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The protective effect of *N*-acetyl cysteine against carbon tetrachloride toxicity in rats

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Abstract

Background: Carbon tetrachloride (CCL4) is used as a solvent for oils and fats, as a refrigerant, and as a dry-cleaning agent. Inhalation of its vapors can depress central nervous system activity and cause degeneration of the liver and kidneys.

Aim: The aim of this study was to investigate whether *N*-acetyl cysteine (NAC) has a protective effect on the toxicity caused by carbon tetrachloride.

Methods: Male rats were used in this experiment. Animals were divided into five groups, 5 animals each: G1 received olive oil, G2 was treated with CCL4 (1 ml/kg body wt) dissolved in olive oil (1:1), G3 received NAC (300 mg/kg body wt), G4 received CCL4 plus NAC, and G5 received NAC for 1 week then administrated to the same treatment of G4. All administrations were performed by gavage and maintained for 4 weeks.

Results: The present study revealed that administration of CCL4 caused a significant increase in liver marker enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and alkaline phosphatase (ALP); also, malondialdehyde (MDA) content in the liver tissue was increased. However, serum total protein showed no change in all groups. Also, histopathological investigation of CCL4 on the liver, testis, and kidney showed different deleterious effects. Additionally, CCl4 induced a highly significant increase in the percentage of sperm shape abnormality and sperm DNA tail moment values. Alternatively, result revealed that the two routes of NAC administrations caused a significant decrease in liver marker enzymes as well as MDA contents; improvement in liver, kidney, and testes architecture; a significant decrease in the percentage of sperm head abnormalities; and decline in the DNA tail moment values.

Conclusion: The present study pointed out the protective effect of NAC against the toxicity of CCL4.

Keywords: *N*-Acetyl cysteine, Carbon tetrachloride, Rats, Liver, Testis and kidney

Background

Carbon tetrachloride (CCl4) is an agent that is commonly used in the dry-cleaning industry, is well known as a typical toxic agent, used to induce oxidative stress in experimental models (Nabeshima et al., 2006; Noori, Rehman, Qureshi, & Mahboob, 2009). A single dose of CCl4 can cause both oxidative stress and acute hepatic injuries (Khan & Alzohairy, 2011). This attributed to the conversion of CCl4 through hepatic microsomal cytochrome P450 into trichloromethyl-free radical ($\cdot CCl_3$ or $\cdot CCl_3OO$) (Stal & Olson, 2000) which sequentially initiate lipid peroxidation process causing injury to the cell plasma membrane and cell organelles, leading to the release of reactive aldehydes with initiation of both pro-inflammatory and pro-fibrotic process (Novo & Parola, 2012). The liver is not the only target organ of CCl4 toxicity, but there are other organs such as the kidney (Ozturk, Ucar, Ozturk, Vardi, & Batcioglu, 2003). CCl4 can lead to acute tubular necrosis in the kidney, lung (Ögetürk, Çolakoğlu, Kuş, Kuş, & Sarsılmaz, 2009), brain (Soliman & Fahmy, 2011), and testis (Abdel Moneim, 2016). Previous data demonstrated that CCl_4 induced decline in rat testicular size and weight and severe irregularities in the testicular architecture, including reduced tubular diameters, loss of the germinal line and reduction in cellular proliferation, degeneration of germ and Leydig cells along with distortions in spermatogenesis (Castilla-Cortazar, Garcia, Quiroga, Diez, & Diez-Caballero, 2000;

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Khan & Ahmed, 2009 and Abdel Moneim, 2016). Moreover, the free radicals of CCl4 bind with the unsaturated fatty acid of sperm plasma membrane forming alkoxy and peroxy radicals; as a result, lipid peroxides are produced, which are extremely reactive leading to alteration in sperm concentration, modification of the hormonal levels, and induction of necrosis (Ogeturk et al., 2005). Free radicals also induce decrease in GSH content, oxidative DNA damages, DNA adducts, DNA fragmentation, chromosomal aberrations and genetic mutations (Jia, Han, & Chen, 2002; Khan, Rizvi, Khan, Khan, & Shaheen, 2009 and Ahmed et al., 2014), germ cells necrosis, and degeneration in the testicular tubules (Guo, Lu, & Hsu, 2005 and Horn et al., 2006). On the other hand, *N*-acetyl cysteine (NAC), a potent antioxidant, has been utilized clinically for the treatment of many diseases (Rodrigues, Eiora, & Schaff, 2004; Akgun et al., 2005; Atkuri, Mantovani, Herzenberg, & Herzenberg, 2007; Sadowska, Manuel, & de Backer, 2007; Baker et al., 2009 and Samuni, Goldstein, Dean, & Berk, 2013). It is a small membrane permeable molecule that can rapidly permeate the intracellular sections. This antioxidant has a diversity of applications, mostly because of the reduced thiol moiety existing in its structure, which can scavenge reactive oxygen species (ROS) directly, and indirectly, NAC protects the liver by being hydrolyzed into cysteine; this, in turn, plays a significant role in the production of glutathione (Pereira-Filho et al., 2008) and enhancement of glutathione-*S*-transferase activity leading to the intracellular defense against oxidative stress and promotes detoxification (Aremu, Madejczyk, & Ballatori, 2008). Previous studies revealed the protective effects of NAC against CCl4-induced hepatotoxicity (Ritter, Reinke, Andrades, et al., 2004; Maksimchik, Lapshina, Sudnikovich, Zabrodskaya, & Zavodnik, 2008, Sahin and Alatas 2010), nephrotoxicity (Hanly et al., 2013 and Ustyol et al., 2017), and genotoxicity (Gurbuz, Ozkul, & Burgaz, 2009). Therefore, the present study was designated to investigate whether NAC has a protective effect on the toxicity of CCl4 by its antioxidant actions through biochemical, histopathological, and genetic investigations.

Methods

Chemicals and reagents

Carbon tetrachloride (CCL4) and *N*-acetyl cysteine (NAC) were obtained from Sigma Company. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphate, and total protein assays kits were purchased from bio-diagnostic company, EL-Doki, Giza, Egypt. All chemicals and reagents used were of analytical grade.

Animals

The present study was carried out using 25 male albino rats weighing 100–120 g which were purchased from the Egyptian Organization for Biological products and vaccines. Animals were housed in plastic cages and maintained in conditions of good ventilation, normal temperatures, and humidity ranges (55 ± 5%), a 12-h light-dark cycle. Rats were fed with standard pellets, containing all nutritive elements, drinking water ad libitum, and were acclimatized for 1 week prior to the treatment. All animal procedures were performed in accordance with the Declaration of Helsinki and the guidelines for the care and use of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Animals procedures were also made in accordance with the National Institutes of Health (NIH) protocol approved by Ain Shams University. Effort were made to minimize animal distress and to reduce the number of animals used in this study.

