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THE POSSIBLE PROTECTIVE EFFECT OF CAPTOPRIL AND NIGELLA SATIVA AND THEIR COMBINATION AGANIST CARBON TETRACHLORIDE INDUCED TESTICULAR TOXICITY IN RATS

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ABSTRACT:

Exposure to carbon tetrachloride may affect male reproductive function. Objectives: This study was designed to elucidate the possible protective effect and the underlying mechanism of the single and combined administration of captopril and nigella sativa oil against CCl4-induced testicular toxicity. Methods: Forty male adult albino rats were used and classified into five group. Goup I (control) were received paraffin oil; group II were injected intrapertonially (I.P.) with CCl4 in a dose of 1 mL/kg on alternative days for 8 weeks; group III were given captopril in a dose of 100 mg/kg; group IV were given nigella sativa oil in a dose of 4 ml/kg; group V were given combination of captopril and nigella sativa oil. Rats of groups (III, IV and V) were received oral (P.O.) daily doses of the drugs for 2 weeks, followed by concurrent administration of CCL4 for 8 weeks. Testicular levels of nitric oxide (NO), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and serum level of the reproductive hormones (FSH, LH and testosterone) were measured. Light microscopic and immunohistochemical examination of testes were done. Results: CCL4 – induced testicular toxicity rats revealed significant increase in NO and MDA level with significant decrease in GSH and GSH-Px levels in testes. A significant decrease in the serum level of the reproductive hormones in CCL4-induced testicular toxicity. Histopathological examination of CCl4- injected rat group revealed degenerative and apoptotic changes in the testes with reduction in the androgen receptor expression. The single and combined pretreatment of the CCL4 - injected rats with captopril and nigella sativa oil, showed significant decrease of the elevated NO and MDA levels, with significant increase in GSH and GSH-Px. In addition, they improved the serum levels of the reproductive hormones. Besides, they improved the histolopathological changes induced by CCL4 in the testes. Conclusion: captopril and nigella sativa oil have protective effect against CCl4 - induced testicular toxicity which attributed to augmentation of the antioxidants and anti-apoptotic effect.

Key Words: ***CCL4, oxidative stress, captopril, nigella sativa oil, testis.***

INTRODUCTION:

Various studies have provided evidence about the decrease in the male fertility in industrialized nations over the last decades. Numerous environmental toxins have been described to induce disruption in the oxidant / antioxidant

balance of the male reproductive system (Rahmouni et al., 2018).

Carbon tetrachloride can lead to toxic effects in many organs such as liver, blood, kidney, lung, brain, and testis (Pirinççioğlu et al., 2012).

Administration of CCL4 has been indicated to cause structural and functional damages in the male reproductive system in experimental animals (Khan and Ahmed, 2009).

The pathogenesis of CCl4 - induced tissue injuries is not completely thoroughly investigated. Oxidative stress is endorsed in the pathogenesis of CCL4 harmful effects that results in the generation of free radicals. That involved in lipid peroxidation and increased nitric oxide (NO) production (Khan et al., 2009). Free radicals are neutralized by an elaborate antioxidant defense system of enzymes such as glutathione and superoxide dimutase (Abdel Moneim and El-Khadragy, 2013).

In addition, It was postulated that CCl4-induced testicular damage through apoptosis in testicular tissue, which is mediated through caspase -3 (Abdel moneim, 2016).

Various natural product extracts showed protective effects against CCl4 - induced oxidative stress damage in organs of experimental animals by diminishing lipid peroxidation, and enhancing the antioxidant enzyme activities (Khan and Ahmed, 2009; Cemek et al., 2010).

Furthermore, numerous herbal products were established to ameliorate CCl4 - induced testicular damage (Rahmouni et al., 2018).

Nigella sativa (family Ranunculaceae) has been used traditionally in the Middle East, Northern Africa and Asia (Ibrahim et al., 2008).

Nigella sativa seeds/oils are used in folk medicine all over the world as anti-inflammatory, analgesic, antipyretic, antimicrobial, antineoplastic, anti asthmatic, antidiarrheal and dyslipidaemiac. Both volatile oil and thymoquinone have been reported to give protection against nephrotoxicity and hepatotoxicity induced by some chemicals (Ali and Blunden, 2003).

It was reported that nigella sativa extract 1has many beneficial effects such as anti-inflammatory, immunomodulator, anti-tumor activity and anti-infertility effects in both human and experimental studies (Ahmed et al., 2013).

Captopril is an angiotensin-converting enzyme (ACE) inhibitor that acts as a scavenger of oxygen derived free radicals. It decreased lipid peroxidation and increased the activity of both glutathione peroxidase (GSH-Px) and glutathione reductase (Ackerman, et al., 2008).

No available data are present relating the protective role of captopril and nigella sativa oil in against CCl4 induced testicular toxicity in experimental studies.

Aim of Work

This study was planned to evaluate the possible protective effect and the underlying mechanism of single and

combined administration of captopril and nigella sativa oil against CCl4- induced testicular toxicity in rats.

MATERIAL AND METHODS:

(1) Chemicals:

Carbon tetrachloride was obtained from ADWIC (Egypt). Captopril, was purchased from Sigma Aldrich Co. (USA).

All other chemicals were of analytical grades. Immuno-histochemical kits for caspase-3 and androgen receptor kits were obtained from Thermo Scientific Co. (USA).

