4)

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 (Fig.5).

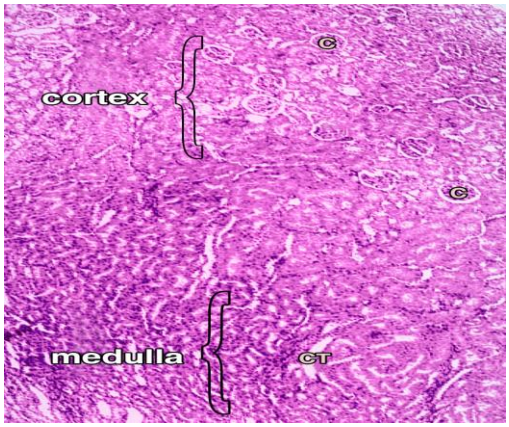


Fig (1): A photomicrograph of a section of control group shows normal kidney structure as outer layer is the cortex and the inner layer is medulla. The cortex is formed of renal corpuscles (C) and medulla contains collecting tubules (CT).

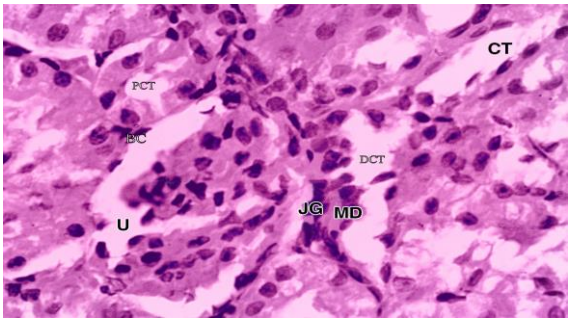


Fig (2): A photomicrograph of a section in the renal cortex of control group shows normal renal corpuscles with a glomerular tuft and urinary space (U) and intact regular Bowman's capsule (BC). Proximal convoluted tubules (PCT) are lined by cuboidal cells with nuclei stained blue and acidophilic cytoplasm. Distal convoluted tubules (DCT) are lined by cuboidal cells. Juxtaglomerular apparatus is formed of juxtaglomerular cells (JG) and macula densa (MD) of DCT. Collecting tubules are lined by light staining simple cuboidal epithelium with distinct boundaries.

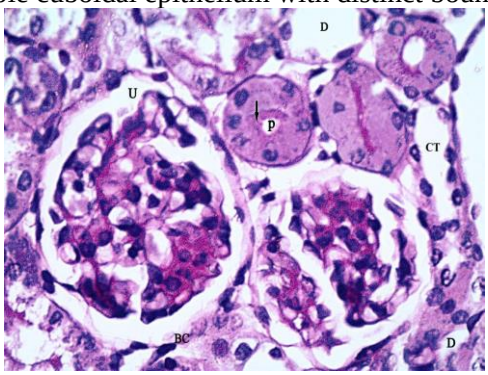


Fig (3): A photomicrograph of a section in the renal cortex of control group shows normal renal corpuscle with intact Bowman's capsule (BC) and urinary space (U). Proximal convoluted tubules (P) show intact cell membranes, narrow lumen and positive apical brush border (arrow). Distal convoluted tubules (D) show intact cell membrane and negative brush border and intact collecting tubule (CT).

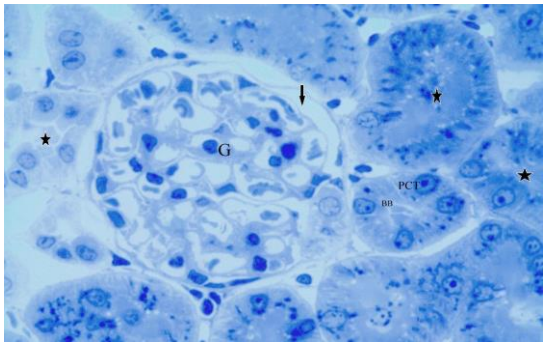


Fig (4): A photomicrograph of a semi thin section in the kidney of control group shows normal renal corpuscles with rounded glomerular tuft (G) and urinary space (arrow). Proximal convoluted tubules (PCT) and apical brush border (BB) and normal lining and lumen of other tubules (star).