Experimental design

Rats were randomly divided into five groups, 5 animals each. Group (1) control group received olive oil only (1 ml/kg) 3 times/week. In group 2, animals received 1 ml/kg of CCl4 dissolved in olive oil (1:1) 3 times/week for 4 weeks; the selective dose was according to Hanafi (2012). Group (3) animals in this group received NAC 300 mg/kg orally according to (Galicia-Moreno et al., 2009). In group 4, rats received CCl4 accompanied with NAC. In group 5, rats received NAC for 1 week then administrated to the same treatment of group 4. All administrations were performed by gavage and maintained for 4 weeks, and all animals survived until the scheduled necropsy in all study groups.

Animals were sacrificed by deep isoflurane inhalation. After deep anesthesia, blood was collected by cardiac puncture; it was withdrawn slowly to prevent the heart collapsing and was processed to serum by allowing it to clot for at least 30 min at room temperature, followed by centrifugation at 2500*g* for 15 min at 4 °C. The liver, kidneys, testes, and cauda epididymis were dissected out for the different investigations which described below.

Assessment of hepatotoxicity

Liver marker enzymes ALT, AST, and alkaline phosphatase were estimated in serum by using kits (bio-diagnostic company, EL-Doki, Giza, Egypt) according to the manufacturer protocol.

Estimation of lipid peroxidation product (MDA)

Lipid peroxidation in tissue liver was estimated calorimetrically by measuring MDA using thiobarbituric acid (TBA) assay modified according to the method of Daper and Hadley (1990). Also, serum total protein was measured.

Histopathological investigation

Immediately, organs such as the liver, kidney, and testes were collected and fixed in 10% buffered formaldehyde solution for at least 24 h before the histopathological study. Then, samples were embedded in paraffin wax, and 5-μm sections were prepared with a rotational microtome. These thin sections were stained with hematoxylin and eosin (H&E); also, liver sections only were stained with masson trichrome. Then, slides were mounted on glass slides with Canada balsam (Sigma, co) and observed for pathological changes under a binocular microscope.

Sperm shape abnormality assay

Assessment of sperm-shape anomaly was made by system portrayed by Wyrobek and Bruce (1978). After the rat was sacrificed, cauda epididymis was dismembered out and set in a Petri-plate containing 0.5 ml of saline solution (0.9%Nacl) at room temperature. The epididymis was sliced into little pieces to permit the sperms to swim out. After that, the smears were prepared using 2–3 drops of the solution, air dried overnight, fixed with absolute methanol for 15 min, and stained with hematoxylin and eosin. One thousand sperms for each rat were analyzed to decide the morphological variations from the norm under oil immersion. Sperm shape abnormalities were classified as sperm head morphology which was categorized as normal, triangular, amorphous, collapsed, deformed orientation, acute curvature, double head, banana shape, without hook and tail abnormality. Data were shown in terms of percentage of abnormal sperms.

Comet assay

DNA damage in sperm was measured using an alkaline Comet assay according to the method described by Singh, Mc Coy, Tice, and Schneider (1988) with some modifications published by Simon and Carrell (2013). Sperm cells were adjusted to a concentration of (6×10^6 spermatozoa/ml) using PBS, and then, 10 μl was mixed with 75 μl of 0.5% low-melting-point agarose. Quickly, 15 μl of the mixture was placed on a precoated slide with (1% normal-melting point agarose) for gel adhesion, covered with coverslips and allowed to solidify on a cold plate at 4 °C for 15 min. Next, coverslips were carefully removed, and slides were submerged in fresh lysis solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, and 1% Triton X-100, pH 10 (was added immediately before use)) for 1 h at 4 °C. Afterward, slides were removed from the lysis buffer and 1.25 ml of dithiothreitol (DTT) was added to lysis buffer and inverted to ensure mixing; then, cells were incubated in lysis buffer with dithiothreitol for a further 30 min at 4 °C, after which lithium diiodosalicyclate (LIS) was added (4 mM final concentration) and incubated for 1.5 h at room temperature. Following lysis, cells were drained off any remaining liquid by standing them vertically on tissue paper against a support. All samples were incubated in chilled alkaline electrophoresis buffer (1 mM EDTA, 0.3 M NaOH) for 20 min. Electrophoresis was carried out for 10 min (25 V, 300 mA). After electrophoresis, slides were drained and washed thrice with neutralizing buffer (0.4 M Tris-HCl, pH 7.4) for 5 min each. The whole procedure was performed in dim light or dark to minimize artefactual DNA damage. Just before visualization, each slide was stained with 50 μl of ethidium bromide (20 μl/ml), rinsed with water, and covered with a coverslip. The slides were examined at 200x magnification using Olympus fluorescent microscope. All slides were coded and examined blindly. A total of 100 randomly selected cells from two replicate slides (50 cells per slide) were examined per sample. The analysis of comet cells was done using TriTek Comet Score Freeware v1.5.

The DNA damage in the treated cells was quantified as the tail moment (the DNA product in tail X the migration distance in the tail) was compared with untreated cells (Olive, Banath, & Durand, 1990).

Statistical analysis

Data from control and treated animals for all tests were analyzed statistically to assess the significant differences using Student's *t* test.

Results and discussion

CCL4 is well known as a typical toxic agent; its toxic effects occur through the production of free radicals. When the generation of the free radicals exceeds the cellular radicals scavenging capacity, the hepatocellular damage might occur. In the present study, the assessment of liver toxicity by CCL4 was done by measuring the marker enzymes AST, ALT, and ALP. Administration of CCl4 caused marked increase in the levels of serum AST, ALT, and ALP as shown in Table 1. This might be due to the release of these enzymes from the cell cytoplasm, into the blood flow quickly after rupture of the plasma membrane and cellular damage (Sallie, Tredger, & Williams, 1991). The present study agrees with Nitin and Khosas (2010); Sahreen, Khan, and Khan (2011); Domitrovic et al. (2015); and Reza, Sagor, and Alam (2015). In addition, CCL4 induced a significant increase in MDA level in liver tissue as compared to control Fig. 1, which in agreement with Lee et al. (2010) and Cai et al. (2015). The high level of MDA is a reflection of the occurrence of lipid peroxidation, leading to liver tissue damage and failure of antioxidant defense mechanisms (Termini, 2000). Alternatively, results showed that administration of NAC before or in combination with CCL4 caused a significant reduction in all liver maker

