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Research paper

Protective effects of *Syzygium aromaticum* oil (Clove) against acrylamide induced hepatic, renal, and testicular toxicity in rats

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Abstract

This study aimed to investigate the possible protective role of clove oil against acrylamide induced oxidative damage and impairment of liver, kidney, and testicular functions in albino rats. The apparent oxidative damage was associated with evident hepatic, renal, and testicular dysfunction, which was confirmed in histopathological lesions, and increased serum aspartate aminotransferase and alanine aminotransferase activities. Acrylamide decreased serum total protein and albumin contents; increased urea and creatinine contents. Acrylamide also reduced testosterone concentration. Treatment of acrylamide intoxicated rats with clove oil minimized liver, kidney, and testicular histopathological changes and normalized their functions. Our findings demonstrate that acrylamide is not only associated with hepatotoxicity but also nephrotoxicity and testicular toxicity. Clove oil administration provided substantial organ protection against hepatic, renal, and testicular dysfunction induced by acrylamide, which was possibly mediated through their antioxidant activities.

Keywords: *Acrylamide,Toxicity, Clove oil, Antioxidant activity, Rats.*

1. Introduction

Acrylamide is an odorless, white crystalline solid at room temperature and has chemical formula: C_3H_5NO and structure $H_2C=CHCONH_2$, and it is a reactive water soluble vinyl monomer (Taha et al., 2013). Acrylamide occurs as a toxicant in carbohydrate rich foods cooked at high temperature, produced through maillard reaction between amino acids, especially asparagine and reducing sugars such as glucose or fructose (Obón-Santacana et al. 2017). The orally consumed acrylamide is absorbed into the general circulation, then distributed to different organs and reacts with DNA, neurons, essential enzymes and hemoglobin and causes different toxic effect (Rayburn and Friedman, 2010). Acrylamide and polyacrylamide used in many applications such as: plastic, aesthetic surgeries, cosmetic industries, opthalmic operations, laboratory processes and oil recovery processes (Schwend et al. 2009).

Scientific application of acrylamide as: water purification, gel electrophoresis and sewage treatment for agricultural sprays, textile printing paste and water retention aids, adhesive and binders for seed coating, monomer acrylonitrite used as a raw material in many manufacturing of acrylic fiber, plastics and synthetic rubbers (Woutersen 1998). Due to acrylamide exposure damage of biological macromolecules and disruption of normal metabolism leads to oxidative stress and imbalance in antioxidant activity (Trevisan et al. 2001).

Medicinal plants and natural herbal products are used as chemotherapeutic agents to provide protection against toxic side effects due to their antioxidant activity (Katrin, 2012). *Syzygium aromaticum* (cloves) are the aromatic dried flower buds of a tree belonging to the family *Myrtaceae*(Mehmet et al. 2007). The essential oil isolated from clove is widely used due to their medicinal properties (Ramadan et al. 2013). Clove considered one of the richest plants of phenolic compounds and has great potential for pharmaceutical, food, cosmetic and agricultural uses. Meanwhile flavonoids are able to counteract the damaging effects of oxidative stress and decrease xenobiotic induced liver toxicity in animals, cooperating with natural systems like endogenous protecting antioxidant enzymes, clove shows antioxidant properties and its extracts could be used as food antioxidants (Kadarian et al. 2002). So this experimental study was conducted to evaluate the protective effect of clove oil against experimentally hepato-renal and testicular toxicity induced by acrylamide in male albino rats.

2. Materials and methods

2.1. Acrylamide

Acrylamide, it was obtained from Sigma-Aldrich (St Louis, Missouri, USA), is available in the form of white powder (99% purity). Rats were orally administered with acrylamide in a dose of 20 mg/kg b.wt once daily for 21 days by stomach tube (Rahangadale et al. 2012).

2.2. Clove oil

Clove oil was obtained from El-Captain Company for extracting natural oils, herbs, and cosmetics, El-Obour City, Cairo, Egypt.It was administered at two doses (100 and 200 mg/kg b.wt orally once daily for 21 days by stomach tube (El-Hadary and Ramadan-Hassanien, 2016).

2.3. Experimental animals

Twenty five adult male Wistar albino rats (185-210 g) were obtained from the laboratory animal's center of Faculty of Veterinary

Medicine, Benha University, Egypt. Rats were housed on standard pellet diet and tap water ad libitum and rats were kept in plastic cages. Rats were acclimatized to the environment for two week before the beginning of the experiment. All rats received standard laboratory balanced commercial diet and water *ad libitum*.

