14

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The Protective Effect of The Methanolic Extract of *Syzgium cumini* L Fruit on Kidney and Testes Tissue Damages induced by Carbon Tetrachloride

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THE PRESENT work investigated the antioxidant effects of *Syzgium cumini* L fruit (jambolan) methanolic extract against Carbon tetrachloride inducing kidney and testes tissue damages in Sprague–Dawley male rats. Twenty - four adult male rats were randomly distributed into 4 groups (six rats for each).Group 1 considered as the normal control; group 2 administered with CCl4 (1.0 ml/kg body weight) in olive oil (1:1, v/v); groups 3 and 4 were treated with jambolan methanolic extract (250 and 500 mg/kg body weight, respectively) + CCl4 (1.0 ml/kg body weight). The rats were treated with limiting doses of methanolic extracts from jambolan fruit continuously for 28 days followed by an administration of CCl4 on the 27t^{h} and 28th days. Rats of the control group (group 1) received an equal volume of olive oil. At the end of the experiment biological data were calculated, blood samples were taken, kidney and testes were collected, weighted. Serum was separated to biochemical analysis. Tissue lipid peroxidation (LPX) and activity of antioxidant enzymes in kidney and testes were also performed. From the results, it could be noticed that the CCl4 may cause high serum levels in kidney functions. TBARS level also significantly increased whereas the levels of antioxidant enzymes as SOD, and CAT decreased in the kidney and testes tissue in rats treated with CCl4. Methanolic extract from jambolan fruits successfully prevented different tissues in rats. Our study demonstrated that the methanolic extract of jambolan fruits contain rich amounts of natural antioxidants which could protect kidney and testes tissues against CCl4-induced oxidative stress.

Keywords: Kidney, Testes, Creatinine, Uric acid, Urea, Testosterone, Antioxidant .

Introduction

Lipid peroxidation initiated by free radicals is considered to be deleterious for cell membranes and has been implicated in a number of pathological situations. These results may be due to environmental pro-oxidant pollutants which induce free radical formation (Szymonik-Lesiuk et al., 2003).

Tissues as liver, kidney, heart, lung, brain and blood happened due to the free radical from carbon tetrachloride which caused poisoning (Dashti et al., 1989). The toxicity from CCl4 is dependent on formation of the trichloromethyl radical (CCl3•), which react with oxygen to give the trichloromethyl peroxyl radical more toxicity (CCl_3O_2•) (Behar-Cohen et al., 1996).

Carbon tetrachloride (CCl_4), an industrial solvent, induces acute and chronic renal injuries (Guven and Gulmez, 2003). Administration of CCl4 causes an increase in lipid peroxidation products and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney (Dogukan et al., 2003).

Carbon tetrachloride is broken by hepatic microsomal cytochrome P450 to trichloromethyl which react with glutathione and protein thiols as sulfhydryl groups and antioxidant enzymes as catalase and superoxide dismutase, eventually leading to different pathological changes (Cemek et al., 2010). The uses of natural antioxidants indicate the role of oxidative stress in CCl4-induced testes damage (Al-Olayan et al., 2014).

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Several researchers showed that the natural antioxidant may protect the testes and kidney against the free radical from lipid peroxidation and weakness of antioxidant case induced by CCl4 (Khan and Ahmed, 2009).

Jambolan Fruit is a good source of water soluble vitamins and free amino acids and like ascorbic acid, thiamine, and niacin like alanine, asparagine, tyrosine, glutamine and cysteine (Paul and Shaha, 2004).

Veigas et al. (2007) studied that the anthocyanin as natural antioxidant inhibits the iron ($FeSO_4$)-induced lipid peroxidation in the different tissues (rat brain, liver, liver mitochondria, testes and human erythrocyte ghost cells) *in vitro*. So, it would be important to confirm the antioxidant effect of Jamun fruits. Fruits contain many different natural antioxidant which are considered beneficial to human health as flavonoids compounds, phenolics acids, carotenoids and many vitamins, therefore, it decreases the risk of degenerative diseases by reduction of oxidative stress and for the inhibition of macromolecular oxidation (Kubola et al*.,* 2011).

"Jamun" is rich in natural antioxidant compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. Therefore, it is effective in the treatment of diabetes mellitus, inflammation, ulcers and diarrhea. Previous studies have shown it to possess chemopreventive, radioprotective and antineoplastic properties (Vikas et al., 2015).

This study was performed to study the effects of jambolan fruits on CCl4-induced oxidative stress to protect the kidney and testes in adult rats.

