Counteracting Effect of Selenium and Vitamin E to Cadmium Toxicity in Rats

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ABSTRACT

The present study was to determine the role of selenium and vitamin E in preventing the harmful effects of cadmium on different organs of experimental animals. Twenty-four male rats were used and divided into 4 groups (6 rats each). Group 1: control group. Group 2: CdCl₂ dissolved in water and administered at the dose of 10 mg / 1000ml. Group 3: CdCl₂ dissolved in water and administered at the dose of 10 mg/1000ml + VE 500 mg /1kg forge. Group 4: CdCl₂ dissolved in water and administered at the dose of 10 mg/1000ml + Se 10 mg/1kg forge. Samples were collected at 2, 4, 6, 8, 10 and 12 weeks from start of the experiment. At the mid-way (day 45) and the terminal of the course of experiment (day 90) rats were sacrificed to obtain liver, kidney and testes. After (45 days) 12 mice were sacrificed and 90 days later 8 mice were sacrificed). Immediately after collection from the target organs small specimens were taken then immersed in formalin 4% for two days. Treatment groups with cadmium chloride (CdCl₂) caused liver, kidney and testes damage which demonstrated functionally by increasing the activity of AST, ALT, urea, and histological alterations. While serum total proteins, albumin and globulin were significantly (P≤0.05) higher in all groups except cadmium group which was significantly (P ≤0.05) lower in serum total proteins, albumin and globulin compared to control and other groups.

Key words: Prickly pear, permeate, Papaya pulp, Sweet whey, probiotic beverages, bifidobacteria

Introduction

Exposure to significant high levels of cadmium occur by smoking. Tobacco smoke transports cadmium into the lungs, then to rest of the body by blood circulation. Cadmium firstly transported to liver through blood. It bound to proteins to form complexes that are transported to the kidneys. Cadmium accumulates in kidney, where it produces damage to filtering system which leads to the excretion of essential proteins and sugars from the body and further kidney damage. Other health effects that can be caused by cadmium are: 1- Diarrhoea, stomach pains and severe vomiting. 2- Bone fracture. 3- Reproductive problems and possibly even infertility. 4- Damage to the central nervous system. 5- Effects on immune system. 6- Possibly DNA damage or cancer development (Suhartono et al., 2014). Also Murugavel and Pari, (2007) reported that cadmium (Cd) accumulation leads to serious changes in the histology of the rats livers, including inflammatory cell infiltration, focal necrosis and sinusoidal dilation. Cd preferentially binds to the membrane and disturbs the redox state of the cells. The increased formation of lipid peroxides and associated reactive oxygen species leads to a collapse in membrane integrity and other pathological changes in the liver. Also Suhartono et al. (2014) reported that exposure to cadmium causes oxidative stress in rats' kidneys. Furthermore, Renugadevi and Prabu, (2009) found that Cd toxicity caused renal dysfunction in rats by increasing lipid peroxidation and disturbing its antioxidant defense system. Furthermore, Koizumi and Li. (1992) reported that Cd induced oxidative stress in testicular tissue, including Leydig cells, which caused severe tissue degeneration just after administration of the metal. Mladenovic et al. (2013) found significant increase in serum AST and ALT while total protein and albumin levels significantly dropped in the Cd group compared to control. Sarkar and Bhatnagar, (1997) reported increase in ALP, serum and blood urea in response to Cd intoxication and indicates liver function impairment due to increased Cd induced oxidative stress.

Vitamin E is a fat soluble vitamin and acts also as an antioxidant, as it prevents the free radical damage to specific fats in the body that are critical for health. Vitamin E is an important vitamin that...
is required for the proper functions of many organs, enzymatic activities and neurological processes. It also extremely useful in naturally slowing blood flow leading to consuming more food with vitamin E. Vitamin E prevents diseases of heart and blood vessels, such as chest pains, high blood pressure, and block hardened of arteries. Vitamin E found only in plant foods including certain oils, nuts, grains, fruits and wheat germ. (Khalil, 2012). Also Mehana, (2008) reported that vitamin E reduced the free radicals generation and improved the antioxidant status in cadmium treated groups of albino rats. Furthermore, vitamin E may be useful in decreasing the toxicity of cadmium. Gupta et al. (2003) reported that vitamins C and E prevent oxidative stress and play vital roles in co-regulating steroid acute regulatory (StAR) gene expression and steroid production in cadmium exposed rats. Filho et al. (2000) demonstrated that vitamin E supplement can protect rats from cadmium-induced renal and cardiac tissue damage. Layachi and Kechrid, (2012) found that when rats treated with Cd, the concentration of serum total protein and serum albumin were diminished as compared with control group. While supplements of vitamin C or vitamin E either alone or together with cadmium, produced recovery in the above mentioned biochemical parameters. Mehana, (2008) reported that cases treated with cadmium chloride and vitamin E showed marked improvement in both biochemical and histological changes comparing to rats treated with cadmium alone as following: A significant reduction in levels of AST, ALT and urea, in comparison with cadmium treated group.