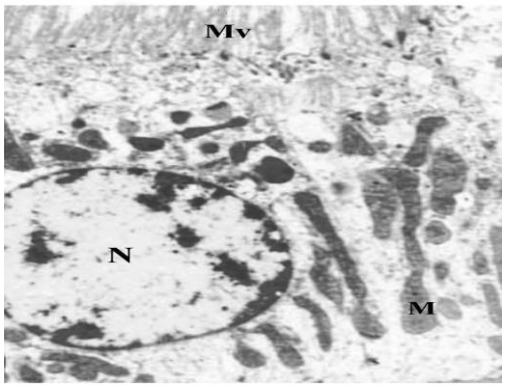


Fig (5) An electron micrograph of the kidney of control group shows the lining cell of the proximal renal tubule, a rounded nucleus (N), regular nuclear membrane, numerous mitochondria (M) arranged longitudinally between the basal infoldings, and apical numerous microvilli (Mv).

The kidney of treated animals:

APM group: In the APM treated group, the renal section showed many degenerative changes. The renal corpuscles showed shrinking and destruction of the glomerular capillaries, irregularity of Bowman's capsule and dilation of the bowman's space (Figs 6, 7). Some tubules were destructed, fused with each other with congestion blood vessel were observed in between. PCT showed loss of parts of brush border (–ve PAS) (Fig. 8).The examination of the semi-thin sections of the kidney showed many degenerative changes, as renal corpuscles showed destruction of the glomerular capillaries, widening of bowman's space and loss of brush border of tubules (Fig.9).

The ultrastructure of the proximal convoluted tubular lining cells revealed an extensive damage of the microvilli forming the brush border (Fig.10).

APM-Se group: there was a marked reduction of the previous changes had observed in the kidney sections of this group. The renal corpuscles appeared nearly similar to the control group, with intact glomeruli, no shrinking, and no congestion of the blood vessels. The tubules appeared similar or less to control group (Figs11, 12, 13.).

The ultrastructure of the proximal convoluted tubular lining cells revealed some cells lining the proximal tubules showed intact microvilli, some vacuoles in their cytoplasm and mitochondria with its cristae (Fig.14).

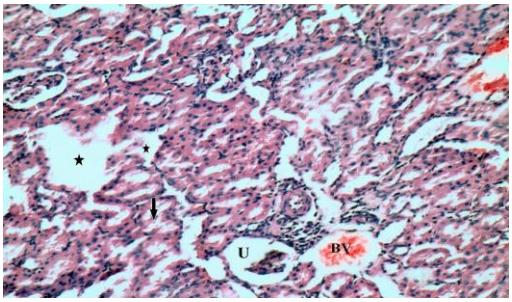


Fig (6): A photomicrograph of a section in the kidney of APM treated group shows destructed of renal corpuscle (star) and tubules, widening of urinary space (U), congested blood vessel (BV) in between.

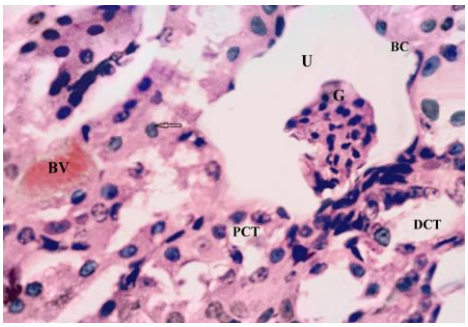


Fig (7): A photomicrograph of a section in the kidney of APM treated group shows irregularity in Bowman's capsule (BC), shrunken of glomerular tuft (G) and widening of urinary space (U). The proximal convoluted tubules (PCT) have dense nuclei, some tubules show dense exfoliated nucleus (arrow) and congested blood vessel (BV).

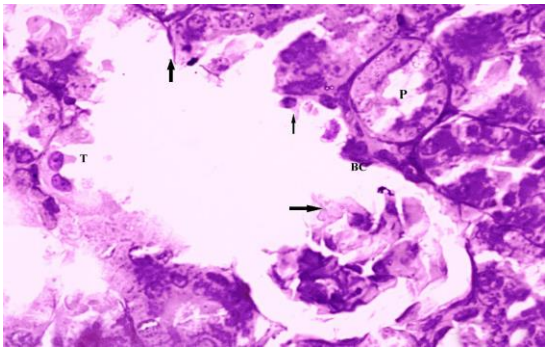


Fig (8): A photomicrograph of a section in the kidney of APM treated group shows destructed renal corpuscle (arrow) and loss of regularity of Bowman's capsule (BC) and destruction of tubules (T).The proximal convoluted tubules (P) show loss of brush border.