2.4. Experimental design

The rats were randomly grouped into five groups (each of 5 rats).
Group 1: Rats which served as the control was orally administered corn oil as vehicle.
Group 2: Rats in this group were served as acrylamide toxic control and were administered an oral dose of acrylamide (20 mg/kg b.wt.).
Group 3: Rats in this group were received clove oil (200 mg/kg b.wt.).
Group 4: Rats in this group were received both clove oil (100 mg/kg) and acrylamide (20 mg/kg b.wt.).
Group 5: Rats in this group were received both clove oil (200 mg/kg) and acrylamide (20 mg/kg b.wt.).
All groups were administrated orally by stomach tube once daily for 21 consecutive days and kept under observation all over the duration of experiment. At the end of experiment blood samples were collected through direct heart puncture in sterile test tubes without anticoagulant for separation of serum which kept at -20^{o}C until biochemical analysis and then all rats were euthanized. For Histopathological studies, liver, kidney and testes were collected and fixed in 10% buffered neutral formalin solution.

2.5. Biochemical analysis

Aspartate aminotransferase (AST) and Alanin aminotranseferase (ALT) in serum were determined by method described by Murray and Kaplan (1984). Total protein concentration in serum was determined according to Koller (1984), albumin concentration in serum was determined according to Doumas et al. (1971), Creatinine concentration in serum was determined by the method described by Murray (1984) and urea concentration in serum was determined according to Kaplan (1984) using all kits from Diamond Company.

2.6. Measurement of antioxidant enzyme activity

Lipid Peroxide (Malondialdehyde) (MDA) was determined by the reaction described by Ohkawa et al. (1979). Superoxide dismutase (SOD) was performed according to the method of Nishikimi et al. (1972).

2.7. Measurement of testosterone

Testosterone determination using ELISA explained by Ekins (1998).

2.8. Histopathological studies

Autopsy samples were taken from the liver, kidney and testes of rats in different groups and fixed in 10% formalin for 24 hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 C in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparffinized (xylene) and stained by hematoxylin and eosin stains (Banchroft et al. 1996) for histopathological examination by light microscope.

2.9. Statistical analysis

The results were expressed as mean ± SE of studied groups using the analysis of variance test (one way ANOVA) followed by Duncan's multiple range test to determine the differences between the averages. All analysis were performed by Statistical Package for Social science Software (SPSS (16) software (SPSS Inc., Chicago, USA).

3. Results

This study was conducted to evaluate the protective effect of Clove oil against experimentally-induced acrylamide toxicity in rats. The concentrations of the liver enzymes (AST & ALT) in serum were significantly increased in acrylamide treated group compared to control group (group I), while in groups (acrylamide + Clove oil 100 mg/kg & acrylamide + Clove oil 200 mg/kg) Liver enzymes (AST & ALT) concentrations were significantly decreased compared to acrylamide treated group and these results were shown in Table (1) . The concentrations of the total protein in serum were significantly decreased in acrylamide treated group compared to control group. While in groups (acrylamide + Clove oil 100 mg/kg & acrylamide + Clove oil 200 mg/kg), total protein concentrations were decreased and this significantly decrease was in (acrylamide + Clove oil 200 mg/kg) compared to acrylamide treated group and these results were shown in Table (1). The concentrations of albumin in serum were significantly decreased in rats treated with acrylamide compared to control group. In groups (acrylamide + Clove oil 100 mg/kg & acrylamide + Clove oil 200 mg/kg), albumin concentrations were increased toward the normal values and these results were shown in Table (1). Values are mean ± SE. Means with different alphabets as superscripts differ significantly ($P<0.05$)
The concentrations of the creatinine and urea in serum were significantly increased in acrylamide treated group compared to control group. In groups (acrylamide + Clove oil 100 mg/kg & acrylamide Clove oil 200 mg/kg), creatinine concentrations were significantly decreased in compared to acrylamide treated group and these results were shown in Table (2). The concentrations of MDA in serum were significantly increased in acrylamide treated group compared to control group. In groups (acrylamide + Clove oil 100 mg/kg & acrylamide + Clove oil 200 mg/kg), MDA concentrations were significantly decreased compared to acrylamide treated group and these results were shown in Table (2). The concentrations of SOD in serum were significantly decreased in acrylamide treated group compared to control group. In groups (acrylamide + Clove oil 100 mg/kg & acrylamide + Clove oil 200 mg/kg), SOD concentrations were increased toward the normal values and these results were shown in Table (2). The concentrations of testosterone in serum were significantly decreased in acrylamide treated group in compared to control group. In clove oil 200 mg/kg, significant decrease in testosterone concentrations compared to control group. In groups (acrylamide + Clove oil 100 mg/kg, significant decrease in testosterone concentrations compared to control group and significant increased compared to acrylamide treated group & acrylamide+ Clove oil 200 mg/kg), testosterone concentrations were decreased compared to control and significant decreased compared to acrylamide treated group these results were shown in Table (2).