Selenium is an element found in soil, water and some foods. Mammals need only a very small amount of selenium as it takes part in the metabolism of different substances. Selenium acts as antioxidant to different toxic products released during the metabolism of different foods, and prevents its harmful effects which may lead to cell death (Tapiero and Tew, 2003). Also Jabeen and Chaudhry, (2010) showed an antagonistic effect of selenium (Se) in cadmium -induced toxicity. It appeared that the subcutaneous administration of Se as sodium selenite was able to curtail the Cd-induced toxic effects in male Sprague–Dawley rats. Also Ognjanović et al. (2007) worked on Wistar male rats showed the effects of the nutritional antioxidant selenium, that ameliorated oxidative stress and loss of cellular antioxidants and suggested that Se efficiently protect liver and kidneys from Cd induced oxidative damage. This protection includes the capability of Se to alter the distribution of Cd in tissues and to induce binding of the Cd-Se complexes to proteins, which are similar to metallothioneins. Yiin et al. (1999) showed that administration of Cd increased the product of lipid peroxidation in testes. Selenium might be very useful in protection against Cd-induced lipid peroxidation, as the presence of Se reduced the Cd effects. Bahraman et al. (2014) found that exposure to cadmium caused a significant increase of AST and ALT activity compared to control rats group (P<0.001). When simultaneously Se and Cd added, it did not induce any significant difference in the transaminase enzymes activity in comparison with Cd treated group (P>0.05). El-Boshy et al. (2014) found that the total plasma protein did not significantly changed in the Cd+Se treated groups compared with the control group, while the albumin serum level was significantly increased in cadmium and selenium treated groups compared with the cadmium treated rats group. Wang et al. (2013) showed a significant increase in level of blood urea nitrogen (BUN) and creatinine in Cd administrated mice group. Treatment with selenium could prevent Cd to induce increase in level of serum urea. The present study planned to determine the effects of selenium and vitamin E in preventing the harmful effects of cadmium on different organs of experimental animals.

Materials and Methods

Materials:

10mg Cadmium (CdCl₂) was added to 1000 ml of water, 500 mg vitamin E was added to 1 kg forage and 10 mg selenium was added to 1 kg forage.

Animals:

The Albino rats used in this study were bought from El- Osman farm, Cairo, Egypt with an average live body weight 225g (ranged from 200-250g).The animals were raised in Animal house belonging to Faculty of Agriculture, Al-Azhar University.
Experimental design:

Animals were divided randomly into 4 equal groups, each contained 6 rats and fed one of the following diets:

**Group 1:** control group.
**Group 2:** CdCl$_2$ dissolved in drinking water at rate 10 mg / 1000ml.
**Group 3:** CdCl$_2$ (10 mg / 1000ml of drinking water) + VE (500 mg / 1kg forge).
**Group 4:** CdCl$_2$ (10 mg / 1000ml of drinking water) + Se (10 mg / 1kg forge).

Quantity of food consumed by rat; 35-60 g forge/day and of drinking water 25–40 ml per day.

Blood samples:

Blood samples were taken from the orbital vein of rats into test tubes and collected into tubes. Samples were collected at 2, 4, 6, 8, and 12 weeks from the start of experiment. A blood sample was taken from 16 mice from the beginning of the experiment to (day 45), then the blood sample was taken from 8 mice and the end of the experiment. Blood samples were centrifuged at 3000 rpm for 15 min to obtain serum. Serum was separated and kept into Eppendorf tubes and stored in a deep freezer (-20ºC) until analyzed.

Biochemical assays:

Serum total protein was determined using colorimetric method according to Koller (1984). Serum albumin was measured using kits according to method of Gindler and Westgard (1973). While Serum globulin was calculated by subtraction of albumin from total protein. Serum AST and ALT was determined using colorimetric method according to Schiele, F. (1982). Serum urea was measured by colorimetric method based on the method of Kaplan, (1984).

Histological Studies:

At the mid-way (day 45) and the terminal (day 90) of the course of experiment, rats were sacrificed to obtain liver, kidney and testes. Small specimens were collected immediately from the target organs then immersed in 4% formalin for two days.

Statistical analysis:

Data were subjected to analysis of variance using SPSS software program package. Also, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significance. Data were analyzed by one way analysis method using the following model. $Y_{ij} = u + N_i + e_{ij}$ were $Y_{ij} =$ the observed value, $u =$ population means, $N_i =$ the effect of treatment, $e_{ij} =$ the standard error.

Results and Discussion

Histopathological examination:

Liver histopathological changes:

The liver structure of the control, cadmium+vitamin E and cadmium+selenium groups showed normal hepatocytic cords and blood sinusoids either after 6 weeks (fig 1, 3 and 4) or after 12 weeks (fig 5, 7 and 8). In rats group treated with cadmium only liver sections showed vacuolarly degenerated hepatocytes with pyknotic nuclei after 6 weeks (fig 2). However after 12 weeks, rats group treated with cadmium only showed severe vacuolarly degenerated hepatocytes in liver section with multinucleated hepatocytes (fig 6). It might be due to cadmium stimulation to form metallothioneins and reactive oxygen species (ROS), thus causing oxidative damage in liver tissues resulting in loss of membrane functions. Also the increase in lipid peroxidation due to cadmium toxicity might alternate
the antioxidant defense system which includes enzymes such as glutathione peroxidase (GPx), glutathione-S-transferase superoxide dismutase (SOD), and catalase (CAT), and nonenzymatic molecule like glutathione, which normally protect against free radical toxicity. Similar findings noticed by Oyinloye et al. (2016) who reported that histological examinations revealed that exposure to cadmium resulted in severe hepatic damage indicated as degeneration of hepatocytes associated with perportal hepatic necrosis as well as cellular infiltration by mononuclear cells and fatty infiltration. Furthermore El-Demerdash et al. (2004) demonstrated the ability of Cd to induce oxidative stress in rat plasma, liver and brain as evidenced by increasing lipid peroxidation after 30 days of Cd treatment. Also Moneim and Ghafeer, (2007) found that treatment with cadmium chloride (CdCl₂) caused liver damage which demonstrated functionally by increasing the activity of SGOT, SGPT, ALP, and histological alterations. These alterations were in the form of dilation of the hepatic sinusoids; dilatation and congestion of central veins; hypertrophy or degeneration of some hepatocytes with either hyalinized or vacuolated cytoplasm with pale nuclei and prominent nucleoli. Pari and Shagirtha, (2010) reported that hepatic histoarchitecture of the Cd-treated rats resulted in severe damage of parenchyma with necrosis, lymphatic infiltration, dilation of sinusoids, cellular degeneration and intracellular vacuolation and pyknotic nuclei. It might be due to the formation of highly reactive free radicals and subsequent lipid peroxidation induced by Cd. The accumulated hydroperoxides can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxides, which is the main cause of hepatocellular damage.