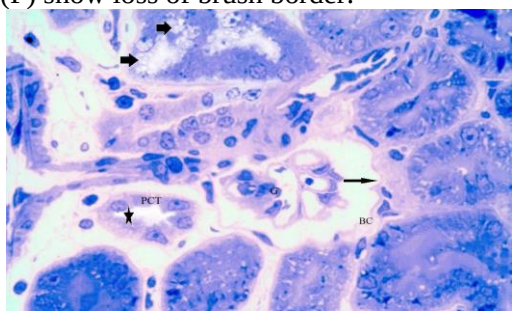


Fig (9): A photomicrograph of a semi thin section in the kidney of APM treated group shows renal corpuscles with shrunken of glomerular tuft (G) and widening of urinary space (thin arrow) and irregularity of bowman's capsule (BC). Proximal convoluted tubules (PCT) show loss of brush border (star), vaculation of the cytoplasm, indistinct nuclear boundaries and irregular outline (thick arrow).

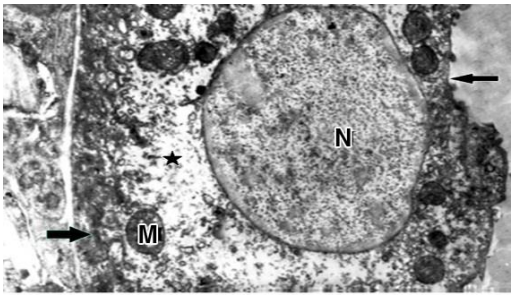


Fig (10): an electron micrograph of APM treated group shows lining cell of the proximal convoluted tubule, its cytoplasm shows scanty cell organelles(stars), decreased number of mitochondria that appeared ballooned with destructed cristae (M), loss of basal infoldings (thick arrow). The nucleus (N) is heterochromatic. The cell also shows loss of apical microvilli (thin arrow).

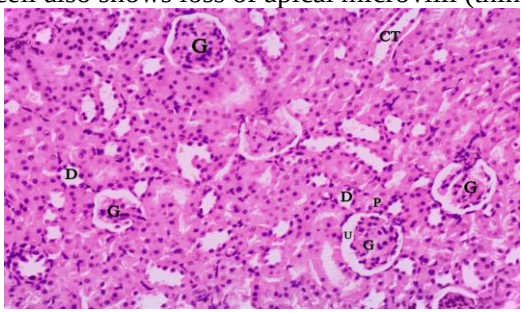


Fig (11): A photomicrograph of a section in the kidney of APM-Se group has normal capillary tuft (G) Bowman's capsule, and urinary space (U). The proximal convoluted tubules (P), distal convoluted tubules (D) and collecting tubules (CT) have normal epithelial lining cells and normal tubular lumen.

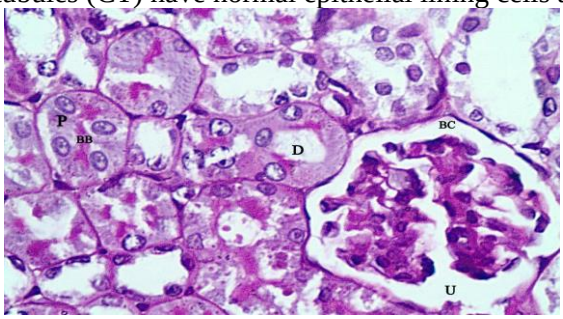


Fig (12): A photomicrograph of a section in the kidney of APM-Se shows similar structure to renal corpuscle of control group with intact Bowman's capsule (BC) and urinary space (U), Proximal Convoluted tubules (P) have intact cell membranes, narrow lumen and positive apical brush border (BB). Distal convoluted tubules (D) have intact cell membrane.

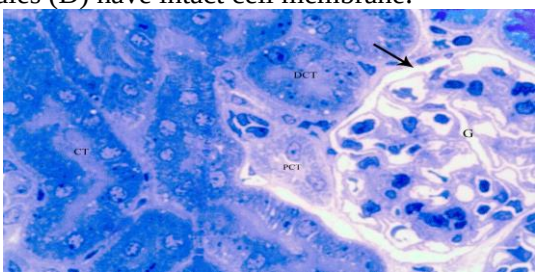


Fig (13): A photomicrograph of a semi thin section in the kidney of APM-Se group shows intact renal corpuscles with glomerular tuft (G) and urinary space (arrow), Normal intact proximal convoluted tubules (PCT), distal convoluted tubules (DCT) and collecting tubules (CT) similar to control group.

