Research Article

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

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Abstract
The aim of the current work is to study the protecting effect of selenium against nephrotoxicity caused by aspartame in adult male albino rat. 45 adult male albino rats, with weight 180 g were arbitrarily separated into 3- groups like so: group-1 (control group) was given distilled water; group-2 was given aspartame /distilled water solvent with a concentration of 500 mg /kg b. wt.; group-3 was given aspartame in addition to selenium. Selenium was given with a day dosage of 0.2 mg/kg (distilled-water solvent) and given intra peritoneal after that aspartame was taken with a 1-hour period for six weeks. Serum urea and creatinine were significantly raised in receiving aspartame rats as well the weight was significantly reduced in receiving aspartame rats. Mixed therapy with selenium significantly restores the functions of kidney, moderate the weight issues.
Keywords: selenium, nephrotoxicity, aspartame, Serum urea, creatinine

Introduction
Aspartame is one of the most commonly taken 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 more than sucrose (Baky, 2016). In 1965, James M. Schlatter, (chemist in G.D. Searle corporation) discovered it. He manufactured aspartame in the sequence of manufacturing an anti-ulcer medication applicant. He detects its sweet-taste when he licked his fingers that have been contaminated with aspartame by accident (Ager et al., 1998).

For at least 30 yrs., aspartame has broadly used as addictive of food due to 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 Aspartame received marketing approval in 1973, but was withdrawn because of doubts related to its carcinogenic effect on rodent brain (Ager et al., 1998).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 worked 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 suitable dose for a day of aspartame is 40 mg/kg of the body weight founded on 1980 joint FAQ/WHO committee on additives of food (Butchko et al., 2002).

Once ingested, 50 % of aspartame is converted to phenylalanine, which was considered 1of 9 fundamental amino acids usually present in food and a portent to tyrosine. Unnecessary magnitude of phenylalanine in the brain may lead to reduce in the level of serotonin in the brain causing a disorder in emotion like the depression (Spiers et al., 1998).

A critical inorganic trace element and foods are chief 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 case with high cholesterol (Duntas and Benvenga, 2015). Selenium seems to have a significant role to preserve the viability of sperm cells (Shahidi, 1997). Recent researches reported that selenium have a significant function in cancer prevention, immunity, heart, and renal disease (Hasanvand et al., 2017 & Mix et al., 2006).

The significance of selenium is well-known as it was considered an antioxidant agent due to its biological role as a forager of reactive oxygen species (Tinggi, 2008 & Ostadalova et al., 2007) and its insufficiency may lead to critical health complications for individuals, such as congestive cardiomyopathy (Keshan disease) (Toufektsian et al., 2000). There was an increased concentration in the study of selenium and its composites as proteins (seleno-proteins) leding to the detection of no less than 30 types of selenoproteins and selenoenzymes like thioredoxin reductase (TrxR), glutathione peroxidase (GPx) and iodothyronine deiodinases (IDD) (Tapiero et al., 2003).

The anti-oxidant action of selenium is primarily described for its function in the development and role of the selenium reliant on 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 employed. They were attained from the Animal Building of Minia University and preserved on standard environment with free to have food and water in an ordinary day light and darkness sequence.

A number of 45 adult male albino rats, their weight range about 180gm were used. Animals were housed in standard clean pens made of plastic and have a regular food & water under controlled conditions. The trial was accepted by the Ethical Committee for animal treatment for research-work in Minia University.

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

Group-I (control): fifteen rats, each of them taken a definite dosage of distilled water every day equivalent to the dosage that was provided to the other studied groups in the experimentation.

Group-II (APM): 15-rats, each of them has aspartame intake, with a daily dosage of 500 mg/kg diluted in distilled-water and provided orally to the rats using intra-gastric tube for six weeks (Saleh, 2014). The ASP tablets are used, each one containing 20 mg.

Group III (APM-Se): fifteen rats, each was coadministered with aspartame and selenium. Selenium was provided with a dosage of 0.2 mg/kg every day diluted in distilled-water and provided intra-peritoneal (Hasanvand et al., 2017) afterward aspartame with a one-hour break (Sadek et al., 2017) within six weeks.