Table 1: Effect of Clove Oil and/or Acrylamide on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) Concentration (U/L), T. Protein Concentrations (mg/dl)and Albumin Concentration (mg/dl) in Serum of Rats (n=5)

Groups	ALT (U/L)	AST (U/L)	Total protein (mg/dl)	Albumin (mg/dl)
Control Group (Corn oil)	117.62±3.61^{c}	52.61±3.57^{c}	6.55±0.24^{a}	4.92±0.25^{a}
Acrylamide treated (20 mg/kg b.wt)	262.58±21.50^{a}	98.18±2.25^{a}	5.10±0.23^{c}	3.47±0.17^{c}
Clove oil (200 mg/kg b.wt)	146.22±6.31^{c}	49.13 ±2.76^{c}	6.37±0.32^{ab}	4.52±0.35^{ab}
Acrylamide (20 mg/kg b.wt) and Clove oil (100 mg/kg b.wt)	227.76±17.94^{ab}	68.43±4.27^{b}	5.65±0.17^{bc}	3.87±0.33^{bc}
Acrylamide (20 mg/kg b.wt) and Clove oil (200 mg/kg b.wt)	199.94±13.06^{b}	59.42±3.15 ^{bc}	6.04±0.25^{ab}	3.99±0.17^{b}

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05).

Table 2: Effect of Clove Oil and/or Acrylamide on Creatinine Concentration (mg/dl) , Urea Concentration (mg/dl), Testosterone Concentration (ng/ml), Malondialdehyde (MDA) Activity (nmol/ml) and Superoxide Dismutase (SOD) Activity (U/ml) in Serum of Rats (n=5)

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Testosterone (ng/ml)	MDA (nmol/ml)	SOD (U/ml)
Control Group (Corn oil)	0.89±0.05^{c}	51.72± 0.3^{bc}	3.97 ±0.18^{a}	8.60±0.54^{c}	108.75± 4.2^{a}
Acrylamide treated (20 mg/kg b.wt)	1.78±0.07^{a}	83.93± 3.57^{a}	1.58 ±0.16^{d}	15.09± 1.36^{a}	36.75±2.69^{c}
Clove oil (200 mg/kg b.wt)	0.91±0.05^{c}	46.7 ± 3.01^{c}	2.90 ±0.05^{c}	7.11±0.49^{c}	97.75±8.05^{ab}
Acrylamide (20 mg/kg b.wt) and Clove oil (100 mg/kg b.wt)	1.05±0.06^{bc}	60.07 ± 2.9^{b}	3.56 ±0.03^{b}	11.61± 0.34^{b}	87.65±2.94^{b}
Acrylamide (20 mg/kg b.wt)and Clove oil (200 mg/kg b.wt)	1.24±0.09^{b}	74.36 ± 5.7^{a}	1.17 ±0.02^{e}	12.27± 0.65^{b}	92.3±1.81^{b}

Values are mean ± SE. Means with different alphabets as superscripts differ significantly (P<0.05)

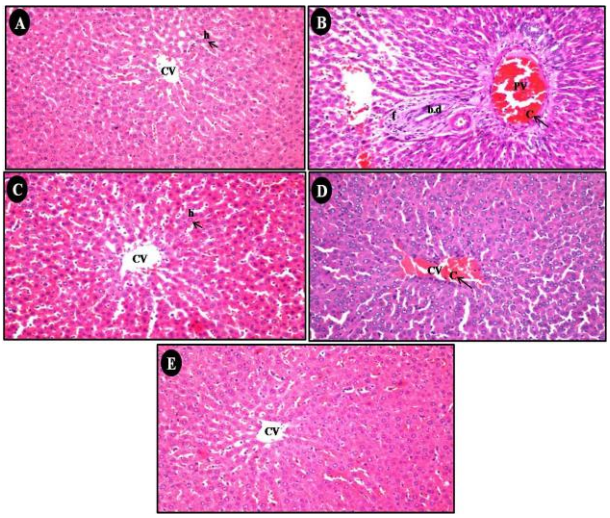

Fig. 1: Histopathological Changes in Liver after Treated with Clove Oil and/or Acrylamide (H&E).