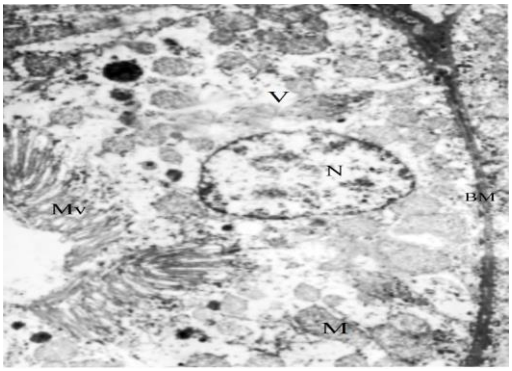


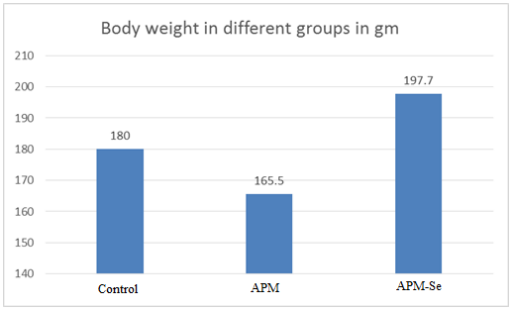
Fig (14): An electron micrograph of APM-Se group shows the lining cell of the proximal renal tubule near or similar to control group; rounded nucleus (N), some vacuoles(V) in cytoplasm, intact mitochondria (M), and the brush border are more or less similar to those of the control group (Mv). Note the thick basement membrane (BM) and the intact intercellular junction**.**

Statistical Results

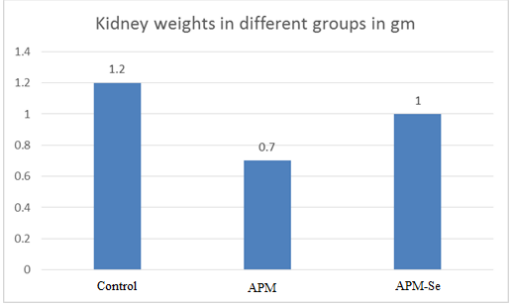
1) Rat body and kidney weights study

Before sacrifice of the rats, body weights of rats were measured. There was a significant difference in total body weights of the APM treated group and APM-Se treated group as compared with control group. The mean ± standard deviation (M±SD) of their body weights were (180±0.1.8) in control group, (165.5± 3) in APM treated group and (195.7±4.3) in APM-Se treated group which showed an apparent change in body weight in the treated group as compared with the control group, as there was a markedly decrease in body weight of APM treated group. This decrease was found to be very highly significant. On the otherwise there was little increase in body weight in APM -Se treated group as shown in histogram1.

After sacrifice, kidneys of each rat were weighted by **Sartorius balance**. The mean ± standard deviation (M±SD) of their kidney weights were (1.2±0.2) in control group, (0.7± 0.1) in APM treated group and (1±0.2) in APM-Se treated group as shown in histogram 2.



Histogram (1): Showing body weights in different studied groups.

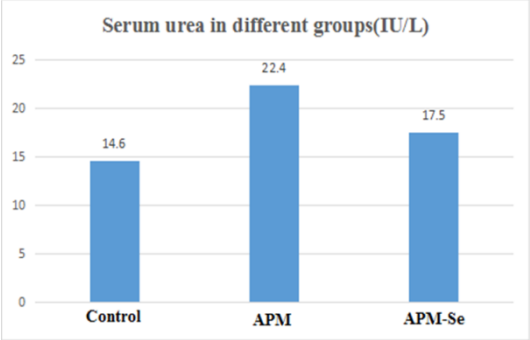


Histogram (2): Showing kidney weights in different studied groups.

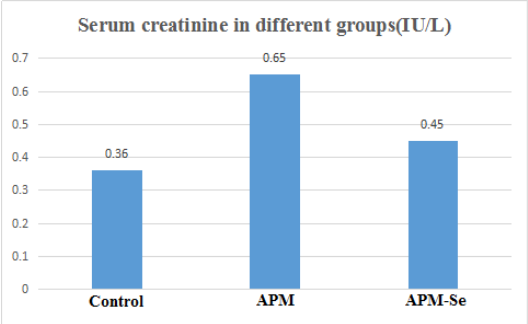
Biochemical study:

The serum level of renal function markers as urea and creatinine (Cr) were measured in blood samples of each group as indicators of nephrotoxicity. The results showed that the administration of aspartame significantly increased serum urea and creatinine compared to the control group. Concomitant treatment of

aspartame and selenium showed significantly decrease in the serum urea and creatinine as shown in histograms 3, 4.