Materials and Methods

Materials

Jambolan Fruits were obtained from a private farm in Tanta Governorate . Sunflower oil and starch were purchased from the local market.

Casein, cellulose, vitamins & minerals, dextrin, L-cysteine, choline chloride, and Ccl4 were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt.

Twenty four male albino rats (Sprague Dawley strain) were obtained from the laboratory animal colony, Helwan, Cairo - Egypt. Weighting were approximately between (150-180g).

Kits used to determine uric acid, urea nitrogen, creatinine, sodium, potassium, LH, FSH, testosterone, are produced by Egyptian American Company for laboratory service and supplied by Alkan Company.

Methods

Plant collection

Jambolan fruits were collected in October 2017 from different farm sites at the Nawag - Elgharbia region (Egypt). The fruits were washed with clean water to remove all debris and sand. All fruits were ripe and blemish-free, the seeds were separated from their pulps and then dark dried for 5 days. The dried sample was ground to fine powder with electric grinder and stored for further analysis.

Chemical analysis of fruits pulp

Moisture, fat, protein, ash, crude fiber and tannins content were determined according to the method outlined in AOAC (2007). Total carbohydrates were determined by difference.

Preparation of Jambolan fruit extract (JFE)

Jambolan fruit extract (JFE) was prepared from freeze-dried Jamun fruit pulp using a methanol extraction method as described by Li et al. (2009) and Lil et al. (2009). The JFE was enriched for anthocyanin content. Briefly, freeze-dried whole fruit powder was sequentially and exhaustively extracted with cold hexane, followed by ethyl acetate, and then acidified methanol (0.1% hydrochloric acid). The extract obtained was reconstituted in water and enriched for anthocyanin content using adsorption chromatography on an XAD-16 Amber lite resin column. Water (5 L) was added to the column to elute sugars and acids, and followed with acidic methanol (0.1% hydrochloric acid) to afford a red-purple JFE. The anthocyanin content was determined using the pH differential method and was calculated as equivalents of cyanidin-3-glucoside, using the extinction coefficient of 26900 L cm−1 mg−1 and a molecular mass of 449.2 g/L (Li et al., 2009 andLil et al., 2009). Individual anthocyanins were identified by high performance liquid chromatography with ultraviolet (HPLC-UV) and tandem mass spectrometry (LC-MS/MS) methods (Li L et al., 2009). JFE contained 3.5% anthocyanins (as cyanidin-3-glucoside equivalents) which occur as diglucosides of five anthocyanidins/aglycons: delphinidin, cyanidin, petunidin, peonidin and malvidin (Li et al., 2009 and Lil et al., 2009).

Experimental design and animal groups

Adult male albino rats, *Sprague Dawley* strain, weighing (150-180g) were obtained from the laboratory animal colony, Helwan, Cairo - Egypt. The animals were kept in wire cages. The diet was introduced to the rats in special food cups to avoid scattering of food. Also water was provided to the rats. Food and water were provided ad-libitum and checked daily. Twenty four adult male albino rats were divided into two main groups: The first main group (6 rats) fed on basal diet as control negative group (C-ve). The second main group (18 rats) fed on basal diet for 26 days. At 27th and 28th all rats were administrated CCl4 by gavage (1ml/kgb.w) (Shah et al., 2017). This second main group was divided into 3 groups each group contained 6 rats as follows:

Group 1: positive control, rats had fed basal diet only (C+ve). Group 2: rats fed on basal diet and received jumbolan fruits extract (250mg/ kg). Group 3: rats fed on basal diet and received jumbolan fruits extract (500mg/kg) orally. At the end of experiments, all rats were fasted overnight sacrificed and the blood samples were collected, a part of blood was centrifuged to obtain the serum. Internal organs were collected and removed (kidney and testis), cleaned in saline solution, dried by filter paper and weighted. The testes and kidneys were rapidly taken on ice bags and frozen at – 18°C till used for assessment of lipid peroxidation and antioxidant activity in testicular and kidney tissue.

Biological evaluation

During the experimental period (28 day), the consumed diet was recorded everyday (feed intake) and body weight was recorded every week. Biological evaluation of the different diets was carried out by determination of body weight gain % (BWG %) feed efficiency ratio (FER) (Chapman et al., 1959).