On the other hand, addition of vitamin E or selenium with cadmium protects against the harmful effect of cadmium toxicity on hepatocytes. The roles of vitamin E on cadmium-induced damage are prevention of oxidative stress and lipid peroxidation activities in liver. Selenium is a cofactor of glutathione peroxidase (GPx), a cyto-antioxidant enzyme. Selenium enhances the availability of glutathione (GSH), which is one of the most abundant intrinsic antioxidants that helps in preventing lipid peroxidation and resultant cell damage (fig. 3, 4, 7 and 8). These results are in agreement with those obtained by Mehana (2008) who indicated improvement in biochemical and histological appearance in rats treated with cadmium chloride and vitamin E comparing to those treated with cadmium chloride alone. The improvement occurred due to the antioxidant power of vitamin E, as scavenger to free radicals, induced by cadmium chloride and enhance immune response as well as prevent lipid peroxidation that cause damage to the cells (liver, kidneys and testes). layachi and kechrid (2012) found that exposure to cadmium provoke liver injury, by inducing lipid peroxidation, led to depletion of liver reduced glutathione, reduction in antioxidant enzyme activities and biochemical parameters variations of rats. However, vitamin E or vitamin C treatments may have partial ameliorative effects on the disturbances caused by cadmium toxicity that increase glutathione (GSH) level and the activities of antioxidant enzymes that ameliorate some biochemical parameters, but vitamin E and vitamin C together perform a more synergistic effect against the observed oxidative stress. Furthermore, Oyinloye et al. (2016) reported that cadmium interfere with cellular components leading to increase generation of free radicals especially, reactive oxygen species (ROS) in Cd-exposed rats. This was evident by the noticeable elevation witnessed in the levels of lipid peroxides in liver. While El- Demerdash et al. (2004) found that Vitamin E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation. Yin et al. (2000) showed that administration of cadmium increased the products of lipid peroxidation in rats' liver, heart, and spleen. Selenium might be very useful protector against Cd-induced lipid peroxidation and might reduce the Cd effects. El-Boshy et al. (2014) demonstrated that cadmium is capable of causing marked oxidative stress in addition to inhibiting the activities of antioxidant enzymes. Treatment with selenium could significantly attenuate the cadmium induced immunosuppressive oxidative stress as well as hepatotoxicity and renal damage. Jihen et al. (2008) found that light microscopic examination indicated severe histological changes in the two organs under cadmium influence. Se or Zn partially alleviated the damage observed in liver.
Fig. 1: Section in hepatic tissue in the liver of control group, showing normal hepatocytic cords, blood sinusoids and central vein (H&E X 400).

Fig. 2: Liver tissue treated with cadmium only after 6 weeks showing vascularity, degenerated hepatocytes with pyknotic nuclei and central vein is dilated (H&E X 400).

Fig. 3: Section in hepatic tissue from rats treated with cadmium + vitamin E after 6 weeks. Normal hepatocytes, normal blood sinusoids and central vein (H&E X 400).
Fig. 4: Section in hepatic tissue treated with cadmium + selenium, after 6 weeks, showing normal hepatocytes, blood sinusoids and central vein (H&E X 400).

Fig. 5: Section in hepatic tissue in liver of the control group showing normal hepatocytic cords, blood sinusoids and central vein.

Fig. 6: Section in hepatic tissue in liver of the cadmium treated group after 12 weeks, showing severe vacuolar and hepatic degeneration with fragmented nuclei or multinucleated hepatic cells (arrows) (H&E X 400).
Kidney histopathological changes:

The kidney structure of the control, cadmium + vitamin E and cadmium + selenium groups showed preserved normal architecture. The cortex showed normal renal glomeruli and renal tubules. The medulla showed normal collecting tubules interstitial tissue and blood vessels after 6 weeks (fig 9, 11 and 12) and 12 weeks (fig 13, 15 and 16). In group treated with cadmium only kidneys showed dilated, congested interstitial blood vessel with thickened muscular wall after 6 weeks (fig 10). However after 12 weeks kidneys showed congestion of glomerular capillaries and peritubular blood capillaries (fig 14). Exposure to Cd could result in apoptosis of the proximal tubular cells and oxidative stress, therefore, disrupt kidney function. Furthermore, the effects caused by Cd toxicity in the tissues may considered associated with the impairment in anti-oxidant defense system. These results supported by Suhartono et al. (2014) who reported that exposure to cadmium causes oxidative stress in the rat kidneys. Also Renugadevi and Prabu (2008) found that cadmium treated rats showed tubular necrosis, inflammatory cell infiltration, tubular degeneration, hemorrhage, swelling of tubules and vacuolization. This could due to the accumulation of free radicals as the consequence of increased lipid peroxidation by free Cd ions in the renal tissues of Cd-treated rats. Renugadevi and Prabu (2009) reported that Cd-treated rats showed tubular necrosis, inflammatory cell infiltration, tubular degeneration, hemorrhage, swelling of tubules and vacuolization. Previous reports suggested that naringenin reduced the histological alterations caused by cisplatin-induced nephrotoxicity. Jemai et al. (2010) showed that cadmium induces nephrotoxicity especially glomerular and tubular damages.
renal cortex showed clear evidence of tubulo-interstitial nephritis. Renugadevi and Prabu (2009) found that cadmium (Cd) toxicity cause renal dysfunction by increasing lipid peroxidation and disturbing its antioxidant defense system. Moneim and Ghafeer (2007) reported that daily administration of cadmium (0.5 mg/kg I.P.) for four weeks induced significant damage in function and structure of kidney assessed by increased creatinine and urea concentrations in plasma, atrophy and swelling of some glomerular capillaries as well as proximal tubular necrosis and apoptosis.