Table 1 Assessment of serum parameters in all groups

Groups	Parameters			
	ALT(IU/l)	AST(IU/l)	ALP(IU/l)	Tp(g/dl)
Control	38 ± 4.98	173.67 ± 9.5	389.5 ± 17.95	8.2 ± 0.66
CCL4	52.33 ± 6.49[c]	229.33 ± 16.89[c]	471.17 ± 12.79[c]	8.8 ± 1.03
NAC	41 ± 5.55	181.33 ± 4.73	408.2 ± 37.59	8.71 ± 0.94
CCL4 + NCA	48.8 ± 7.41[b]	192.67 ± 13.34[a]	425.8 ± 15.94[b]	8.8 ± 0.84
NAC before CCL4	46.3 ± 5.6[a]	188.5 ± 9.99[a]	415.8 ± 14.6[a]	8.5 ± 1.03

Values are given as mean ± SD ($n = 6$)
[a]Significant compared with control at $P < 0.05$
[b]Significant compared with control at $P < 0.01$
[c]Significant compared with control at $P < 0.001$

enzymes (Table 1). These results were in accordance with Wong, C-Ooi, and Wong (2003); Kamalakkannan et al. (2005); Priya, Vijayalakshmi, Vivekanandan, and Karthikeyan (2011); and Sahin and Alatas (2013). NAC is an excellent source of sulfhydryl compound, which converted in hepatocytes into metabolites capable of stimulating GSH synthesis; it also provides sufficient cysteine to promote detoxification and eliminates directly reactive oxygen species. Previous studies showed the protective effect of NAC against liver damage induced by various hepatotoxic chemicals such as methanol (Raza, Ahmad, Gado, & Alshabanah, 2003) and ethanol (Ronis, Butura, Sampey, Shankar, & Prior, 2005). Interestingly, animals received NAC before or in combination with CCl4 showed a significant decrease in the MDA levels when compared with those received CCL4 alone; this result is in accordance with Sahin & Alatas, 2010. Also, Wang et al. (2014) reported that NAC was able to ameliorate oxidative stress and cytotoxicity in HepG cells. Additionally, Morsy, Abd Alla, Mahmoud, Abdel Wahab, and Mahmoud (2012) showed that curcumin, α–lipoic acid and NAC caused a significant decrease of hepatic MDA levels as compared to CCL4 fibrotic rats. However, results revealed insignificant change in serum total protein in all groups as compared to control; this was context with Hanafi (2012).

Histological examination of liver sections from control and NAC groups stained with H&E showed normal architecture (Fig. 2), and Masson trichrome staining revealed the minimal amount of thin and fine collagen fibers around the central vein and portal tract area (Fig. 3). Liver sections of rats that received CCL4 showed vacuolated cytoplasm and congestion (Fig. 4), dense amorphous material in eosinophilic between and around hepatocytes vacuolar degeneration (Fig. 5), aggregations of inflammatory cells and marked inflammatory changes associated with fatty changes (Fig.6), focal necrotic areas, congestion and pyknotic nuclei were also observed (Fig.7). These observations agree with Manjrekar, Jisa, Bag, and Adikary (2008); Morsy et al. (2012); Morakinyo, Oludare, Anifowose, and Adegoke (2012); Domitrovic et al. (2015); and Hismiogullari et al. (2015). Masson trichrome stained sections showed congested thickened portal tract with dense fibrous tissues radiating from the portal vein and extending into the parenchyma (Fig. 8). Conversely, liver sections from animals

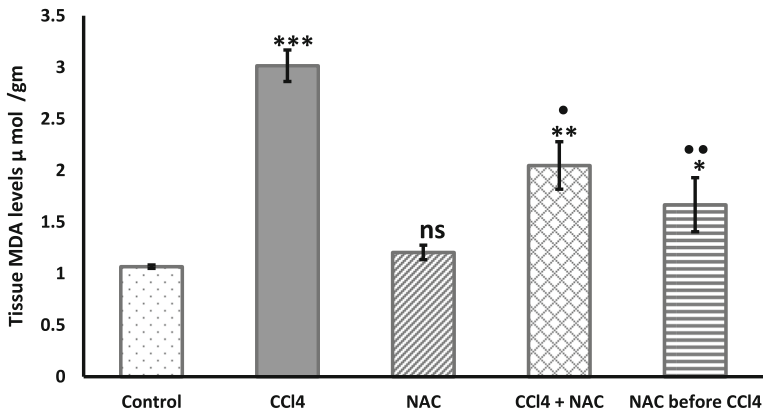

Fig. 1 Mean, SEM, and significance of MDA content in liver tissue in different groups. $^{ns}P > 0.05$ compared with control. $^{*}P < 0.05$ compared with control. $^{**}P < 0.01$ compared with control. $^{***}P < 0.001$ compared with control. $^{\bullet}P < 0.01$ compared with CCL4. $^{\bullet\bullet}P < 0.001$ compared with CCL4

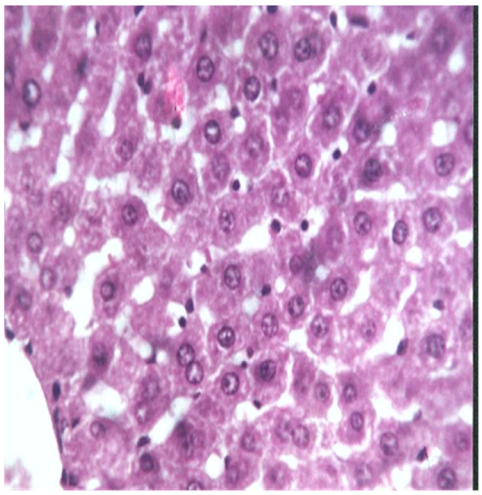

Fig. 2 Photomicrograph of the control liver showing cords of hepatocytes radiating from the central vein and separated by blood sinusoids (H&E; × 200)

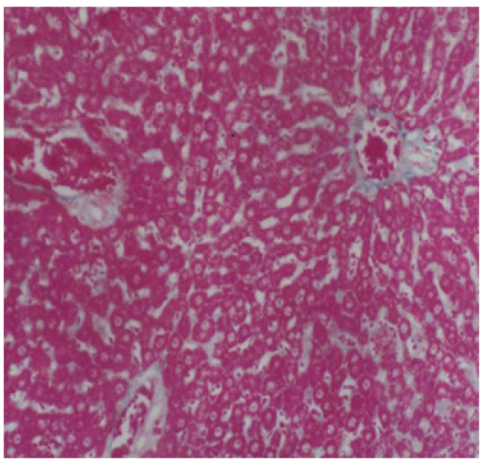

Fig. 3 Photomicrograph of the control liver showing minimal amount of connective tissue around the portal area (Masson's trichrome × 200)