Biochemical analysis

Serum creatinine (Faulkner and King, 1976), serum uric acid (Barham & Trinder, 1972. and Fossati et al., 1980) and urea nitrogen (Patton and Crouch, 1977) ,sodium and potassium according to Sonnen wirth and Jaret (1980) were determined . Serum testosterone concentration was determined according to Wilke (1987). Serum levels of FSH and LH were determined according to Loraine and Bell (1976).

Assessment of oxidant/antioxidant activity

Kidneys and testes were removed, homogenized and centrifuged at 10,000 rpm at 0°C for 20 min. The supernatant was used for estimation of different antioxidant levels by calorimetric method using spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam), Lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) methods (Esterbauer and Cheesman 1990), Superoxide dismutase (SOD) by method developed by Sun et al. (1989) and Catalase (CAT) by colorimetric assay (Aebi,1984).

Statistical analysis

Data were presented as means ± SD Statistical analysis of data were tested for significance using a one way analysis of variance (ANOVA) followed by Duncan's multiple range test using computerized SPSS program (Snedecor, and Cochran 1986). Values were considered significant at $P < 0.05$.

Results and Discussion

The exposure can come from the air, drinking water, foodstuffs and soil. It could also be from certain industrial sites where carbon tetrachloride is still used or where previously industrial contamination had occurred. It could be concluded that the increase of carbon tetrachloride may be due to environmental pollution. (ATSDR, 2005). To the best of our information, this is the first study to estimate the effects of jambolan to protect kidney and testes from CCl4. Therefore, in this study, we found that the fruits extract of *Syzgium cumini* L contains high amounts of antioxidant and anti-inflammatory and it could be used to protect kidneys and testes from CCl4-induced injury. The tested parameters as chemical composition, biochemical parameters and antioxidants enzymes in tissue of kidney and testes were determined in negative control rats and CCl4-induced injury rats and the results are tabulated.

Proximate analysis

Jambolan fruits were investigated on dry weight basis. The following parameters in Table 1 were determined for moisture, fat, crude protein, ash, total carbohydrate and crude fiber, the ratios were 82.01, 1.31, 1.56, 5.72, 6.97 and 2.43(g/ 100g DW), respectively. Jambolan could be considered as a very good nutrient. These results agree with Raza et al. (2003). Jambolan fruits contain many different natural antioxidants which are considered beneficial to human health as flavonoids compounds, phenolics acids, carotenoids and many vitamins, therefore, it

decreases the risk of degenerative diseases by reduction of oxidative stress and used for the inhibition of macromolecular oxidation (Kubola et al., 2011).

TABLE 1. Proximate composition of the jambolan fresh fruit pulp (g in 100 g pulp).

Constituent	Pulp
Moisture	82.01±0.04 g
Crude Protein	1.56±0.01g
Crude Fiber	2.43±0.01g
Fats/Oils	1.31±0.11g
Ash	5.72±0.017 g
Carbohydrates	6.97±0.7 g
pH	3.09±0.03
Food Energy g/calories	39.67±3.82

*Values are means ± standard deviation of three determinations (n=3)

*Energy was calculated by summation of (fat x 9 k cal) + (carbohydrate x4 k cal).

Effects on body weight gain and feed intake

Effects of fruits extract of *Syzgium cumini* L on (BWG%, FER and Feed intake) against carbon tetrachloride toxicity in rats are recorded in Table 2. Results revealed that protective group which fed on basal diet and received jambolan fruits extract (250mg/kg) & (500 mg/kg) then administrated ccl 4 after 28 days, improved the BWG %, FER and FI compared with the control positive group. The mean values for protective group received jambolan fruits extract (250mg/kg) and (500 mg/kg) (24.05±3.7, 10.91±.75 and 509.83±7.7 respectively) and (23.59±1.94, 9.6±1.07 and 506.16±10.4 respectively) compared to the control positive group 24.05±3.7, 10.91±.75 and 505.16±8.6, respectively. In the current study, the failure kidney and testes damage by CCl4 is induced in rats, the results showed that the BWG % and FER decreased compared to the negative control rats group. These results are consistent with Lee et al. (2007) who reported that the injection of CCl4 significantly decreased body weight gain and food intake. Moreover, Khan et al. (2012) found the CCl4 may cause liver failure groups in rats showing a significant reduction in body weight compared to the control negative rats which showed a significant increase in body weight gain and feed intake having received jambolan fruits extract (250mg/kg) & (500 mg/kg). This may be due to presence of polyphenols present in jambolan fruits which improved and protected liver cells against damage via increasing both the levels and activities of antioxidant enzymes in liver and kidneys according to Jachec et al. (2002) leading to improving the appetite and increasing feed intake.