On the other hand, addition of vitamin E and selenium with cadmium resulted in protection of kidney from damage. Selenium is thought to enhance antioxidant capacity of cells, through increased superoxide dismutase (SOD) and glutathione reductase (GR) activities and elevated GSH content following selenium and vitamin E supplementation (fig. 11, 12, 15 and 16). The obtained results are similar to those reported by Filho et al. (2000) who demonstrated that vitamin E supplement can protect rats from cadmium-induced renal and cardiac tissue damage. Furthermore, Wang et al. (2013) observed that selenium may protect renal function against cadmium induced toxicity in mice. Furthermore, pretreatment of mice with Se significantly inhibited the apoptosis due to ROS and mitochondrial dysfunction. Adi et al. (2016) showed that normal nephritic ultrastructure in the control and Vitamin E co-treated groups. The Cd and Vit.-E groups displayed the normal appearance of microvillii, smooth rounded nucleus, and undamaged mitochondria with regular structure in the renal tubular epithelial cells. Cd treatment caused wide-range of kidney injury with injured microvillus and dilated cisternae of the smooth endoplasmic reticulum (SER). The glomerular epithelial cells showed nuclear membrane damage, nuclear chromatin condensation, and margination in Cd-induced kidneys. Shaikh et al. (1998) found that oxidative stress induced by cadmium administration was reduced by vitamin E co-treatment. Furthermore, hepatic and renal cortex malondialdehyde (MDA) levels were significantly lower in the Vit. E co treated rats than in the Cd alone group. Abarikwu et al. (2015) administration of selenium and rutin (RUT) alone or in combination prevented the histological alterations induced by Cd in the rats showing almost normal appearance of kidney glomeruli and tubules. This could attributed to the antioxidant and chelating properties of Se and RUT, which significantly reduced the oxidative threat leading to reduction of pathological changes and restoration of normal physiological functions. Sharaky et al. (2007) reported that selenium treatment could protect the rats’ kidney tissues against the toxicity of cadmium and increased the activities of antioxidant enzymes in these tissues.

Fig. 9: Section in the kidney tissue in the control group after 6 weeks, shows normal renal glomeruli and renal tubules. (H&E X 400).
Fig. 10: Section in the kidney tissue after 6 weeks. The cadmium group show dilated necrosis and congested interstitial blood vessel with thickened vascular wall (arrow) (H&E X 400).

Fig. 11: Section in the kidney tissue in the cadmium + vitamin E group after 6 weeks, show normal renal glomeruli and normal renal tubules. (H&E X 400).

Fig. 12: Section in the kidney tissue in the cadmium + selenium group after 6 weeks, show normal renal glomeruli and renal tubules. (H&E X 400).
Fig. 13: Section in the kidney tissue in the control group after 12 weeks, show normal renal glomeruli and renal tubules. (H&E X 400).

Fig. 14: Section in the kidney tissue in the cadmium group after 12 weeks, show congestion of glomerular capillaries (arrow) and peritubular blood capillaries (arrow head) (H&E X 400).

Fig. 15: Section in the kidney tissue in the cadmium + vitamin E group after 12 weeks, show normal renal glomeruli and renal tubules (H&E X 400).
**Fig. 16:** Section in the kidney tissue in the cadmium + selenium group after 12 weeks, show normal renal glomeruli and renal tubules (H&E X 400).

**Testes histopathological changes:**

The testes structure of the control, cadmium + vitamin E and cadmium + selenium groups revealed normal architecture, normal seminiferous tubules with healthy spermatogonia cells, and normal interstitial leydig cells that found after 6 weeks (fig 17, 19 and 20) and after 12 weeks (fig 21, 23 and 24). In cadmium treated group, the testes showed degenerated seminiferous tubules with necrosed spermatogonic cells after 6 weeks (fig 18) while after 12 weeks the testes showed depletion of spermatogonia cells (figs 22) which probably due to increased lipid peroxidation levels in plasma and tissues with alterations in antioxidant defenses (GPx and GSH). Thus it possible that oxidative stress and disturbance in antioxidant defenses were the causes of testes damage induced by cadmium in this experimental model. These results are in agreement with the findings of Ekhoye et al. (2013) who indicated that cadmium chloride (CdCl₂) treated groups showed damage and degeneration of seminiferous tubules in testes. Cadmium is very dangerous to testicular function through increasing oxidative stress. Cadmium chloride causes testicular damage and impairs male fertility. Adamkovicova et al. (2014) found that subchronic cadmium exposure at rate 30mg/L resulted in moderate to severe testicular injury. Regarding the tissue constituents of the testis, many irregularly outlined seminiferous tubules showed disarranged epithelial layers and necrotic cellular debris. El-Shahat et al. (2009) found that exposure to cadmium induces histopathological and biochemical effects on rat testes. The increased oxidative stress, resulted from Cd intoxication in testicular tissue, might be responsible, at least partially, of histopathological changes. On the other hand addition of vitamin E and selenium to cadmium resulted in reducing and/or preventing both the oxidative stress and damage in the testis (that caused by cadmium) (fig. 19, 20, 23 and 24). The obtained results are similar to those reported by Hussein et al. (2014) who used α-tocopherol and Cd and showed that seminiferous tubules and the leydig cells almost retained their normal structure with normally arranged seminiferous epithelium and a healthy interstitium. Mohamed et al. (2014) showed that exposure to cadmium led to obvious degenerative changes in testicular tissue accompanied by decreasing level of serum testosterone hormone. Simultaneous administration of vitamin E and Zinc have ameliorated most of induced testicular damage. Thus dietary agents like Vitamin-E and Zinc can be recommended as effective protectors against cadmium intoxication. Ognjanovic et al. (2009) reported acute intoxication with cadmium causes a significant increase in lipid peroxidation in the testes of rats. Pretreatment with ubiquinone (CoQ10) and/or Vit E were very effective in the prevention of oxidative damage induced by Cd, which resulted in significant decrease in lipid peroxidation in the testes. Yang et al. (2005) found the same protective effects of α-tocopherol, apparent also in our cytometry experiments, at cadmium dose of 2 mg/kg BW. In contrast to rats receiving cadmium alone at rate 2 mg/kg BW, the cell distribution in rats receiving α-tocopherol was
similar to those of controls. However, when cadmium dose increased beyond 2 mg/kg, α-tocopherol had no protective effects. Yiin et al. (1999) showed that administration of cadmium increased the products of lipid peroxidation in testes. Selenium might be very useful in protection against Cd-induced lipid peroxidation, as presence of Se reduced the Cd effects.