Histogram (3): Showing levels of serum urea in different studied groups.



Histogram (4): Showing levels of serum creatinine in different studied groups

Discussion

In the control group, histological examination of this group using magnifying lens revealed normal architecture of the kidney, as the kidney was formed of outer cortex and inner medulla. The cortex contained renal corpuscles, medulla had collecting tubule, and similar observations of this result were reported by Eroschenko and Di Fiore, 2013.

The light microscopic examination showed that the renal cortex was formed of renal corpuscles, renal tubules and minimal interstitial tissue in between. The renal corpuscles were composed of glomeruli surrounded by Bowman's spaces. The proximal convoluted tubules appeared to be lined by acidophilic cuboidal epithelium with an apical brush border and enclosing a narrow lumen. The distal convoluted tubules were lined with acidophilic cuboidal epithelium surrounding a wider lumen as in Eroschenko, 2005.

Ultrastructurally, the renal corpuscles were formed of glomerular fenestrated capillaries surrounded by podocytes with its processes that share in the formation of the filtration barrier. The lining cells of the proximal tubules rested on a thin basement membrane. The apical membrane had apical numerous microvilli. The intercellular junction between adjacent cells was observed at the lateral membrane. Their cytoplasm contained euochromatic rounded nuclei, and numerous longitudinally arranged mitochondria between the basal infoldings. The distal renal tubule lining cells rested on a thin basement membrane. Their cytoplasms had euochromatic rounded nuclei, numerous mitochondria, and basal infoldings. These results are similar to those reported by Young and Health, 2003.

The renal sections of APM treated group revealed degenerative changes by light microscopic examination in the form of shrinking of the glomeruli and widening of the urinary space and loss of the brush border of proximal convoluted tubular epithelium, with widening of their lumens and vaculation of their cytoplasm. Some cells of the renal tubules had exfoliated nuclei. These changes appeared in the proximal tubular epithelial cells specifically more than distal tubules and this was explained by some authors by the fact that PCTs are the first to come into contact with the toxic agent after its filtration by the glomeruli as in Mourad, 2011.

Ultrastructurally examination of renal sections confirms destructive changes demonstrated by L.M. as most of the proximal convoluted epithelial cells showed heterochromatic nuclei

with vacuolation of cytoplasm and contained ballooned mitochondria with partially destroyed cristae. The apical microvilli were partially destroyed, with loss of the basal infoldings, similar results that reported by Saleh, 2014 and Mohamed, 2011.

The mitochondrial changes observed might be considered as early manifestations of apoptosis and an adaptive process to unfavorable environments such as excess exposure of the cell to free radicals as in Alleva et al., 2011. Some authors agreed with this explanation as they proved that methanol significantly increased the Malondialdehyde (MDA) level and caspase-3 activity. This elevated level caused an increase in the level of lipid peroxidation and activation of the intrinsic pathway of apoptosis as in Kurcer et al., 2010.

These destructive changes were similar to El Haliem et al., 2011 who reported that aspartame had destructive toxic effect on the kidney and liver of adult albino rats.

In the APM-Se treated group, the light microscopic examination of renal sections showed a marked reduction of the previous changes had observed in the kidney sections of APM group. The renal corpuscles appeared similar to the control group, with intact glomeruli, no shrinking, and no congestion of blood vessel. The tubules appeared similar or less to the control group as in Saleh, 2014.

Ultra structurally examination of renal sections revealed that the filtration barrier thickness was more or less similar to that of the control group. However, some cells lining the proximal tubules showed vacuoles in their cytoplasm. The distal convoluted tubules appeared more or less similar to those of the control group as most of the renal corpuscles and renal tubules were more or less similar to those of the control group. These results were similar to Sedighi et al., 2014.

The results of the current work agree with Traber and Stevens, 2011 who reported that Se has a role in activating antioxidant enzyme system e.g. vitamin E, vitamin C and glutathione peroxidase that remove produced reactive oxygen species, so plasma membrane lipid peroxidation is prevented. GPx (Selenium-dependent glutathione peroxidase) is one of the main anti-oxidative enzymes in the cells and it has been shown that Se is a structural component of GPx. Glutathione peroxidases detoxify hydro peroxidases and prevent oxidative damage to cell membrane and it prevents apoptosis of the cell as in Penglase et al., 2014& Hagiwara et al., 2011& Orun et al., 2008& Kaur et al., 2003.