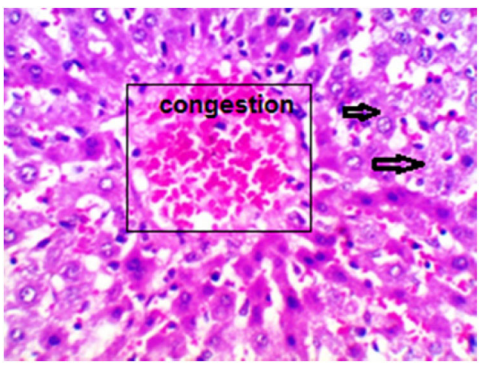

Fig. 4 Photomicrograph of CCl4-treated liver sections showing vacuolated cytoplasm and congestion and dilation of blood vessels (H&E; × 400)

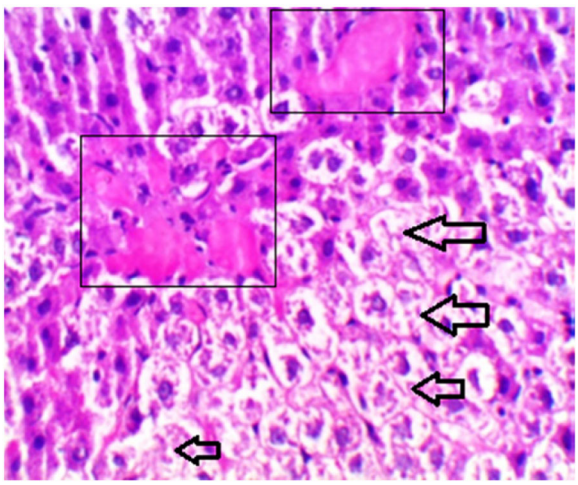

Fig. 5 Photomicrograph of CCl4-treated liver sections showing dense amorphous eosinophilic material between and around hepatocytes and vacuolar degeneration (H&E; × 400)

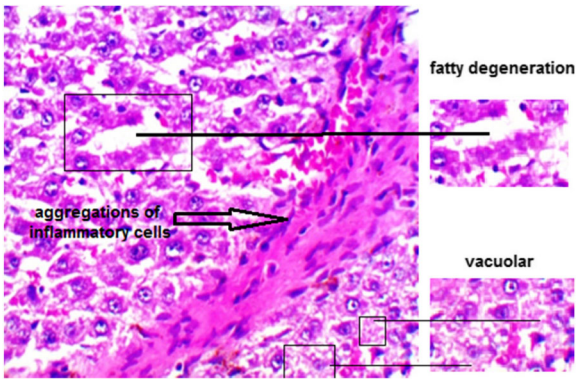

Fig. 6 Photomicrograph of CCl4-treated liver sections showing aggregations of inflammatory cells, associated with vacuolar and fatty degeneration (H&E; × 400)

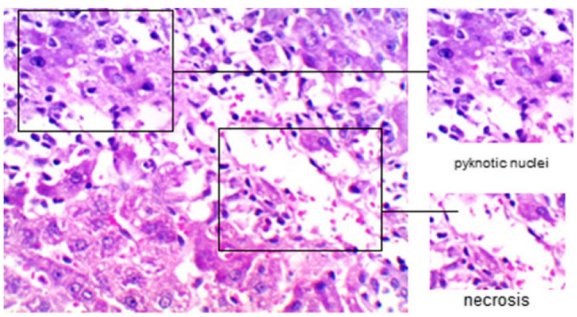

Fig. 7 Photomicrograph of CCl4-treated liver sections showing necrosis area with pyknotic nuclei (H&E; × 400)

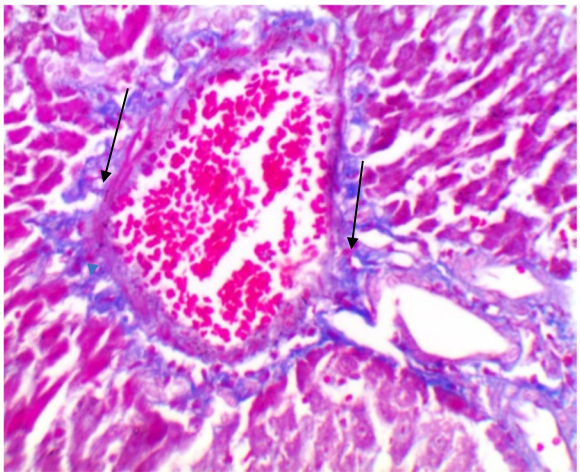

Fig. 8 Photomicrograph of CCl4-treated liver sections showing congested thickened portal tract with dense fibrous tissues radiating from the portal vein and extending into the parenchyma (Masson,s trichrome × 400)

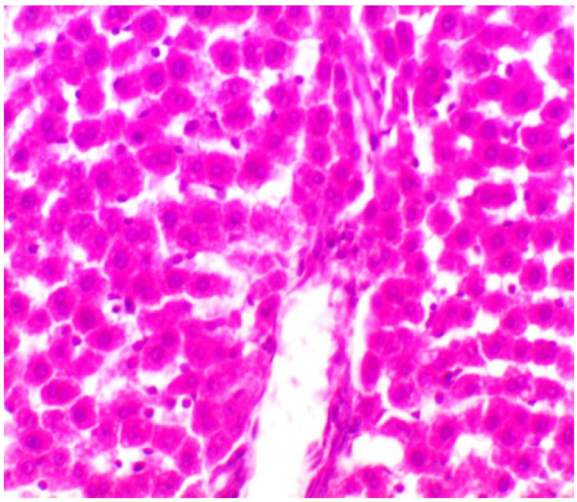

Fig. 10 Photomicrograph of liver section after CCL4 + NAC treatment showing improvement in liver architecture (H&E) × 400

treated with NAC 1 week before CCL4 or in combination with CCL4 stained with H&E (Figs. 9 and 10) and Masson showed improvement in liver architecture sections. NAC prevented the accumulation of collagen fibers as shown in Fig. 11; these results agree with Galicia-moreno et al. (2009) and Pereira-Filho et al. (2008) who found that administration of NAC preserved the normal levels of collagen in CCl4-intoxicated rats. The effects of CCl4 were prevented completely or partially by NAC antioxidant activity as it prevented the increase in lipid peroxidation products (Manibusan, Odin, & Eastmond, 2007; Morsy et al., 2012 and Demiroren et al., 2014). The development of CCl4-induced liver fibrosis is usually associated with oxidative stress and lipid peroxidation (Manibusan et al., 2007). Bedossa, Houglum, Trautwein, Holstege, and Chojkier (1994) reported that hepatocyte lipid peroxidation plays a major role in the regulation of collagen α1(I) gene expression and that it may be a link between hepatocyte injury and hepatic fibrosis. Additionally, Galicia-Moreno et al. (2009) reported that NAC increased liver GSH levels, reduced lipid peroxidation products and transforming growth factor (TGF) levels, and prevented collagen