Fig. 17: Section in the testicular tissue of control group after 6 weeks, show normal seminiferous tubules with healthy spermatogonia cells (H&E X 200).

Fig. 18: Section in the testicular tissue of cadmium group after 6 weeks, show degenerated seminiferous tubules with necrosed spermatogonic cells (arrow) (H&E X 400).
Fig. 19: Section in the testicular tissue of cadmium + vitamin E group after 6 weeks, show healthy seminiferous tubules with normal spermatogenic cells (arrow) (H&E X 200).

Fig. 20: Section in the testicular tissue of cadmium + selenium group after 6 weeks, show normal seminiferous tubules with healthy mature spermatogenic cells (H&E X 400).

Fig. 21: Section in the testicular tissue of control group after 12 weeks, show normal seminiferous tubules with healthy spermatogonia cells (H&E X 400).
**Fig. 22:** Section in the testicular tissue of cadmium group after 12 weeks, shows depletion of spermatogonia cells (arrow) (H&E X 400).

**Fig. 23:** Section in the testicular tissue of cadmium+ vitamin E group after 12 weeks, show normal seminiferous tubules with healthy spermatogonia cells (H&E X 200).

**Fig. 24:** Section in the testicular tissue of cadmium+ selenium group after 12 weeks, show normal seminiferous tubules with healthy spermatogonia cells (H&E X 400).
Blood serum parameters:

Serum (AST) and (ALT) activities.

The results showed increase in AST and ALT in rats treated with cadmium only, in comparison with the other groups and their standard (tables 1 and 2). There was an increase in AST and ALT in the group treated with cadmium and vitamin E comparing to the control group. Moreover, there were increase in AST and ALT in the group treated with cadmium + selenium comparing to the control group or cadmium + vitamin E group. The results of administration of cadmium, vitamin E + cadmium and selenium + cadmium to rats on serum AST and ALT activities are shown in Tables (1 and 2). The achieved results at 2, 4, 6, 8, 10 and 12 weeks showed that all values of both AST, ALT were in normal range for all experimental groups except cadmium group which recorded significantly ($P \leq 0.05$ ) higher AST and ALT values compared to all other groups. This means that cadmium treatment significantly ($P \leq 0.05$) increased serum AST and ALT compared to all other groups.

These results may be referred to damage occurred in liver due to treatment of rats with cadmium as observed in histopathological results of liver. Also, the increment observed in AST and ALT activities by cadmium administration in this study can indicate bad or altered function of liver and disturbed metabolism of the liver which may reflect a bad health of rats subjected to the treatment of this group. Pari and Shagirtha (2010) showed that serum enzymatic activities of AST, ALT and ALP were increased after 21 days of exposure to cadmium. The increased activities of these enzymes represent biomarkers for liver damage. In addition, increased levels of serum hepatic markers suggest an extensive liver injury, which occasioned by Cd due to increasing lipid peroxidation which have the ability to cause membrane damage. Cadmium causes structural and functional damage to the cell membrane and increase the membrane permeability leading to the leakage of hepatic enzymes in to the blood. El-Demerdash et al. (2004) found that decrease in liver AST and ALT activities may be due to liver dysfunction and disturbance in the synthesis of these enzymes. Furthermore, the liver damage by oral administration of cadmium chloride (CdCl$_2$) could be confirmed through the increase in level of plasma bilirubin. Therefore, the increase in the activities of AST and ALT in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. Elgaml and Hashish. (2014) reported that lysosomal instability caused by cadmium chloride (CdCl$_2$) resulted from leakage of hepatic enzymes (ALT, AST and ALP) into the blood stream. A highly significant increase in (ALT, AST and ALP) was detected in cadmium treated rats. The increase in the transaminases (ALT and AST) may be attributed to hepatic damage. Meanwhile, the alteration in serum ALP level may be attributed to cholestasis and acute hepatocellular necrosis. Moneim and Ghafeer. (2007) showed that liver enzymes (SGOT, SGPT and ALP) in the cadmium (Cd) treated group were significantly elevated compared with the control group, denoting the presence of liver dysfunction. Oyinloye et al. (2016) found significant elevation in serum transaminases activity (AST and ALT), which clearly indicates the loss of cellular integrity and the leakage of hepatic membrane. Hepatocellular injury was associated with exposure to cadmium. El-Kady et al. (2009) found that rats treated with cadmium (Cd$^{2+}$) ions alone showed a significant increase in ALT and AST activities accompanied with a significant decrease in total protein (TP). The disturbances in the activities of these enzymes in serum represent biomarkers for liver damage. Murugavel and Pari (2007) found that the increase in activities of these enzymes (AST, ALT and ALP) in serum, after Cd treatment, reflect the destructive effect of Cd on cell membrane, resulting in increasing release of functional enzymes from intracellular locations, which gives the indication of the hepatotoxic effect of Cd. Sarkar et al. (1997) showed that increase in SGPT, SALP, serum bilirubin and blood urea were in response to Cd intoxication which indicates liver function impairment due to increase of Cd induced oxidative stress. Tables (1 and 2) also showed that treatment of rats with vitamin E or selenium plus cadmium significantly stopped the increase in serum AST and ALT while exposure of rats to cadmium alone produce increase in all enzymes of liver. These results indicate that vitamin E or selenium have a hepatoprotective effect against cadmium hepatotoxicity. Layachi and kechrid (2012) revealed that activities of serum GOT, GPT and alkaline phosphatase significantly increased, compared to their normal levels. It could be attributed to the hepatic damage resulted with increasing release and leakage out of these enzymes from liver cytosol into the blood stream which gives an indication to the hepatotoxic effect of this
Table 1: Means ± S.E of the effect of selenium, Vit E and cadmium on serum AST concentrations (U/L)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>12 weeks</th>
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<tr>
<td></td>
<td>mean± S.E</td>
<td>d.t</td>
<td>mean± S.E</td>
<td>d.t</td>
<td>mean± S.E</td>
<td>d.t</td>
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<tr>
<td>G1</td>
<td>5.335± 0.522</td>
<td>d</td>
<td>6.037± 0.437</td>
<td>d</td>
<td>7.262± 0.481</td>
<td>d</td>
<td>8.355± 0.305</td>
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<tr>
<td>G2</td>
<td>24.737± 0.759</td>
<td>a</td>
<td>29.165± 0.915</td>
<td>A</td>
<td>33.275± 0.864</td>
<td>a</td>
<td>34.180± 1.460</td>
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<tr>
<td>G3</td>
<td>10.330± 0.360</td>
<td>c</td>
<td>11.580± 0.288</td>
<td>C</td>
<td>12.257± 0.340</td>
<td>c</td>
<td>12.835± 0.815</td>
</tr>
<tr>
<td>G4</td>
<td>14.482± 0.355</td>
<td>b</td>
<td>15.925± 0.273</td>
<td>B</td>
<td>16.990± 0.562</td>
<td>b</td>
<td>17.840± 1.520</td>
</tr>
</tbody>
</table>