TABLE 2. The effect of *Syzgium cumini L*extract on (BWG%, FER and Feed intake) against carbon tetrachloride toxicity in rats (n=6).

Parameters / groups	BWG%	FER	Feed intake (g)
Control (-)	31.16±2.20[a]	14.54±1.31[a]	510.83±8.6[a]
Control (+)	17.6±1.43[c]	8.19±.97[c]	505.16±8.6[a]
Fruits extract (250)	24.05±3.7[b]	10.91±.75[b]	509.83±7.7[a]
Fruits extract (500)	23.59±1.94[b]	9.6±1.07[b]	506.16±10.4[a]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

Effects of CCl4 on relative kidney and testes weight

Concerning relative kidney weight "as a part of body weight" as shown in Table 3, there was a significant increase in the relative weight of the kidney for CCl4 group (.806±.03) compared to the normal group (.630±.033). There was no difference in relative kidney weight in rats received jambolan fruits extract (250 mg/kg) and (500 mg/kg) (786±.06 and .776±.06 respectively compared to the control + v group (.806±.03). Also, there was a significant increase in the relative weight of testes for CCl4 group (1.20±.166) compared to the normal group (.943±.027). The obtained data agree with Lin et al. (2006) and Fang et al. (2007) showing that the injection of CCl4 in rats may cause liver fibrosis and lead to increase the weight of kidney. Lee et al. (2007) who reported that organs weights were significantly increased after injection with CCl4. The rats fed with jambolan fruits extract may be the lowest CCl4 toxicity and significantly improved testes weight than the CCl4 rats group which helps in reducing edema in the testes due to fluid accumulation (Al-Olayan et al., 2014).

TABLE 3. The effect of *Syzgiumcumini L*extract on relative kidney and testes weight against carbon tetrachloride toxicity in rats.

Parameters / groups	Kidney	Testes
Control (-)	.630±.033[b]	.943±.027[c]
Control (+)	806±.03[a]	1.20±.166[a]
Fruits extract (250)	.786±.06[a]	1.08±.075[b]
Fruits extract (500)	.776±.06[a]	1.07±.065[b]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

Effects on serum biomarkers related to kidney functions

It was obvious from Table 4 that Serum creatinine, Uric acid and urea concentrations were significantly higher in serum of rats with CCl4 (.625±.10, 5.56 ±.45 and 24.16±1.16 respectively) compared to the normal group. Rats received jambolan fruits extract (250mg/kg) & (500 mg/kg) + Ccl4 showed a significant decrease in createnine and uric acid concentration. Serum urea concentration was lower in the serum of rats given jambolan fruits extract (250mg/kg) & (500 mg/kg) with+ CCl4 (13.00±.89 and 14.15±.69 respectively) than the control +ve group (24.16±1.16) and close to the normal group. Toxic chemicals, certain drugs, infectious agents can induce damage to the kidney that ultimately leads to the imbalance of electrolyte (Rajakrishnan et al., 2017).

TABLE 4. The effect of *Syzgiumcumini L* extract on kidney functions against carbon tetrachloride toxicity in rats.

Parameters / groups	Createnine (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)
Control(-)	.286±.04[c]	2.25±.35[c]	14.53±1.82[b]
Control(+)	.625±.10[a]	5.56±.45[a]	24.16±1.16[a]
Fruits extract(250)	.400±.02[b]	3.78±.46[b]	13.00±.89[b]
Fruits extract (500)	.360±.02[b]	3.46±.05[b]	14.15±.69[b]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

These results agree with Jie-QiongMaa et al. (2018) who found that mice receiving CCl4 alone showed kidney injury as evidenced by elevation in serum biochemical markers, inflammation, caspase-3 activity and apoptosis in kidney, while jambolan fruit extracts were effective in reduction of blood urea, creatinine, and uric acid levels in rats with kidney damage induced by ccl4. These data agree with Chaudhary and Prasad (2014) who found that kidney and liver functions decrease significantly after Jamun wine consumption. This may be because jambolan fruit had high anthocyanin content which attributes to its antioxidant and free radical scavenging activity and a diuretics effect with an increase in glomerular filtration rate due to its antioxidant ability which strengthens the renal antioxidant system and eliminates oxidation reactions. The mechanism of the beneficial effect of anthocyanins on kidneys is undoubtedly heterogeneous. Anthocyanin decrease the inflammatory process (they inhibit cyclooxygenase-1 and-2) (Subarnas and Wagner, 2000), decontract blood vessels and improve microcirculation (Bertuglia, 1995).