A. Normal liver section from control rat shows no histopathological alteration and the normal histological structure of the central vein (CV) and surrounding hepatocytes (h) in the parenchyma (X10).
B. Acrylamide treated rats showed, sever dilatation and congestion (C) were detected in the central and portal veins vein (PV) associated with periductal fibrosis (f) surrounding the bile ducts (b.d) at the portal area.
C. Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration.
D. Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed mild congestion (C) was observed in the central vein (CV).
E. Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration.

The histopathological changes in liver were shown in Figure (1). Normal liver section from control rat shows no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (Figure 1A). Acrylamide treated rats showed, sever dilatation and congestion were detected in the central and portal veins vein associated with periductal fibrosis surrounding the bile ducts at the portal area (Figure 1B). Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration (Figure 1C). Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed mild congestion was observed in the central vein (Figure 1D). Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration (Figure 1E).

The histopathological changes in kidney were shown in Figure (2). Normal kidney section from control rat showed no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex (Figure 2A). Acrylamide treated rats showed, sever congestion in the cortical blood vessels (Figure 2B). Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration (Figure 2C). Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed Congestion was detected in the tufts of some individual glomeruli (Figure 2D). Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration (Figure 2E).

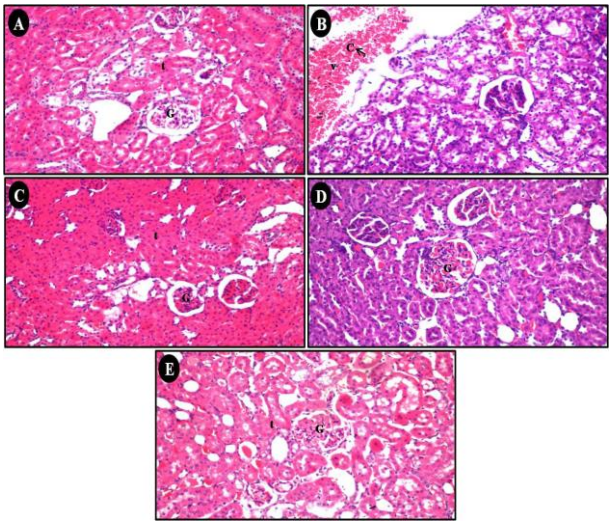

Fig. 2: Histopathological Changes in Kidney after Treated with Clove Oil and/or Acrylamide (H&E).

A. Normal kidney section from control rat showed no histopathological alteration and the normal histological structure of the glomeruli (G) and tubules (t) at the cortex.
B. Acrylamide treated rats showed, sever congestion (C) in the cortical blood vessels (v).
C. Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration.
D. Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed congestion was detected in the tufts of some individual glomeruli (G).
E. Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration.

The histopathological changes in testes were shown in Figure (3). Normal testes section from control rat showed no histopathological alteration and the normal histological structure of the mature

active seminiferous tubules with complete spermatogenic series (Figure 3A). Acrylamide treated rats showed, degeneration with lose of spermatogenic series were observed in some individual seminiferous tubules (Figure 3B). Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration (Figure 3C). Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed Oedema with congested blood vessels were noticed in the interstitial stromal tissue between the seminiferous tubules (Figure 3D). Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration (Figure 3E).

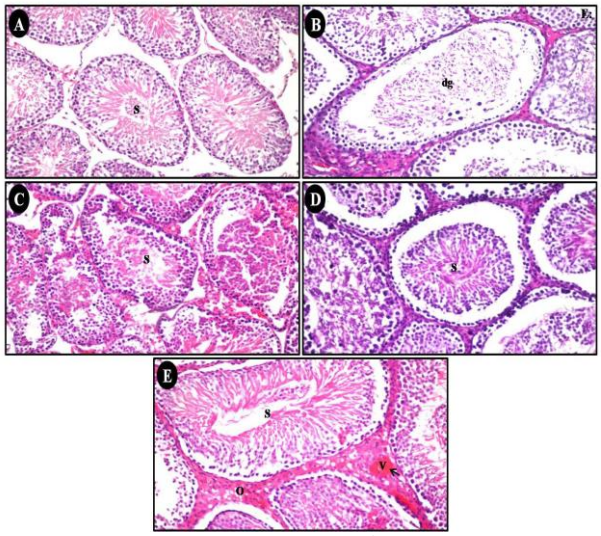

Fig. 3: Histopathological Changes in Testes after Treated with Clove Oil and/or Acrylamide (H&E).