S.E: Standard error.  d.t: Duncan’s multiple range test between groups. Means with the same letter are not significantly different.

G1: Control group. G2: Group treated with Cadmium only. G3: Group treated with Cadmium + Vitamin E. G4: Group treated with Cadmium + selenium. The results showed increase in AST in rats treated with cadmium only in comparison with other groups and other criteria. There was increase in AST in the group treated with cadmium and vitamin E comparing to the control group.

Table 2: Means ± S.E for the effect of selenium, Vit E and cadmium on serum ALT concentrations (U/L)

<table>
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<tr>
<th>Treatment</th>
<th>Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>12 weeks</th>
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<tr>
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<td>mean± S.E</td>
<td>d.t</td>
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<tr>
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<td>c</td>
<td>1.440± 0.065</td>
<td>b</td>
<td>1.600± 0.065</td>
<td>c</td>
<td>1.600± 0.160</td>
</tr>
<tr>
<td>G2</td>
<td>12.400± 0.336</td>
<td>a</td>
<td>15.412± 0.526</td>
<td>a</td>
<td>16.617± 0.388</td>
<td>a</td>
<td>17.340± 0.850</td>
</tr>
<tr>
<td>G3</td>
<td>1.680± 0.103</td>
<td>bc</td>
<td>1.840± 0.103</td>
<td>b</td>
<td>1.920± 0.065</td>
<td>c</td>
<td>2.160± 0.080</td>
</tr>
<tr>
<td>G4</td>
<td>2.080± 0.172</td>
<td>b</td>
<td>2.280± 0.136</td>
<td>b</td>
<td>2.640± 0.103</td>
<td>b</td>
<td>2.880± 0.160</td>
</tr>
</tbody>
</table>

S.E: Standard error.  d.t: Duncan’s multiple range test between groups. Means with the same letter are not significantly different.

G1: Control group. G2: Group treated with Cadmium only. G3: Group treated with Cadmium + Vitamin E. G4: Group treated with Cadmium + selenium. The results show increase in ALT in rats treated with cadmium only in comparison with other groups and other criteria.

metal. Therefore, the supplementation of vitamin E or vitamin C had protected liver function from cadmium intoxication as indicated by the significant restoration of serum total protein, albumin, serum glucose, GOT, GPT and alkaline phosphatase. The decrease of glutathione level in cadmium treated animals may be a result of oxidative stress, which has occurred with cadmium toxicity. Mohamed et al. (2014) showed that Cd and Se groups treated with low dose show none significant change in ALT and AST serum level when compared with the control group. Cadmium (Cd) is capable of causing marked oxidative stress besides inhibiting the activities of antioxidant enzymes. The treatment with Se could significantly attenuate Cd immunosuppressive oxidative stress as well as hepatotoxicity and renal damage. Alhazza (2008) found that selenium reduced the accumulation of cadmium in liver cells which may lead to a decrease in hydroperoxide level. He showed that selenium alleviated the deleterious effects of cadmium on liver enzymes. The ameliorating effect of selenium on biochemical parameters (ALT, AST and ALP) might be due to an interaction of selenium with cadmium forming biologically inactive cadmium selenide complexes. El-Boshy et al. (2014) found that rats group treated with cadmium plus selenium did not show significant changes in serum ALT and AST levels as compared with control group. They also indicated that selenium ameliorated cadmium hepatotoxicity in rats treated with cadmium plus selenium. Several mechanisms could be operating.
with the protective action of selenium, which could result, for example, into changing absorption of cadmium or change in their action and distribution in the organism and within target organs.