Kidney specimens representing all groups were processed for light microscopic examination using Haematoxylin and Eosin stain *(*Drury & Wallington, 1980*)*. Similarly, semithin sectors (1micron) were arranged from half of group B kidney specimens and were marked with toluidine blue to be studied by microscope.

Ultrathin sectors (0.1micron) were made for transmission electronic microscopic examination using uranyl-acetate and lead citrate (Bozzola & Russel, 1992).

Statistical analysis

This was made for the numbers of rats' progeny of each gravid female, their body and kidney weights in both the control and studied sub-groups of group-A. The variables were presented in the form of M±SE (Mean± Standard error). ***Student t-test*** was employed for matching the means of the variables among the control and treated sub-groups of group-A.

Results

Histological Results:

The kidney of control animals:

The light microscopic examination of the kidney demonstrated standard histological picture. It showed normal renal corpuscles with rounded glomeruli and regular intact Bowman's tablet. The Bowman's tablet was consisted of dual layer of epithelial cells, the internal visceral layer, the external parietal layer and bowman's space in between. The juxta-glomerular apparatus (JGA) lied in between the glomerulus and distal convoluted tubule which formed of JG 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 brushed border (+ve)

PAS. The distal convoluted tubular coating 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 coating cells of the proximal involuted tubules had Normal nuclei& cytoplasm with standard brush border. (Fig. 4)

The ultrastructure of proximal convoluted tubular lining cells exposed the typical appearance of the microvilli forming the brush border. The cell had normal euchromatic nucleus limited by a regular nuclear membrane with one nucleolus. The cytoplasm was rich in healthy lightly marked oval or rounded mitochondria by a definite crista (Fig.5).

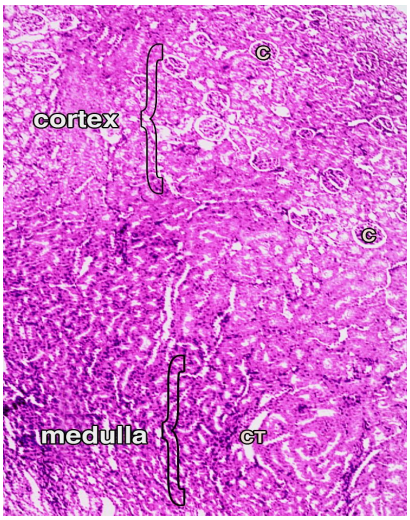


Fig (1): A micrographic photo of a sector of controls displays typical kidney assembly as external layer is the cortex and the internal layer is medulla. The cortex is molded of renal corpuscles (C) and medulla comprises collecting tubules (CT). H&E, X 250.

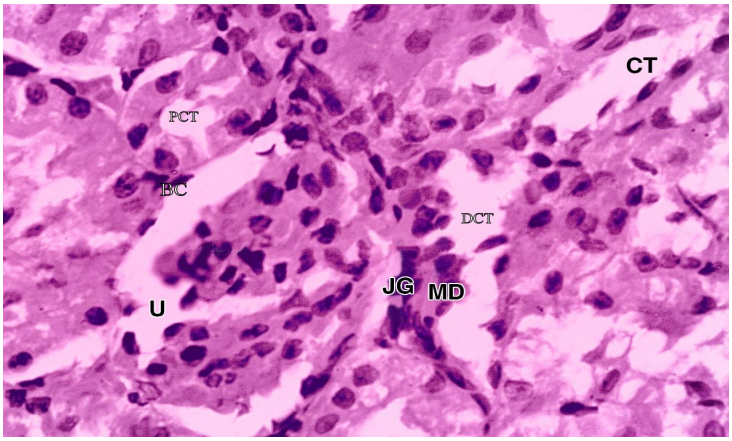


Fig (2): A micrographic photo of a section in the renal cortex of controls displays typical renal corpuscles with a glomerular tuft and urinary space (U) and intact regular Bowman's capsule (BC). Proximal convoluted tubules (PCT) are lined via cuboidal cells by nuclei stained blue and acidophilic cytoplasm. Distal convoluted tubules (DCT) are lined via cuboidal cells. JGA is shaped of JG cells and macula densa (MD) of DCT. Gathering tubules are lined by light discoloration humble cuboidal epithelium with separate borders. H&E, X 400.