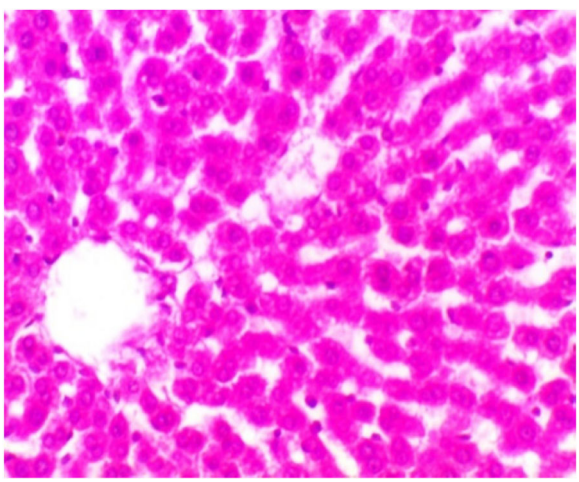

Fig. 9 Photomicrograph of the liver section after NAC treatment 1 week before CCl4 showing improvement in the liver architecture (H&E; × 400)

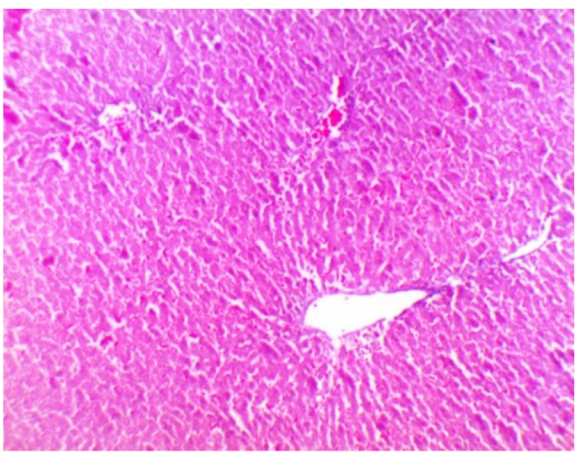

Fig. 11 Photomicrograph of treated liver sections treated (CCL4 + NAC) group showing less thickened fibrotic tissue (Masson's trichrome × 200)

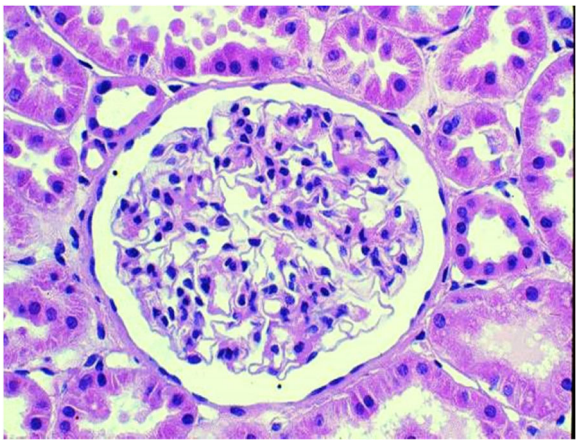

Fig. 12 Photomicrograph of control kidney showing normal architecture (H&E; × 400)

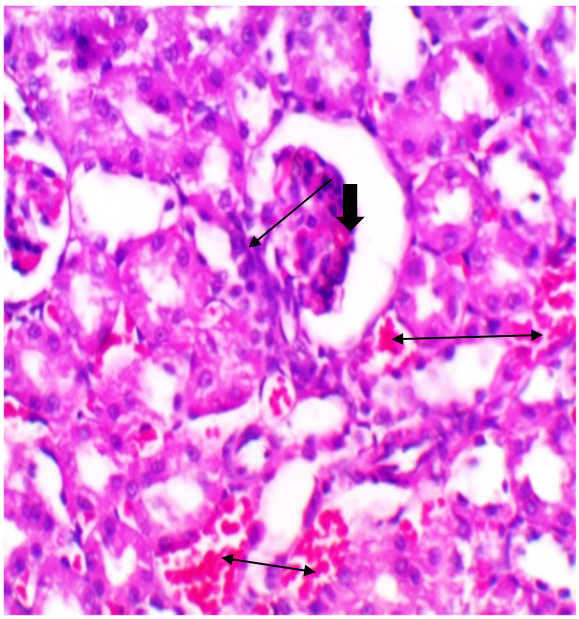

Fig. 14 Photomicrograph of CCl4-treated kidney sections showing sever congestion (2head arrow), vacuolation, inflammatory changes (arrow) and dilatation of Bowman's space with glomerular atrophy (thick arrow) (H&E; × 400)

accumulation. Additionally, Pereira-Filho et al. (2008) reported that NAC decreases the fibrosis scores, increases glutathione per-oxidase levels, and reduces inducible nitric oxide synthase levels. The reduction of glutathione (GSH) plays a key role in detoxifying the reactive toxic metabolites of CCl4, and the fibrotic process begins when the GSH stores are markedly depleted (Annadurai, Vigneshwari, Thirukumaran, Thomas, & Geraldine, 2011).

Kidney sections from control rat stained with H&E showed normal histological morphology (Fig. 12). While treated group with CCL4 showed that the structure of individual tubular lining cells are enlarged and filled with eosinophilic granules in cytoplasm (Fig. 13), severe congestion, vacuolation, inflammatory changes, and dilatation of Bowman's space with glomerular atrophy and periglomerular space (Figs. 14, 15, 16, and 17), this agree with Manjrekar et al. (2008); Morakinyo et al. (2012); and Hismiogullari et al. (2015). Kidney sections obtained

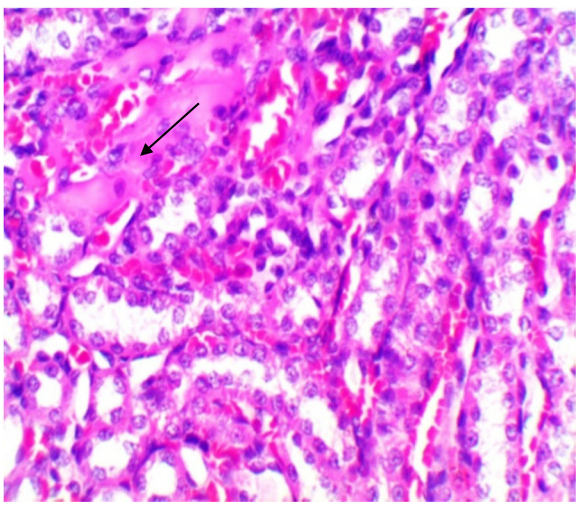

Fig. 13 Photomicrograph of CCl4-treated kidney sections showing individual tubular lining cells are enlarged and filled with eosinophilic granules in cytoplasm (H&E; × 400)