Effects on serum electrolytes

A significant reduction in the mean value of serum Na and K for control (+) group occured; 144.83±2.99 and 6.8±.33 mmol/l, respectively, as compared to the control (-) group (155.50±3.01 and 8.13±.25 mmol/l, respectively) as a result of salt –losing nephritis. Also, there was a significant increase in the mean value of serum Na and K for treated groups received ccl4 (152.5±4.92, 152.5±4.92**,** 7.73±.98 and 7.16±.29 respectively) compared to the control (+) group.

TABLE 5. The effect of *Syzgiumcumini L* extract *on* Sodium and Potassium against carbon tetrachloride toxicity in rats.

Parameters / groups	Sodium (mmol/l)	Potassium (mmol/l)
Control (-)	155.50±3.01[a]	8.13±.25[a]
Control (+)	144.83±2.99[c]	6.8±.33[b]
Fruits extract (250)	152.5±4.92[a]	7.73±.98[a]
Fruits extract (500)	148.50±1.87[b]	7.16±.29[a]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

The administration of jambolan fruit significantly increased the serum sodium and potassium concentration toward normal values in CCl4-treated animals, which indicates the potentiality of jambolan fruit to overcome electrolyte imbalance.

Effects on the antioxidant enzymes kidney and renal TBARS levels

In aspect of kidney tissue estimation, it is shown as an increase in the lipid peroxidation (242±.03 n Mol/gm) and a decrease in the level of SOD (.113±.01) and CAT (.113±.01) for CCl4 group compared with the control (-) group (.082±.01, .220±.02and .224±.02 respectively). Rats received jambolan fruits extract (250mg/kg) & (500 mg/kg) with administrated Ccl4 showed a significant decrease in the lipid peroxidation (.123±.01 and.143±.01 and an increase in the level of SOD (.149±.02 &.180±.02 respectively) and CAT (.143±.02 and.232±.02 respectively) compared with positive control. Our data showed a significant decrease in antioxidant enzymes activities in all studied organ following acute exposure to CCl4. Ko et al. (1995) mentioned that the CCl_4 distributed and deposited to tissues as the liver, brain, kidney, lung and heart.The results showed that the conversion of CCl_4 by cytochrome P-450 to produce form trichloromethyl radical ($\bullet CCl_3$) and trichloromethyl peroxide radical ($CCl3O_2\bullet$). The greatest fraction of •CCl3 reacts very rapidly with O_2 and more reactive free radicals as CCl3OO• is produced from •CCl3. These free radicals may cause firstly the peroxidation poly unsaturated fatty acids (PUFA) for cell membrane, cell necrosis, GSH depletion, membrane damage and loss of antioxidant enzyme activity. The perturbations of these antioxidants can be explained by the high production of H_2O_2 and O_2 resulted by CCl_4 toxicity.The pretreatment with jambolan fruits extract can prevent the occurrence of oxidative damage. These findings are in line with those of Benherlal and Arumughan (2007), who estimated the natural antioxidant as DPPH•, OH•, O2•- and lipid peroxidation in the ethanolic extract of the fruit pulp, kernel and seed coat were evaluated as gallic acid, quercetin and trolox. The results showed that the kernel extract was the best in the DPPH scavenging assay and lipid peroxidation followed by the seed coat and pulp extract.

TABLE 6. The effect of *Syzgiumcumini L* extract on the lipid peroxidation and antioxidant enzyme of kidney.

Parameters / groups	n Mol MDA/gm. kidney tissue	Unit SOD /gm) kidney tissue	CAT gm) kidney tissue
Control(-)	.082±.01[c]	.220±.02[a]	.224±.02[a]
Control(+)	.242±.03[a]	.113±.01[d]	.113±.01[d]
Fruits extract(250)	.123±.01[b]	.149±.02[c]	.143±.02[b]
Fruits extract (500)	.143±.01[b]	.180±.02[b]	.232±.02[a]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