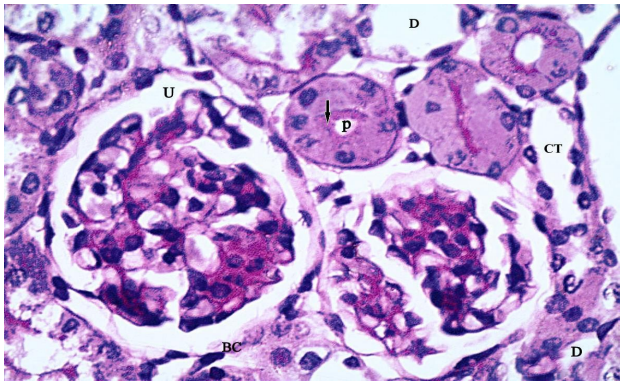


Fig (3): A micrographic photo of a section in the renal cortex of controls giving typical renal corpuscle with intact BC and urinary space (U). Proximal convoluted tubules (P) show complete cell membranes, narrow lumen and positive apical brush border (arrow). Distal convoluted tubules (D) show intact cell membrane and negative brush border and intact collecting tubule (CT). PAS, X 400.

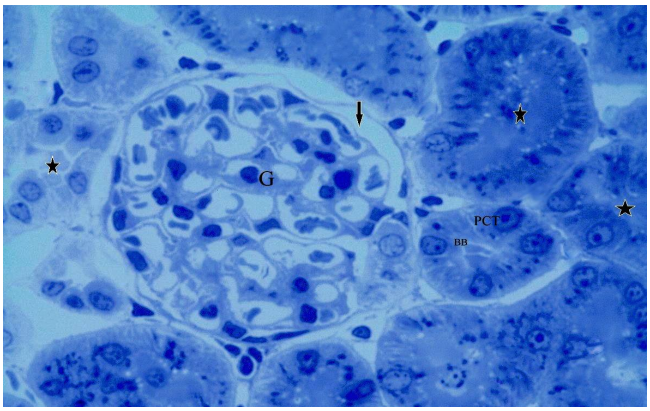


Fig (4): A micrographic photo of a semi thin section in the kidney of controls displays typical renal corpuscles with curved glomerular tuft (G) and urinary space (arrow). PCT and apical brush border (BB) and ordinary lining and lumen of other tubules (star), Toluidine blue, X 400.

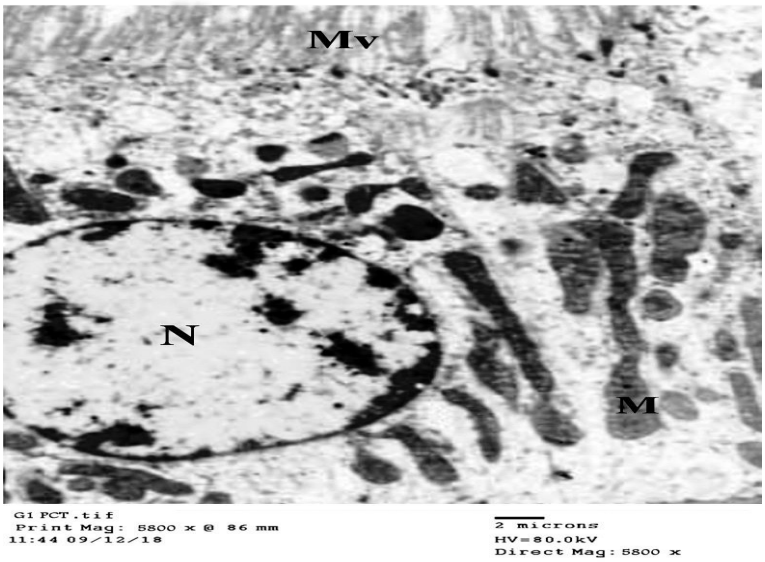


Fig (5) An electronic micrographic image of the kidney of controls displays the lining cell of the proximal renal tubule (PRT), a curved nucleus (N), standard nuclear membrane, numerous mitochondria (M) organized longitudinally among the basal infoldings, and apical numerous microvilli (Mv). X 5800.

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-capsule (BC) and dilation of bowman-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 tubes, broadening of bowman-space and damage of brush border of tubules (Fig.9).

The ultrastructure of the proximal convoluted tubular lining cells exposed an extensive harm 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 controls (Figs11, 12, 13.).