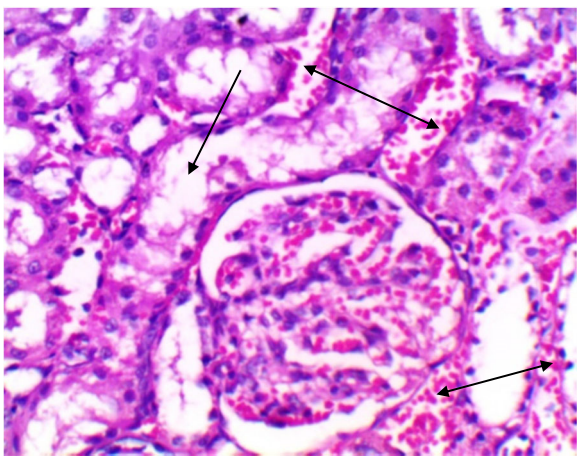

Fig. 15 Photomicrograph of CCl4-treated kidney sections showing Congestion (2head arrow) & vacuolar deg. (arrow) (H&E; × 400)

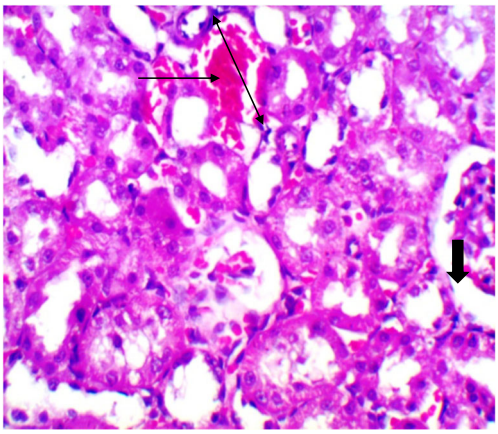

Fig. 16 Photomicrograph of CCl4-treated kidney sections showing congestion (arrow), vacuolation, inflammatory changes (2head arrow) and periglomerular space (thick arrow) (H&E; × 400)

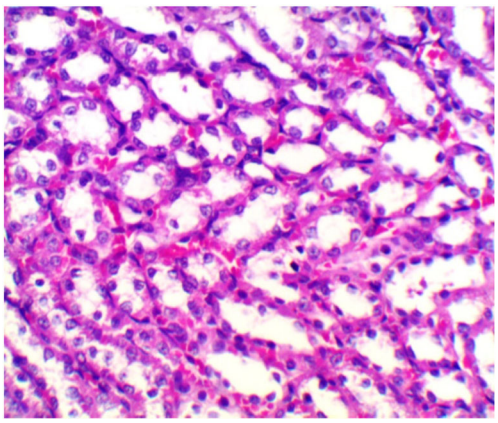

Fig. 18 Photomicrograph of treated kidney sections treated (CCL4 + NAC) group showing improvement in architecture sections kidney (H&E; × 400)

after the two routes of NAC treatment stained with H&E showed improvement in the architecture (Figs. 18 and 19). The powerful antioxidant effect of NAC appeared in the histological recovery of kidney; this attributed to its potential to eliminate free radicals from CCl4-induced oxidative stress. (Ustyol et al., 2017).

Sections of testes from control rats stained with H&E showed normal histological structure (Fig. 20). CCl4-treated group showed sloughing and destruction of walls of seminiferous tubules necrosis, edema, and increase in fibers deposition (Figs. 21, 22, and 23), in accordance with Manierea et al. (2005); Moustafa et al. (2007)); Manjrekar et al. (2008); Khan and Ahmed (2009); Hanafi (2012), and Khan (2012). These effects are due to the production of oxygen radicals in excess of the antioxidant capacity of the stressed tissue Yousef and Salama (2009). Fig. 24 shows improvement in the testis architecture after treatment with NAC; this agrees with Asadpour, Shahbazfar, Kianifard, Azari, and Nzaboli (2013).

Moreover, a highly significant increase ($P < 0.0001$) in the percentage of abnormality in sperm head morphology was observed in the CCl4 group (2.78 ± 6.02) when compared to control group (0.72 ± 2.28). This result agrees with Sonmez et al. (2014). Additionally, results revealed that abnormalities such as amorphous and without hook were the most frequently observed types after CCL4 exposure which coincides with Abdou, Salah, Hoda, and Abdel Rahim (2012). On the contrary, deformed orientation, triangular, acute curvature, and

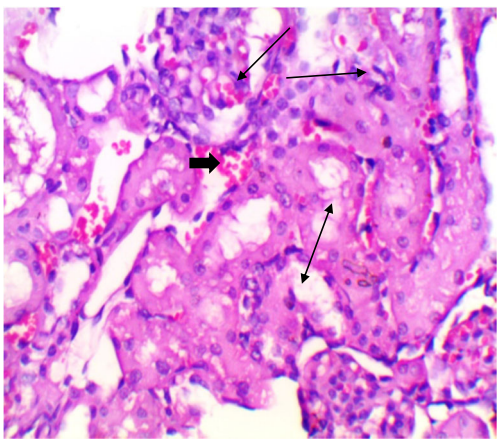

Fig. 17 Photomicrograph of CCl4-treated kidney sections showing congestion (thick arrow), vacuolation (2head arrow) and, inflammatory changes (arrow) (H&E; × 400)

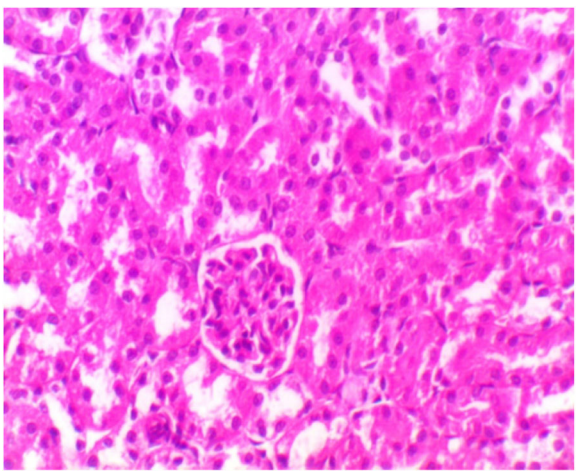

Fig. 19 Photomicrograph of treated sections of kidney with (CCL4 + NAC) showing improvement in architecture of sections (H&E; × 400)