Effects on serum biomarkers related to testes functions

From the results in Table 7, it could be noticed that the mean values of serum hormones; testosterone, luteinizing hormone and follicle stimulating hormone after treatment of rats with CCl4, the mean values of testosterone, LH and FSH (15±.16, .85±.10 and 6.13±.45 ng/ml) decreased as compared to the control (-ve) group (4.75±.28, 1.56±.32 and 8±.28 ng/ml). In the treated group (jambolan fruits extract +CCl4), the results showed that the testosterone levels were restored to control values. From the statistically analysis it could be noticed that the levels of testosterone, LH and FSH were significantly increased in rats fed with jambolan fruits extract compared to the control (+ve) group.The mean values of testosterone, LH and FSH in the rats' after treatment with CCl4 decreased as compared to the control (-) group.Secretion of testosterone is probably impaired due to excessive oxidative stress and the degeneration of Leydig cells (Santos et al., 2004). The suprachiasmatic hypothalamic nucleus (SCN) might be affected by the toxic effects of CCl4 that may cause the failure of the pituitary gland to secrete FSH and LH and will result in testicular dysfunction leading to infertility (Khan et al., 2011). Treatment of rats with jambolan fruits extract ameliorated the toxic effects of CCl4 and the levels of testosterone, FSH and LH increased. Jambolan fruit contains polyphenolic anthocyanin derivatives, such as delphinidin-3,5-diglucoside and petunidin-3,5-diglucoside (Li et al., 2009), that inhibit cyclooxygenase activity, an enzyme that plays a key role in inflammation.

TABLE 7. The effect of *Syzgium cumini L*extract on Testosterone, LH and FSH against carbon tetrachloride toxicity in rats.

Parameters / groups	Testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control(-)	4.75±.28[a]	1.56±.32[a]	8±.28[a]
Control(+)	.15±.16[d]	.85±.10[c]	6.13±.45[c]
Fruits extract(250)	1.84±.41[b]	1.41±.24[a]	6.96±.21[b]
Fruits extract (500)	1.42±.36[c]	1.10±.34[cb]	6.65±.41[b]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant.

Effects on the antioxidant enzymes testes and TBARS levels

Oral administration of CCl4 to rats for 2 days significantly ($P<0.05$) increased the activity of the lipid peroxidase (MDA) and decreased the activity of superoxide dismutase (SOD), and catalase (CAT) enzymes in testes (.214±.02, .166±.01 and .158±.02 respectively) when compared to the negative control group (.073±.01, .166±.01 and .158±.02 , respectively). Pretreatments with jambolan fruits extract significantly ($P<0.05$) increased the activity of tissue SOD and CAT enzymes when compared with positive control group (Table 8). our data showed a significant decrease in antioxidant enzymes activities in all studied organ following acute exposure to CCl4. These findings are in line with those of Shereen et al. (2015) and Rahmouni et al. (2017). Ojo et al., (2016) and Shah & Khan (2017) found that the tested rat was exposure to CCl4, the results showed a significant decrease in CAT, POD, SOD and GPx profile, and also the GSH level was exhausted and TBARS was elevated. Moreover, higher production of H2O2 and nitrite in testes samples on account of CCl4 treatment suppresses the antioxidant defense while enhances the cellular injuries (Sahreen et al., 2013). Moreover, Veigas et al. (2007) found that the anthocyanin is rich in fruit peel extract which to free radical scavengers in the DPPH• scavenging assay. Zhang and Lin (2009) reported that the hydrolysable and condensed tannins in the fruit had contained highly amounts from antioxidant activity and DPPH radical scavenging. Whilst, Veigas et al. (2007) showed that the pulp extract is rich in anthocyanin which inhibition the lipid peroxidation in the different organs in rat brain, liver, liver mitochondria, testes and human erythrocyte but the degree of protection was variable.

TABLE 8. The effect of *Syzgiumcumini L* extract on the lipid peroxidation and antioxidant enzyme of testes.

Parameters / groups	n Mol MDA/gm. testes tissue	Unit SOD /gm) testes tissue	CAT gm) testes tissue
Control (-)	.073±.01[c]	.166±.01[b]	.158±.02[b]
Control (+)	.214±.02[a]	.071±.01[c]	.074±.01[c]
Fruits extract (250)	.125±.01[b]	.162±.02[b]	.158±.01[b]
Fruits extract (500)	.140±.03 [b]	.229±.02[a]	.237±.02[a]

Values denote arithmetic means ± SD of the mean. Means with different letters (a, b, c) in the same column differ significantly at (p 0.05) using one way ANOVA test, while those with similar letters are non-significant

Conclusion

From the obtained results, it could be concluded that the jambolan fruits extract has a protecting effect on CCl4-induced failure kidney and testes damage in rats due to its antioxidant properties. Therefore, it could be recommended that the jambolan fruits is beneficial in reducing tissue damage in patients exposed to toxic doses of CCl4.

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