The ultrastructure of the proximal convoluted tubular lining cells shows that a few cells lining the proximal tubules displayed complete microvilli, a few vacuoles in their cytoplasm and mitochondria with its cristae (Fig.14).

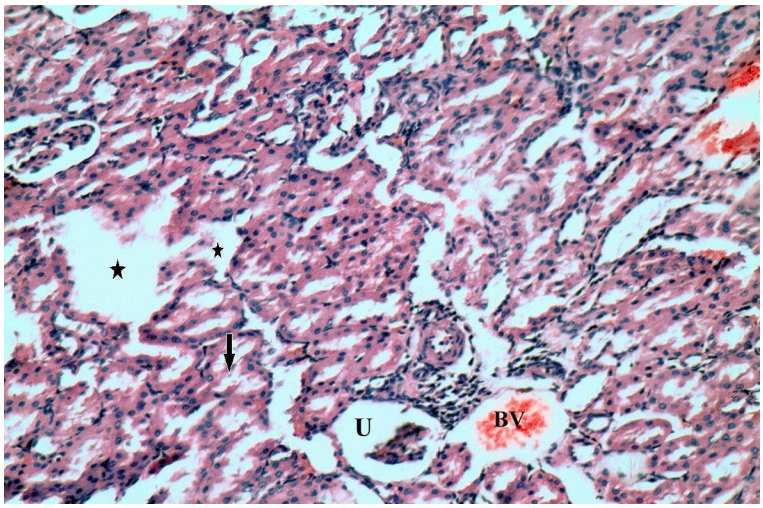


Fig (6): A micrographic photo of the kidney sector of APM -group shows destructed of renal corpuscle (star) and tubules, widening of urinary space (U), congested blood vessel (BV) in between H&E, X 250.

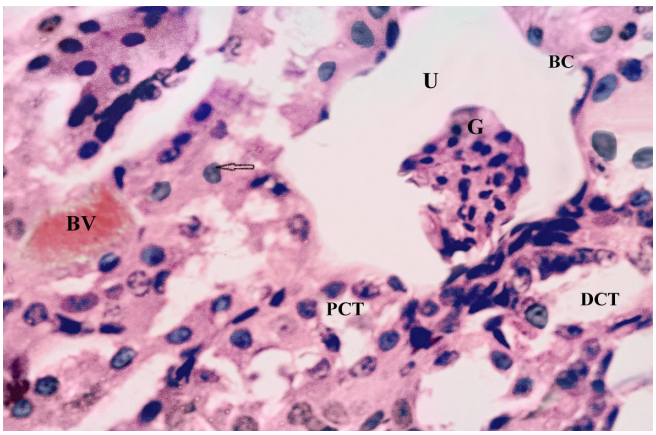


Fig (7): A micrographic photo of the kidney sector of APM -group displays anomaly in Bowman's capsule (BC), shrunken of glomerular tuft (G) & widening of urinary space (U). The PCT have dense nuclei, some tubules show dense exfoliated nucleus (arrow) and congested blood vessel (BV) H&E, X 400.

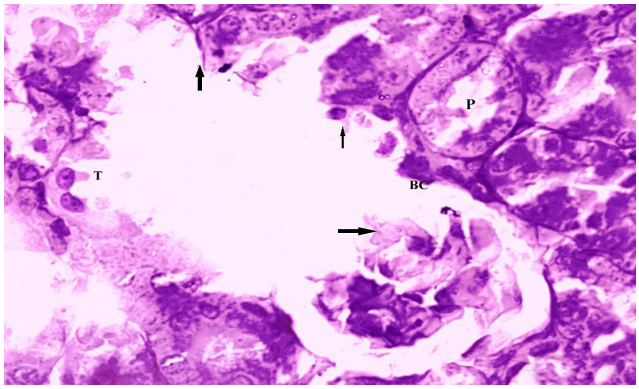


Fig (8): A micrographic photo of the kidney sector of APM -group displays destructed renal corpuscle (arrow) and loss of regularity of the BC & destruction of tubules (T). The PCT display damage of brush border. PAS, X 400.