In APM treated group, there was a significant decrease in the rat body and kidney weight, this agree with many previous studies that encourage the use of aspartame in regime as in De la Hunty et al., 2006 who reported that using foods and drinks sweetened with aspartame instead of sucrose resulted in a significant reduction in both energy intakes and body weight about 0.2kg/week.

Also Anton et al., 2010 revealed that diet containing aspartame leads to increase satiety and decrease food intake, decrease postprandial glucose compared to when sucrose used.

The reduction of kidney weight agree with Mourad, 2011 who reported that six week of aspartame administration (40 mg/kg body weight) leads to a significant increase in lipid peroxidation level. Lipid peroxidation is an auto catalytic mechanism leading to oxidative destruction of cellular membranes as it damages polyunsaturated fatty acids tending to reduce membrane fluidity which is essential for proper functioning of the cell. An increase in free radicals causes overproduction of malondialdehyde (MDA) level observed, which is an index of lipid peroxidation, indicated kidney cell membrane damage after APM administration. This is in accordance with Parthasarathy et al., 2006 who investigated that methanol administration significantly increased MDA level in the lymphoid organs; also, Zararsiz et al., 2007 recorded a significant increase in MDA level in the kidney of rats after treatment with formaldehyde.

The results of body weight reduction of APM treated group are in contrast to De Matos Feijó et al., 2013 as greater weight gain was promoted by the use of aspartame or saccharin, compared with sucrose, and this weight gain was unrelated to caloric intake. This might occur because of a decrease in energy expenditure or increase in fluid retention might be involved.

In APM-Se treated group, there was slightly increase in the body and kidney weight in

comparison to APM treated group. This might be due to the effect of Se on the thyroid hormones as serum T3 decrease in administration of Se in high doses and decrease in low Se doses as Se is one of the structure of IDD that is one of seleno-enzymes and key tissue specific regulators of intra cellular thyroid hormone availability and signaling and this agree with the study of Hawkes and keim, 2003. However these finding are in contrast with Schulze et al., 2004 as it revealed that stable consumption of Se had no difference in weight gain.

The kidney function can be detected by measuring of serum creatinine as it is the most widely used as a marker in estimating glomerular filtration rate as in Nitescu et al., 2006.

In APM treated group, there was a significant increase in serum urea and creatinine concentration. These results also reported by Odabasi et al., 2009 & Saleh, 2014. This elevation of urea and creatinine reflect the severity of renal insufficiency with fall in glomerular filtration rate because of the majority of methanol and formic acid that affect the tubular epithelial cells by the formation of superoxide anion, hydrogen peroxide and increased levels of free radicals production. Oxidative stress induced by aspartame leads to these degenerative changes due to depletion of antioxidants inducing abnormalities in the function and metabolism of multiple intracellular organelles as in Zararsiz et al., 2007 & Parthasarathy et al., 2006 and Roldan et al., 2003.

Also, the present results are in contrast with Boj et al., 2003 who reported that systemic doses of formaldehyde in rats had resulted in significant alterations in urea and creatinine levels 24-48 hours following the application but producing no inflammation or tissue lesion in kidney tissues due to short application time.

In APM-Se treated group, there was a marked reduction in the level of serum urea and creatinine in comparison to APM treated group. These results are in agreement with Hasanvand et al., 2016 who reported that administration of Se after ischemic reperfusion of the kidney leads to decreased renal injury and lipid peroxidation that is appear in keeping normal levels of serum urea and creatinine.

Orun et al., 2008, also reported that Se had renoprotective effects in lead intoxication and gentamicin induced acute renal failure. It had been observed that Se inhibits injury induced by free radicals that destroy fatty acid of the subcellular membrane.

These results of the current work agree with El Haliem and Mohammad, 2011 who reported that co-administration of Pimpinella anisum oil was effective in decreasing the toxic effect of aspartame on both the kidney and liver of adult albino rats. This improvement might be secondary to the antioxidant ability of anise, which attacks reactive oxygen species (ROS) as Se act.

So administration of Se reduces oxidative damage that induced by aspartame in both histopathological and biochemically studies in our rat model.

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