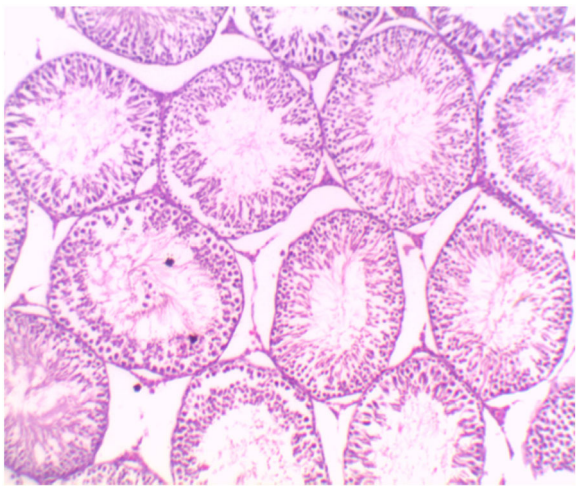

Fig. 20 Photomicrograph of control testis showing normal architecture (H&E;400)

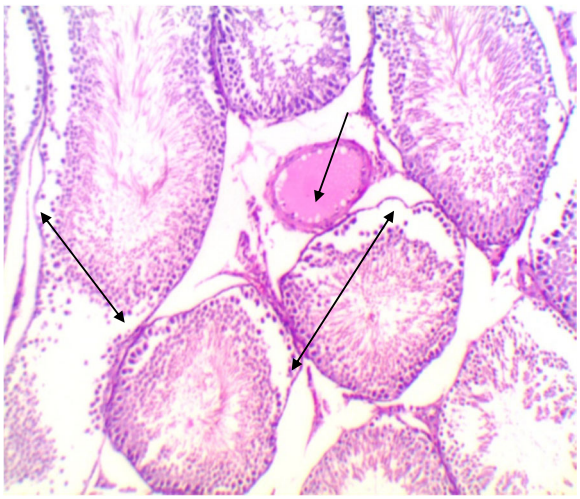

Fig. 23 Photomicrograph of CCl4-treated testis sections showing disrupted basement membrane, spermatogenic layer, and intertubular oedema (H&E; × 400)

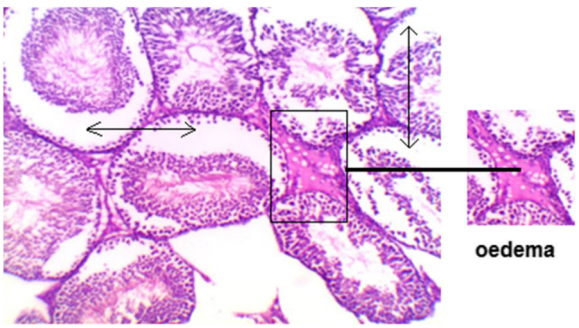

Fig. 21 Photomicrograph of CCl4-treated testis sections showing sloughing and destruction of walls of seminiferous tubules and oedema (H&E; × 400)

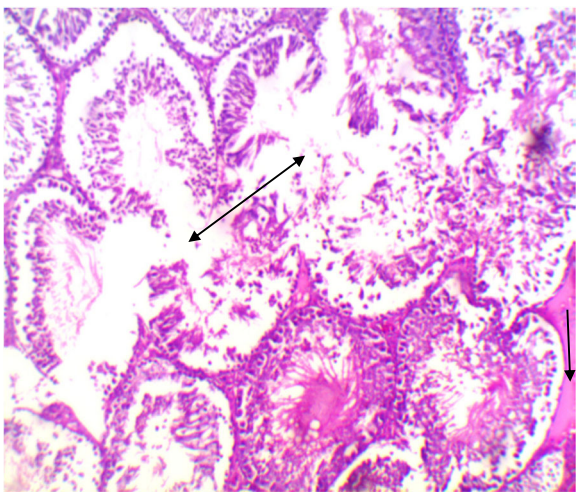

Fig. 22 Photomicrograph of CCl4-treated testis sections showing destruction of walls of seminiferous tubules and oedema (H&E; × 400)

double headed sperms were the least common morphological abnormalities (Table 2 and Fig. 25). In addition, CCl4 induced damage in sperm head DNA which represented as a significant ($P < 0.05$) increase in the tail moment values (Table 2). This result concurs with Khan (2012), who declared that the Free radicals of carbon tetrachloride cause testicular DNA fragmentation qualitatively and quantitatively in testicular tissue.

The oxidative stress is considered as one of the main cause of DNA damage in the germ cells. In normal condition male, reproductive system has a balance between ROS creation and antioxidant activity. But the increase of ROS in semen can affect sperm or seminal plasma

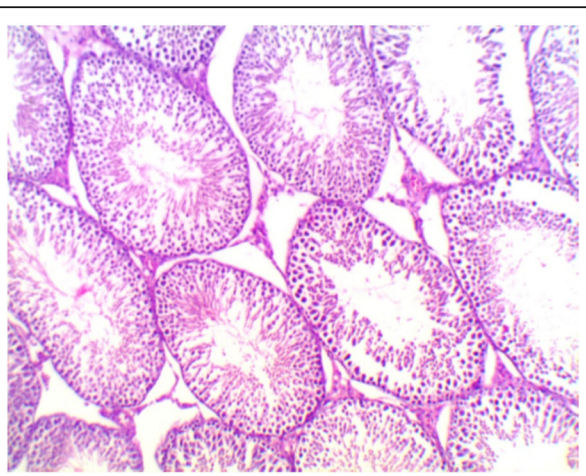

Fig. 24 Photomicrograph of treated sections of testis with (CCL4 + NAC) showing improvement in architecture of sections (H&E; × 400)

Table 2 Percentages of abnormal sperms and tail moments in rats post-treatment with CCL_4 and/or NAC

Treatment	No of counted sperms	Types of sperm head abnormalities								Tail abnormalities	Total			Tail moment%	
		t	col	d-o	b-s	wh	am	ac	dh		Total	%	S.D.	Mean	S.D.
Control	5000/group	–	–	–	2	18	4	4	0	8	36	0.72	2.28	0.1505	0.036
CCL_4		12	–	14	–	31	68	5	3	6	139	2.78	6.02[d]	1.642	0.976[b]
NAC		10	–	9	7	8	5	4	4	7	54	1.08	5.3 [a]	0.3939	0.336[a]
CCL4 + NAC		9	5	15	–	6	11	3	–	8	57	1.14	4.34[af]	0.3036	0.265[ae]
NAC then CCL4		12	6	6	3	16	13	5	1	7	69	1.38	6.14[af]	0.2474	0.04[ce]