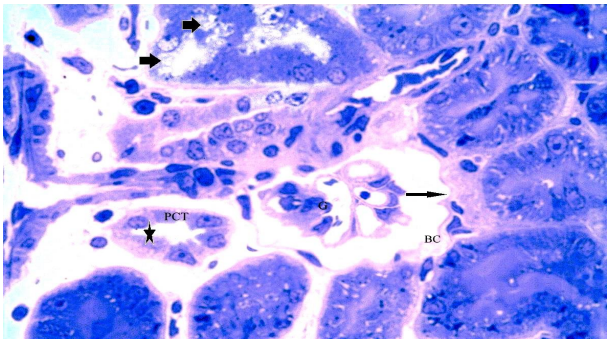


Fig (9): A micrographic photo of the kidney sector of APM -group displays renal corpuscles with shrunken of glomerular tuft (G) and widening of urinary space (thin arrow) and irregularity of BC. PCT display damage of brush border (star), vacuolation of the cytoplasm, indistinct nuclear boundaries and irregular outline (thick arrow). Toluidine blue, X 400.

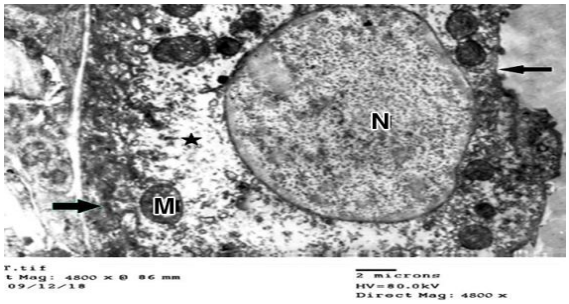


Fig (10): an electron micrograph of APM treated group shows lining cell of the proximal convoluted tubule, its cytoplasm shows scanty cell organelles(stars), decreased number of mitochondria that appeared ballooned with destructed cristae (M), loss of basal infoldings (thick arrow). The nucleus (N) is heterochromatic. The cell also shows loss of apical microvilli (thin arrow). X 4800.

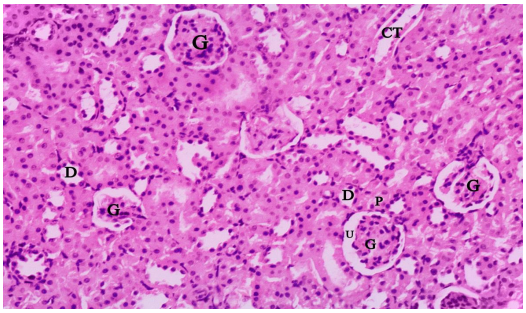


Fig (11): A micrographic photo of the kidney sector of APM-Se-group has normal vessel tuft (G) BC, & urinary space (U). The PCT (P), DCT (D) and collecting tubules (CT) have ordinary epithelial lining cells and ordinary tubular lumen. H&E, X 250.

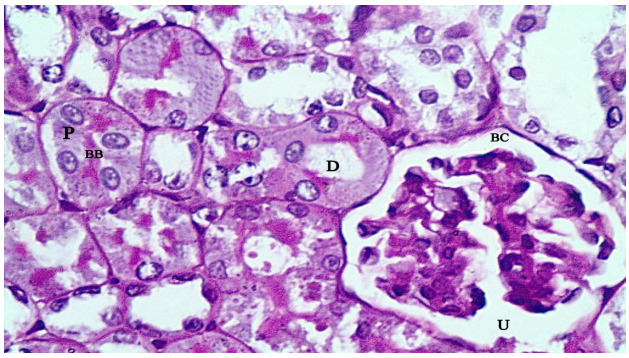


Fig (12): A micrographic photo of the kidney sector of APM-Se-group shows similar structure to renal corpuscle of control group with intact BC and urinary space (U), PCT (P) have intact cell membranes, thin lumen and positive apical brush border (BB). DCT (D) have complete cell membrane. PAS, X 400.

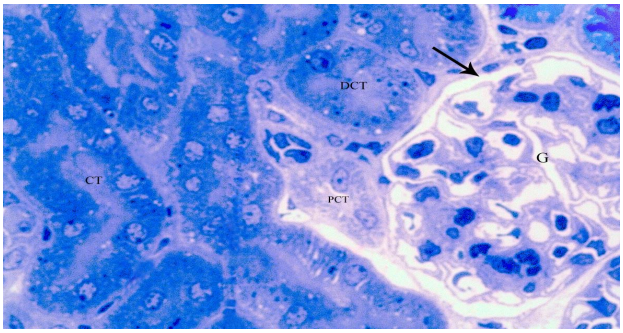


Fig (13): A micrographic photo of the kidney sector of APM-Se-group displays intact renal corpuscles with glomerular tuft (G) and urinary space (arrow), Normal intact PCT, DCT and CT like to control group. Toluidine blue , X 400.