**Serum total protein, albumin and globulin levels.**

The results showed decrease in total protein, albumin and globulin in rats treated with cadmium only in comparison with other groups and other standard. Effect of administration of cadmium, vitamin E + cadmium or selenium + cadmium to rats on serum total protein, albumin and globulin at 2, 4, 6, 8, 10 and 12 weeks of experiment are presented in Tables 3, 4 and 5. The levels of serum total proteins, albumin and globulin were significantly (P ≤ 0.05) higher in all groups of experiment, except cadmium group which had significantly (P ≤ 0.05) lower serum total proteins, albumin and globulin compared to control and other groups. Treatment of rats with vitamin E or selenium plus cadmium significantly stopped the reduction in serum total protein and albumin due to exposure to cadmium to reach the normal range of the control group.

The significant reduction in serum total protein, albumin and serum globulin due to cadmium intoxication caused a significant decrease in serum total protein, albumin and globulins as compared with control rats.

**Table 3:** Means ± S.E for the effect of selenium, Vit E and cadmium on serum total protein concentrations (g/dl).

<table>
<thead>
<tr>
<th>Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
</tr>
<tr>
<td>G1</td>
<td>7.555±0.015</td>
<td>a</td>
<td>7.900±0.110</td>
<td>A</td>
<td>7.985±0.020</td>
<td>A</td>
</tr>
<tr>
<td>G2</td>
<td>6.340±0.040</td>
<td>d</td>
<td>6.290±0.040</td>
<td>C</td>
<td>5.940±0.035</td>
<td>D</td>
</tr>
<tr>
<td>G3</td>
<td>7.360±0.010</td>
<td>b</td>
<td>7.500±0.050</td>
<td>B</td>
<td>7.690±0.020</td>
<td>B</td>
</tr>
<tr>
<td>G4</td>
<td>7.140±0.080</td>
<td>c</td>
<td>7.375±0.025</td>
<td>B</td>
<td>7.425±0.045</td>
<td>C</td>
</tr>
</tbody>
</table>

S.E: Standard error. d.t: Duncan's multiple range test between groups. Means with the same letter are not significantly different. G1: Control group. G2: Group treated with Cadmium only. G3: Group treated with Cadmium + Vitamin E. G4: Group treated with Cadmium + selenium. The results showed decrease in total protein in rats treated with cadmium only in comparison with other groups and other criteria.

**Table 4:** Means ± S.E for the effect of selenium, Vit E and cadmium on serum Albumin concentrations (g/dl).

<table>
<thead>
<tr>
<th>Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
</tr>
<tr>
<td>G1</td>
<td>4.270±0.010</td>
<td>a</td>
<td>4.635±0.085</td>
<td>A</td>
<td>4.735±0.015</td>
<td>a</td>
</tr>
<tr>
<td>G2</td>
<td>3.240±0.010</td>
<td>d</td>
<td>3.210±0.010</td>
<td>C</td>
<td>3.075±0.035</td>
<td>d</td>
</tr>
<tr>
<td>G3</td>
<td>4.155±0.005</td>
<td>b</td>
<td>4.305±0.035</td>
<td>B</td>
<td>4.510±0.010</td>
<td>b</td>
</tr>
<tr>
<td>G4</td>
<td>3.945±0.035</td>
<td>c</td>
<td>4.160±0.020</td>
<td>B</td>
<td>4.265±0.035</td>
<td>c</td>
</tr>
</tbody>
</table>

S.E: Standard error. d.t: Duncan's multiple range test between groups. Means with the same letter are not significantly different. G1: Control. G2: Cadmium. G3: Cadmium + Vitamin E. G4: Cadmium + selenium. The results showed decrease in albumin in rats treated with cadmium only in comparison with other groups and other criteria.

Elgaml and Hashish. (2014) reported highly significant decrease in serum total protein and albumin levels while non-significant change noticed on globulin level in the cadmium chloride (CdCl₂) treated
group of rats. This was reflected on a significant decrease in the albumin globulin ratio (A/G ratio). These results may be due to the impaired liver function or protein synthesis as a result of damage in hepatic cells. The decrease in A/G ratio may be due to a great reduction in albumin, which cannot be compensated by the serum globulin level. Oyinloye et al. (2016) found significant decrease in serum total protein and albumin of animals exposed to cadmium. This can be attributed to impairment in hepatocyte functions causing decrease of cytochrome P-450 activity and inhibition of protein metabolism in the liver. Ghonim et al. (2017) suggested that reduction in serum total protein and albumin, possibly due to the reduced ability of liver to synthesize proteins because of cadmium insult. The reduction in serum total protein and albumin levels indicate that cadmium caused poor liver function that impaired synthesis of albumin in the liver which clearly appeared in histopathological examination of liver. Meanwhile, treatment with vitamin E and selenium can repair or treat the disorder of liver caused by cadmium. El-Demerdash et al. (2004) revealed that treatment of rats with cadmium chloride (CdCl₂) caused a significant (P < 0.05) decrease in plasma total protein (TP) and albumin (A), while globulin (G) and A/G ratio were not affected. Administration of β-carotene, vitamin E and/or their combination did not cause any significant change in biochemical parameters, while alleviated the harmful effects of CdCl₂. Layachi and Kechrid (2012) found a significant decrease in serum total protein and albumin. The decrease in serum total protein and albumin due to cadmium treated mice might refer to changes in protein synthesis and/or metabolism. While the supplementation of vitamin E or vitamin C protected liver function from cadmium intoxication as indicated by the significant restoration of serum total protein and albumin. Mohamed et al. (2014) reported that hypoproteinemia and hypoalbuminemia could attributed to liver damage and proteinuria as renal dysfunction is common in laboratory rats had Cd toxicity. While Se ameliorate Cd hepatotoxicity in Cd and Se treated group.

Table (6) showed that treatment of rats with cadmium for 2, 4, 6, 8, 10 and 12 weeks significantly (P ≤ 0.05) increased serum urea as compared with the control group. While treatment of rats with cadmium + vitamin E and cadmium + selenium for 2, 4, 6,8,10 and 12 weeks significantly (P ≤ 0.05) decreased serum urea as compared with the cadmium group.