A. Normal testes section from control rat showed no histopathological alteration and the normal histological structure of the mature active seminiferous tubules (S) with complete spermatogenic series.
B. Acrylamide treated rats showed, degeneration (dg) with lose of spermatogenic series were observed in some individual seminiferous tubules.
C. Clove oil (200 mg/kg b.wt) treated rats showed, no histopathological alteration.
D. Acrylamide + Clove oil (200 mg/kg bwt) treated rats showed no histopathological alteration.
E. Acrylamide + Clove oil (100 mg/kg bwt) treated rats showed oedema (O) with congested blood vessels (v) were noticed in the interstitial stromal tissue between the seminiferous tubules (S).

4. Discussion

Clinical chemical analysis is an essential tool used in human and veterinary medicine for diagnosis and prediction the outcome of disease (Smith and Reynard 1992).Little information about the effect of medicinal plants as clove on metabolism and biochemistry following hepatic and renal toxicity was recorded. Screening studies must be carried out to evaluate the possible protective and drawbacks of some medicinal plants that will aid in therapeutic or prophylactic regimens of these plants and changing the outlook towards treatment measures in the future. This work done for studying the effect of clove oil on hepatic, renal and testicular injury induced by acrylamide in albino rats.

4.1. Biochemical effects of clove oil and/or acrylamide on liver

Alterations in serum levels of hepatic transaminases (AST and ALT) were used as markers for liver damage and disease. In our study, there was a significant increase in AST and ALT levels in acrylamide administered rats. Clove oil administration ameliorated both liver and kidney changes confirming the protective role of clove oil against hepatic toxicity induced by acrylamide overdose. The effect of acrylamide administration on AST and ALT levels were in accordance with (Sawk et al. 2007; Sharma and Jain 2008; Gabr et al. 2010; Teodor et al. 2011). The present results for the effect of acrylamide upon the serum liver enzymes activity also agreed with Siahkoohi1 et al. (2014) and Osman et al. (2016) who reported a significant increase in serum AST and ALT levels in acrylamide -treated adult male rats compared with control.

The elevation in serum AST and ALT activities may be resulted from the increase permeability of hepatocyte cell membrane which may occur due to the toxic effect of acrylamide which cause cellular damage and in turn leakage of the enzymes outside the cellular structure to extracellular fluid. The elevation of serum AST and ALT activities may occurred due to the bipolar nature of acrylamide the CH2=CH-part may undergo hydrophobic interaction and the $–CONH_2$ part can form hydrogen bonds with the cell component, this property may enhance its ability to alter the structure of cell membarne structure and make the paranchymal cell membrane of the liver more permeable by causing the active retention of enzymes and making them appear first in the extracellular space and then in the blood (Chinoy and Memon 2001).

Significant decreases of the total protein and albumin levels reported in the current study after exposure to Acrylamide are going in line with study by Hammad et al. (2013) reported significant decreases of the total protein and albumin, levels following acrylamide administration in rats. The hypoproteinaemia in rats given different concentrations of acrylamide might have resulted from hepatocellular dysfunction. Evidence of liver damage was characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration. Acrylamide caused a significant decrease in serum total protein and total albumin (Khalifa et al. 2016). A steady decrease in hepatic protein levels with higher doses of acrylamide could be attributed to retarded protein synthesis, change in protein metabolism, or to the leakage of protein reserves from hepatocytes (Asha et al. 2008).

4.2. Biochemical effects of clove oil and/or acrylamide on kidney

The levels of urea and creatinine in serum are used as indicators for determination of kidney functions; however, concentration of serum creatinine is a more effective indicator than urea concentration at the initial stages of kidney diseases (Donadio et al. 1997). Evaluation of serum chemistries, including blood urea nitrogen, creational and uric acid is required to confirm renal dysfunction (Susan and May 1998). From our results concerning the effect of acrylamide on serum urea and creatinine, a significant increase were noticed in both urea and creatinine. These results were confirmed by Alturfan et al. (2008) who reported the relationship between cancer of the bladder and kidney with acrylamide intake. Also agreed with Teodor et al. (2011) who reported that acrylamide toxicity caused a significant increase in creatinine, urea and uric acid indicating kidney toxicity. Consequently, it was determined in this study that acrylamide, having some negative effects on health, significantly increased serum urea and creatinine levels of male and female rats at low dosages and for long time intake (Yener et al. 2016).