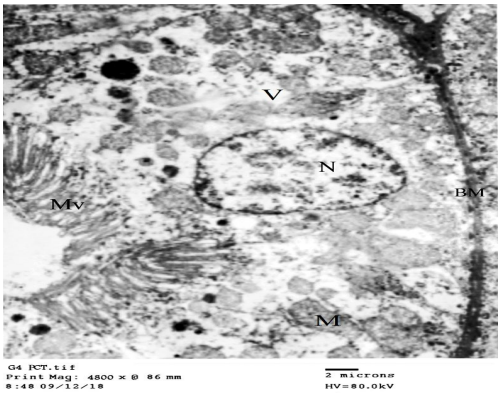


Fig (14): An electron micrograph of APM-Se group shows the lining cell of the PRT near or similar to controls; rounded nucleus (N), some vacuoles (V) in cytoplasm, intact mitochondria (M), and the BB are relatively comparable with those of the controls (Mv). Note the dense basement membrane (BM) and the intact intercellular junction**.** X 4800.

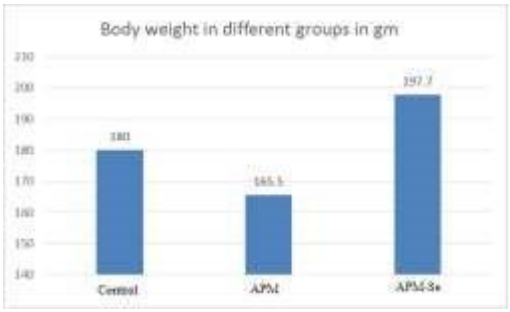
Statistical Results

1) Rat body and kidney weights study

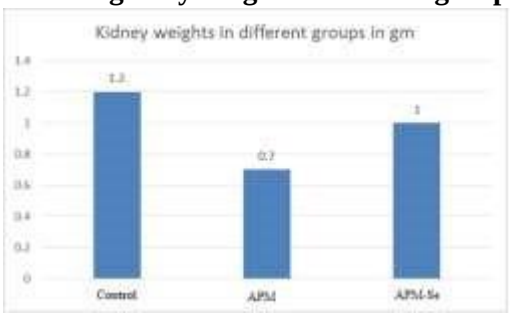
Before the start of rats sacrificing, body weights of rats were measured. There was a significant change in the whole-body weights of the APM treated group and APM-Se-group in comparison with controls. The mean ± standard deviation (M±SD) of their body weights were (180±0.1.8) in controls, (165.5± 3) in APM treated group and (195.7±4.3) in APM-Se-group which showed an apparent change in body mass in the treated group in comparison with the controls, and a markedly reduction in body weight of APM treated group. This decrease was found to be very highly significant. On the otherwise there was slight growth in body mass for APM -Se treated group as shown in histogram1.

Afterward the rates sacrificing, kidneys mass of every one was determined by **Sartorius balance**. The mean ± standard deviation (M± SD) of their kidney weights were (1.2±0.2) in

the controls, (0.7± 0.1) in APM-treated-group and (1±0.2) in APM-Se-treated-group as indicated in histogram 2.



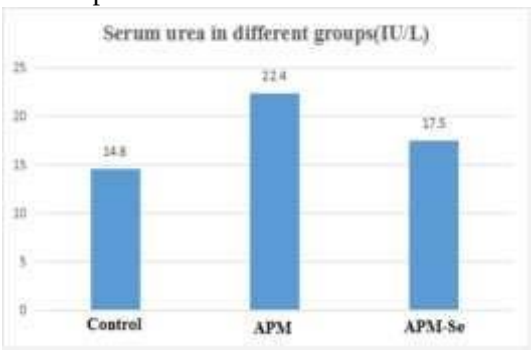
Histogram (1): Showing body weights in various groups of the study.