4 animals were used for comet assay
t triangular, *col* collapsed, *am* amourphous, *bs* banana shape, *wh* without hook, *do* deformed orientation, *ac* acute curvature, *dh* double head, *S.D.* standard deviation
[a]$P > 0.05$ compared with control
[b]$P < 0.05$ compared with control
[c]$P < 0.01$ compared with control
[d]$P < 0.0001$ compared with control
[e]$P < 0.05$ compared with CCL4
[f]$P < 0.01$compared with CCL4

antioxidant defense mechanisms and cause oxidative stress (Sabeti, Pourmasumi, Rahiminia, Akyash, & Talebi, 2016). The different CCL4 metabolic pathways result in ROS generation and alteration of antioxidant defense systems in animals (Stal & Olson, 2000). ROS have negative effects on sperm function where sperm plasma membrane contains unsaturated fatty acids that are inclined to free radical attack leading to lipid peroxidation and DNA fragmentation. Compromising the integrity of the sperm membrane may lead to damage in sperm DNA (Agarwal, Saleh, & Bedaiwy, 2003) and decline the sperm's capacity to repair damage (Ryan, Weil, Newburger, Haugland, & Simons, 1990). Seminal ROS decreases sperm motility (Kao et al., 2008) and distorts sperm morphology. On the other hand, NAC is a free radical scavenger which encourages cell glutathione production and could counteract oxidant-mediated tissue injury (Zembron-Lacny, Slowinska-Lisowska, Szygula, Witkowski, & Szyszka, 2009). In the present study, NAC treatment has been shown to lower the percentages of abnormal sperms and sperm DNA damage. Treatment with NAC accompanied with CCL4 caused a decrease in the percentage of abnormal sperms from 2.78 with CCl4 alone to 1.14% and decreased the tail moment percentage to 0.3036 (Table 2 and Fig. 26), while pre-treatment with NAC for 1 week followed by administration of NAC plus CCL4 caused a decrease in the percentages of abnormal sperms and tail moment into 1.38 and 0.2474 respectively. These values were found to be significant when compared with those which received CCL4 alone. Earlier studies showed that NAC lowers the concentration of damaging ROS within semen, improving semen volume and viscosity, increasing motility (Ciftci, Verit, Savas, Yeni, & Erel, 2009), and increasing the percentage of sperm with normal morphology (Safarinejad & Safarinejad, 2009). Moreover, Yedjou, Tchounwou, Haile et al. (2010) indicate that NAC treatment increases cell viability and affords protection from DNA damage in HepG2 cells exposed to PbNO3. NAC also reduced DNA damage induced by ionizing radiation exposure during cardiac catheterization procedures (Andreassi et al., 2012).

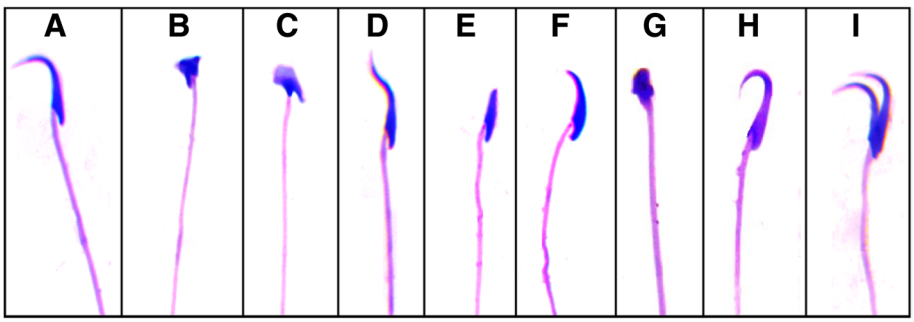

Fig. 25 Different types of head shape anomalies after CCl4 and/or NAC treatment. **a** Normal. **b** Triangular. **c** Collapsed. **d** Deformed orientation. **e** Banana shape. **f** Without hook. **g** Amorphous. **h** Acute curvature. **i** Double head

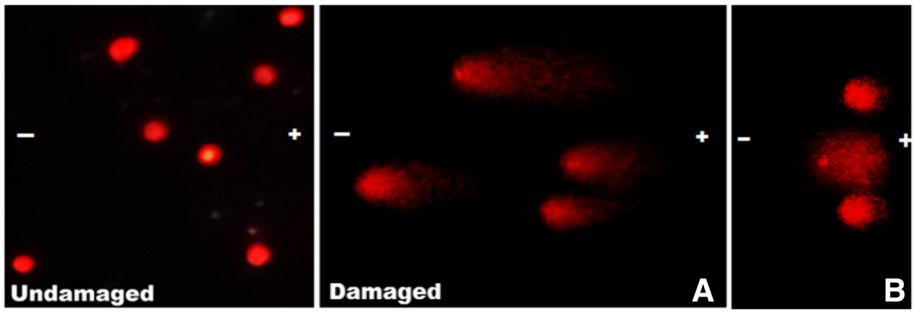

Fig. 26 Sperms DNA in control (undamaged) and after CCl4 treatment (Damaged (**a**)) and CCL4 with NAC (**b**)

Conclusions

In conclusion, NAC remarkably reduced serum liver marker enzymes and MDA content in liver tissue, also it has ameliorative effects on the liver, kidney, and testis tissues. Additionally, it caused a decrease in the percentage of sperm shape abnormalities and DNA damage induced by CCL4 exposure.

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CCL4: Carbon tetrachloride; DNA: Deoxyribonucleic acid; DTT: Dithiothreitol; EDTA: Ethylene diamine tetraacetic Acid; GSH: Reduced glutathione; H&E: Hematoxylin and eosin; HepG2: Human liver carcinoma; LIS: Lithium diiodosalicyclate; MDA: Malondialdehyde; Na2EDTA: Sodium salt of Ethylenediaminetetraacetic acid; NAC: *N*-Acetyl cysteine; PbNO3: Lead nitrate; PBS: Phosphate-buffered saline; ROS: Reactive oxygen species; TBA: Thiobarbituric acid; TGF: Transforming growth factor; Tris–HCl: Tris (hydroxymethyl) aminomethane Hydrochloride

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions

All authors participated in all parts of the research. All authors read and approved the final manuscript.

Ethics approval

This study follows the Declaration of Helsinki and the guidelines for the care and use of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Animals procedures were also made in accordance with the National Institutes of Health (NIH) protocol approved by Ain Shams University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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