4.3. Antioxidant effect of clove oil

Acrylamide caused significant increases in MDA and decrease of SOD activity due to the oxidative stress induced by acrylamide (Mohamed-Sadek. 2012).Acrylamide is able to increase lipid peroxidation by inducing oxidative stress with generation of free radicals (Jiazhong et al. 1998). These results are in agreement with other reports that showed an increase in lipid peroxidation in brain and liver upon administration of acrylamide (Srivastava et al. 1983).

4.4. Biochemical effects of clove oil and/or acrylamide on testes

Testis is a target organ of acrylamide action as it caused severe damage in seminiferous tubules and caused decrease in plasma free and total testosterone in a dose dependent manner (Drury and Wallington, 1980). Testosterone is a steroid hormone regulates sperm production within the testes. It is produced by interstitial cells and its secretion is regulated by pituitary hormone (Karen and Thomas, 1996)

In the present study testosterone level in sera of rats treated with acrylamide significantly decreased, and co-treatment with clove oil elevate this decrease. This result is in consistence, other authors found that administration of acrylamide caused a significant reduction of serum testosterone level (Yassa et al. 2013). This significant reduction of testosterone may be a result from the direct damage of acrylamide to the Leydig cells (Song et al. 2008). Moreover, acrylamide may alter the androgen biosynthesis of interstitial cells in the testes (Fowler et al. 1987) or induces the enzymes activity of hepatic biotransformation, which is capable of metabolically transforming androgens into products with low androgen receptor binding activity (Sonderfen et al. 1987). The reduction in serum testosterone was accompanied by the histopathologicaly changes that represented by congestion and interstitial edema, necrosis, calcification and degeneration of spermatogenic cells in the seminiferous tubules with formation of spermatid gaint cells (Elghaffar et al. 2016)

In the present study, treatment with clove oil along with acrylamide resulted in moderate attenuation of the histopathological changes in testes that induced by acrylamide. These findings are supported by Tajuddin et al. (2003) who indicated that the 50% ethanolic extract of clove produced a significant and sustained increase in the sexual activity of normal male rats, thus the resultant aphrodisiac effectively of the extract lends support claims for its traditional usage in sexual disorders.

4.5. Histopathological changes in liver, kidney and testes

Liver of acrylamide treated rats showed, sever dilatation and congestion were detected in the central and portal veins vein associated with periductal fibrosis surrounding the bile ducts at the portal area. These results were accorded with liver of rats treated with acrylamide showed frequent necrosis and bleeding indicated hypertrophy of nuclei, pyknotic nuclei, proliferation of sinusoidal bile duct and hemorrhage (Nagao et al. 2007). The vacuilation of the hepatocytes cytoplasm may be occurring due to progressive ischemia, hypoxia and accumulation of lipid in the hepatocytes (Moustafa and Abdul Hamid, 2007).

Kidney of acrylamide treated rats showed; sever congestion in the cortical blood vessels. The kidneys of acrylamide treated rats showed infiltration of few mononuclear inflammatory cells as well as degenerative changes of some cells lining the renal tubules, other cells showed necrosis. These findings may be due to the fact that kidneys are the way of excretion of acrylamide and its metabolites. These results were similar to those reported by Totani et al. (2007).

Testes of acrylamide treated rats showed; degeneration with lose of spermatogenic series were observed in some individual seminiferous tubules. Christina et al. (2017) mentioned that administration of varied doses of acrylamide to mature male Sprague-Dawley rats caused general distortion in the histoachitecture of the reproductive tissues of the animal models in a dose dependent manner relative to the control. The seminiferous tubules of the testes showed marked depletion of the spermatogenic series in the treated animals, while the epididymal tubules also showed focal areas of cell degeneration. These defects seen in this study could be due to alterations in the normal reproductive hormonal levels and is a clear indication that acrylamide may have the ability to impair the process of spermatogenesis and also may have the propensity to cause low sperm count (Zenick et al. 1986) which is a potent pointer to reduced fertility and/or infertility.

5. Conclusions

Clove act as natural antioxidants and effective in prevention of acrylamide induced oxidative stress. Clove would be used as dietary supplements for a beneficial application in protection against acrylamide toxicity.

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