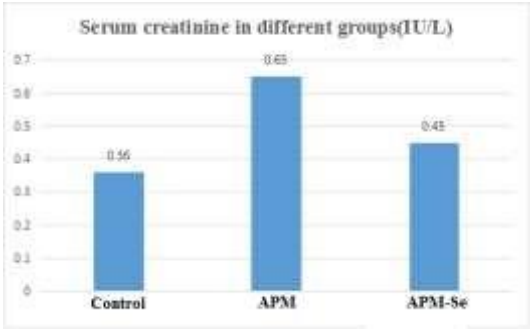
Histogram (2): Showing kidney weights in various groups of the study.

Biochemical study:

The level of the renal function serum indicators as creatinine & urea were measured in blood compared samples of each group as indicators of nephrotoxicity. The results showed that the administration of aspartame a significant raise serum creatinine & urea in comparison with the controls. Concomitant treatment of aspartame and selenium showed a significant reduction in the serum urea and creatinine as shown in histograms 3, 4.



Histogram (3): Showing levels of serum urea in various groups of the study.



Histogram (4): Showing levels of serum creatinine in various groups of the study

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 external cortex and internal medulla. The cortex confined renal corpuscles, medulla had collecting tubule, and analogous explanations of this consequences were described by Eroschenko & Di Fiore in 2013.

The light microscopic investigation showed that the renal-cortex was consisted of renal corpuscles, renal tubules and negligible interstitial tissue among them. The renal corpuscle was consisted of glomeruli enclosed by Bowman's spaces. The proximal convoluted tubules (PCT) seemed to be coated by acidophilic cuboidal epithelium with an apical brush border (BB) and enclosing a narrow lumen. The distal convoluted tubules were coated by acidophilic cuboidal epithelium surrounding a broader lumen as in Eroschenko, 2005.

Ultra-structurally, the renal corpuscle was consisted of glomerular fenestrated capillaries enclosed by podocytes by its procedures that part in the foundation of the barrier of filtration. The coating cells of the PCT was resting on a thin cellar membrane. The apical membrane has many apical microvilli. The intercellular cone-ction among neighboring cells was detected at the adjacent film. The cytoplasm confined euochromatic curved nuclei, and many longitudinally organized mitochondria among the basic infoldings. The distal renal tubule coating cells refreshed on a tinny basement film. Their cytoplasms had euochromatic round nuclei, many mitochondria, and basic infoldings. These results are similar to those reported by Young and Health, 2003.

The renal sections of the group treated by APM revealed degenerative changes by light microscopic examination in the form of shrinking of the glomeruli and broadening of the urinary space and loss of the BB of PCT epithelium, with broadening of their lumens and vacuolation of their cytoplasm. A number of cells of the renal tubules have exfoliated cores. These variations in the PCT epithelial cells precisely higher than distal tubules and this was clarified by several researchers in the basis that PCTs are the initially contacting the toxic agent afterward it was filtrated by glomeruli (Mourad, 2011).

Ultra-structurally examination of renal sections confirms destructive changes demonstrated by L.M. as the majority of the proximal intricate epithelial cells displayed heterochromatic core by vacuolation of cytoplasm and confined ballooned mitochondria with partially smashed cristae. The apical microvilli were incompletely smashed, with damage of the basic infoldings, like the consequences that described by (Saleh, 2014) & (Mohamed, 2011).

The mitochondrial variations detected may be measured as primary appearances of apoptosis and adaptive procedure to negative atmospheres like extra contact of the cell to free-radicals (Alleva et al., 2011). Several researchers approved this clarification as they demonstrated that methanol significantly raised the level of Malondialdehyde (MDA) and the activity of caspase-3. This raised level produced from a rise in the lipid peroxidation level and beginning of the fundamental apoptosis pathway as in Kurcer et al., 2010.

These destructive changes were similar to El Haliem et al., 2011 who reported that aspartame had damaging toxic effect on the adult albino rat's liver &kidney.

In an 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 seemed equivalent to the controls, by intact glomeruli, no shrinking, and no congestion of vessel of the blood. The tubules looked similar or fewer to the controls as in Saleh, 2014.

Ultra-structurally examination of renal sections revealed that the purification barrier width was relatively analogous to that of controls. But, a number of cells coating the PCT displayed vacuoles in the cytoplasm. The DCT appeared comparatively like that of the controls as the majority of the renal corpuscles and renal

tubules were essentially like that of the controls. (Sedighi et al., 2014).