The study of kidney dysfunction was performed by estimating blood urea levels, which significantly increased in the group exposed to cadmium, suggesting renal dysfunction and oxidative damage in comparison to the control group. Meanwhile, histological examination revealed that Cd caused dilated, necrosis and congested interstitial blood vessel with thickened muscular wall. Nowadays there is no doubt that Cd induces kidney toxicity and cell death. The mode of cell death is typically apoptosis. Moneim and Ghafeer (2007) reported that levels of plasma creatinine and urea were significantly increased after cadmium treatment compared to the control group, indicating the impairment in kidney function. Ghonim et al. (2017) recognized a toxic effect of Cd on the kidney.

| Table 5: Mean ± S.E for the effect of selenium, Vit E and cadmium on serum globulin concentrations (g/dl). |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | 2 weeks                     | 4 weeks                     | 6 weeks                     | 8 weeks                     |
| Treatment                  | mean± S.E                   | mean± S.E                   | mean± S.E                   | mean± S.E                   |
|                            | d.t                          | d.t                          | d.t                          | d.t                          |
| G1                         | 3.285±0.005                 | 3.265±0.025                 | A                            | 3.245±0.005                 |
|                            | 3.195±0.015                 | 3.175±0.025                 | a                            | 3.165±0.025                 |
|                            | 3.215±0.004                 | 3.195±0.020                 | ab                           | 3.170±0.020                 |

S.E: Standard error. d.t: Duncan’s multiple range test between groups. Means with the same letter are not significantly different. G1: Control group. G2: Group treated with Cadmium only. G3: Group treated with Cadmium + Vitamin E. G4: Group treated with Cadmium +selenium. The results showed decrease in globulin in rats treated with cadmium only in comparison with other groups and other criteria.

Serum Urea levels:

The results showed increase in urea in rats treated with cadmium only in comparison with other groups and other standard Table (6) showed that treatment of rats with cadmium for 2, 4, 6, 8,10 and 12 weeks significantly (P ≤ 0.05) increased serum urea as compared with the control group. While treatment of rats with cadmium + vitamin E and cadmium + selenium for 2, 4, 6,8,10 and 12 weeks significantly (P ≤ 0.05) decreased serum urea as compared with the cadmium group.

The study of kidney dysfunction was performed by estimating blood urea levels, which significantly increased in the group exposed to cadmium, suggesting renal dysfunction and oxidative damage in comparison to the control group. Meanwhile, histological examination revealed that Cd caused dilated, necrosis and congested interstitial blood vessel with thickened muscular wall. Nowadays there is no doubt that Cd induces kidney toxicity and cell death. The mode of cell death is typically apoptosis. Moneim and Ghafeer (2007) reported that levels of plasma creatinine and urea were significantly increased after cadmium treatment compared to the control group, indicating the impairment in kidney function. Ghonim et al. (2017) recognized a toxic effect of Cd on the kidney.
Cadmium increased the levels of serum creatinine and urea. Alteration in the kidney function probably regarded to the oxidative damage induced by Cd, since Cd impaired the glomerular filtration, so that creatinine and urea accumulated in the blood. Renugadevi and Prabu (2008) reported that urea is the major nitrogen-containing metabolic product of protein metabolism. Uric acid is the major product of purine nucleotides, adenosine and guanosine. It is well established that cadmium inhibits the incorporation of amino acid into protein causing an increase in urea level. Mehana (2008) reported that rats treated with cadmium chloride and vitamin E showed marked improvement in both biochemical and histological changes comparing to rats treated with cadmium alone as a significant reduction in levels of AST, ALT and urea were occurred, in comparison with cadmium treated group. Obianime and Roberts (2009) found that vitamin C, E and Selenium, individually and collectively, caused inhibition of increasing the effect of cadmium or changes in phosphatase, urea creatinine and hormonal parameters thus reversing histological distortions in the liver, kidney and testis of the male Wistar rats. Wang et al. (2013) showed a significant increase in level of blood urea nitrogen (BUN) and creatinine in cadmium administrated mice group. Treatment with selenium, however, could prevent Cd to induce increasing level of serum and urine biochemical parameters. Mohamed et al. (2014) reported that urea and creatinine in the Cd and Se treated groups were none significantly changed when compared with the control group. There is evidence that Se ameliorate the renal damage in Cd intoxicated rat by reducing the urea and creatinine serum level.

<table>
<thead>
<tr>
<th>Time</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
<td>mean±S.E</td>
<td>d.t</td>
</tr>
<tr>
<td>G2</td>
<td>64.065±1.285</td>
<td>a</td>
<td>64.950±1.850</td>
<td>a</td>
<td>67.355±2.895</td>
<td>a</td>
</tr>
<tr>
<td>G3</td>
<td>29.495±1.285</td>
<td>b</td>
<td>30.020±2.050</td>
<td>c</td>
<td>29.700±0.520</td>
<td>c</td>
</tr>
<tr>
<td>G4</td>
<td>32.190±0.360</td>
<td>b</td>
<td>37.375±1.045</td>
<td>b</td>
<td>38.215±3.415</td>
<td>b</td>
</tr>
</tbody>
</table>

S.E: Standard error. d.t: Duncan’s multiple range tests between groups. Means with the same letter are not significantly different. G1: Control group. G2: Group treated with Cadmium only. G3: Group treated with Cadmium + Vitamin E. G4: Group treated with Cadmium + selenium. The results showed increase in urea in rats treated with cadmium only in comparison with other groups and other criteria.

**Conclusion**

This study demonstrates that exposure to cadmium either provoke oxidative liver and kidney; cause testes injury by inducing lipid peroxidation or changes biochemical parameters of rats. However, vitamin E or selenium treatment may have protective effects on the disturbances caused by cadmium toxicity due to activities of the antioxidant enzymes that ameliorate biochemical parameters.

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