The present study has the same results of as Traber & Stevens, 2011, who concluded that Se has a function in the antioxidant enzyme system activation e.g. vitamin-E &-C and glutathione peroxidase that get rid of the formed active oxygen radicals, so plasma film lipid peroxidation is protected. GPx (Se-depending glutathione peroxidase) is a major anti-oxidative enzyme in the cell and it was showed that Selenium is a physical constituent of GPx. Glutathione peroxidases detoxify hydro peroxidases and avoid oxidative damage to membrane of the cell and it avoids the cell apoptosis (Penglase et al., 2014& Hagiwara et al., 2011& Orun et al., 2008& Kaur et al., 2003).

In the group treated by APM, there was a significant reduction in the body and kidney mass of the rats, this agreed with several earlier investigations that inspire the aspartame usage in diet (De la Hunty et al., 2006). De la Hunty et al., concluded that the usage of aspartame in foods and drinks as an alternative of sucrose lead to a significant decrease in energy consumptions in addition to body mass about 0.2kg/week.

In the same way, 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 decrease of kidney weight agrees with Mourad, 2011 who reported that six week of aspartame administration (40 mg/kg body mass) leads to a significant rise in lipid peroxidation level. Lipid peroxidation is considered as an automatic catalytic method guiding to oxidative destructive effect of the cellular membranes as it destructs polyunsaturated fat-acids in order to decrease the fluidity of the membrane that was critical for suitable function of the cell. A rise in free radicals causes overproduction of MDA level observed, which was an indication of the lipid peroxidation, specified kidney cell membrane damaging afterward APM administration. This result agree that of Parthasarathy et al., 2006 who examined that methanol management significantly raised level of MDA in the organs of lymphoid; similarly, Zararsiz et al., 2007 find a significant rise in level of MDA in the rats kidney afterward treating by formaldehyde.

The outcomes of body mass decrease of group treated by APM are in disagreed to De Matos Feijó et al., 2013 that found great mass increase was resulted from aspartame or saccharin usage, in comparison with sucrose, and this mass increase was unconnected to caloric consumption. This might occur because of a reduction in energy spending or rise in fluid holding may be the reason.

In APM-Se treated group, there was slightly increase in the body and kidney mass compared to those treated by APM. This might be due to the outcome of Se on the hormones of the thyroid 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 results were 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 greatest commonly used as indicator in the estimation of filtration rate of glomerular as in Nitescu et al., 2006.

In the group treated by APM, a significant rise in serum creatinine& urea levels were exist. These consequences also concluded by Odabasi et al., 2009 & Saleh, 2014. This elevation of urea and creatinine reflect the severity of renal insufficiency with decrease in the filtration rate of glomerular due to almost all of the methanol and formic acid that affect the tubular epithelial cells by the creation of superoxide anion, hydrogen peroxide and amplified amount of free radicals' formation. Oxidative stress induced by aspartame leads to these degenerative changes due to depletion of antioxidants encouraging anomalies in the role and uptake of several

intra-cellular organelles as in (Zararsiz et al., 2007, Parthasarathy et al., 2006 & Roldan et al., 2003).

Also, the current results are in contrast with Boj et al., 2003 who concluded that the systemic dosages of formaldehyde in rats was give rise to a significant variation in the levels of creatinine & urea after 24-48 hours of application but not producing kidney inflammation or tissue lesion because of the short interval of application.

In the group that treated by APM-Se, there was a marked reduction in the level of serum creatinine & urea in comparison to those treated by APM. These was an agreement with Hasanvand et al., 2016 results who concluded that administration of Se after kidney ischemia reperfusion 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 reno-protective effects in lead (Pb) poisoning and acute renal failure caused by gentamicin. It was found that Selenium prevents injury caused by free-radicals that destruct the fat-acid of the sub-cellular membrane.

The results of the current work agree the El Haliem and Mohammad, 2011 who concluded that co-administration of Pimpinella anisum oil was operative in reducing the aspartame toxic effect on kidney and liver of adult albino rats. This outcome may be secondary to the anti-oxidant ability of anise, which attacks reactive oxygen species (ROS) as selenium 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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