(2) Nigella sativa oil:

Nigella sativa seeds, obtained from the local market in Egypt, were authenticated by Pharmacognosy Department, Faculty of Pharmacy, Assiut University. The total crude oil was extracted by cold pressing (hydraulic press).

The oil was analyzed to match the known standard specifications of its fixed and volatile oils.

Reversed-phase thin layer chromatography (TLC) was carried out following the method outlined in the British Pharmacopoeia for identification of the fixed oil. The oil contained saturated fatty acids, mainly; myristic, palmitic and stearic as well as unsaturated fatty acids, primarily; oleic, linoleic and linolenic. Thymoquinone, the major active component of the volatile oil, was identified and quantified by HPLC using an isocratic mobile phase of water: methanol: 2-propanol. The concentration of thymoquinone was about 0.103% (w/v) in the total crude oil.

(3) Animals and Experimental design:

Adult male albino rats (200-250 g) were obtained from animal house of faculty of Medicine, Assiut university, Egypt. Rats were housed in stainless steel cages at constant temperature of 25 ± 2^{O}, illumination (12 h light/dark) and had free access to standard pellet chow and water ad Libitum. Dealing with the animals followed the regulations of the animal house of Faculty of Medicine, Assiut University. One week after acclimatization, rats were randomly divided into five groups (8 rats/each).

Group I (negative control) rats were injected with liquid paraffin oil I.P. for 8 weeks; group II (positive control) rats were injected I.P. with CCL4 in a dose of 1 mL/kg b.w. diluted 1:6 with paraffin oil on alternative days for for 8 weeks to induce testicular toxicity and double distalled water P.O. (Noureen et al., 2017), group III rats were given captopril in a dose of 100 mg/kg/day P.O. (Hrenák et al., 2013), group IV rats were given nigella sativa oil in a dose of 4 ml/kg/day P.O. (Abdel-Zaher et al., 2011) and group V rats were given captopril and nigella sativa oil in the same previously mentioned doses. Groups (III, IV and V) were pretreated with oral daily dose of captopril or nigella sativa oil for 2 weeks and continued with the concurrent administration of CCL4 for 8 weeks (Ahmed, et al., 2011).

(4) Blood and tissue samples collection:

The rats were sacrificed by decapitation under light ether anesthesia and dissected at the end of 10th weeks.

Blood was collected by cardiac puncture from all rat groups. The collected blood samples were centrifuged at 3000 rpm for obtaining sera which stored at -20 until required for estimation of the hormonal assay. The testis was removed and divided into two parts; the first part was homogenized in ice cold saline. The testicular tissue homogenates were centrifuged at 3000 RPM for 15 minutes for measurement of oxidative stress markers.

The resulting supernatant was stored at -80 for biochemical analysis. The second part was fixed in 10% formalin and processed for histopathological examination (Khan et al., 2009).

(5) Biochemical assays:

(I) Measurement of oxidative stress markers in the testis:

(i) Nitric oxide (NO):

Nitric oxide level was measured in the testicular tissue homogenates by assaying nitrate, one of the stable end-products of NO oxidation. Nitrite concentration was measured using Griess reagents as described by Green and his coworker (1982).

(ii) Malondialdhyde (MDA):

Malonaldehyde content in the testicular tissue homogenates as an indicator of lipid peroxidation was measured by a colorimetric method according to the method described by Ohkawa, et al. (1979).

(iii) Reduced glutathione (GSH):

Estimation of intracellular GSH content of neutralizing supernatant testicular tissue was assayed using Ellman's reagent, which was measured spectrophotometrically according to the method described by Griffith (1980).

(iv) Glutathione peroxidase (GSH-Px):

Glutathione peroxidase (GSH-Px) activity was measured spectophtometrically in the supernatant of tissue homogenate by the method described by Paglia and Valentine (1967).

(II) Hormonal assay in the serum:

The serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by ELISA (enzyme - linked immunosorbent assay) using (Accu-Bind ELISA) kits according to manufacturer's structure (Monobind Inc., Lake Forest, CA, USA).

(6) Histopathological Examinations

For light microscopic investigations, specimens from testis were fixed in 10% formalin, dehydrated in alcohols and embedded in paraffin. Five micron tissue sections were stained with hematoxylin and eosin stain (H&E) and Periodic acid–Schiff (PAS) and masson's trichrome. Semithin sections of the testes were stained with toludine blue stain for histopathological examination (Bancroft and Stevens, 1996).

For immunohistochemistry examinations, a series of slides (3–5 µm) were prepared. To identify the presence of androgen receptors in the seminiferous tubules and interstitial tissue, the immunohistochemical reactions with specific antibodies: mouse monoclonal

antibody anti-androgen receptor (AR) (AR441, polyclonal antibody, thermo scientific Co., USA) were carried out. To identify apoptosis in testicular tissues anti-caspase-3 (CPP32- Ab4 polyclonal antibody, thermo scientific Co., USA) were added for 1 hour at room temperature in a dilution of 1:100 then washed 4 times in phosphate buffer saline (PSB).

The deparaffinized sections of the paraformaldehyde - fixed paraffin-embedded organ cultures were reacted to 3% hydrogen peroxide to inhibit endogenous peroxidase activity. The sections were microwaved in citrate buffer (pH 6.0). After cooling to room temperature, the slides were washed in PSB four time and then incubated for 60 min with mentioned above primary antibodies.

Then, Biotinylated Goat Anti-Polyvalant (secondary antibody) was applied and incubated for 30 minutes at room temperature and washed 4 times. Next, streptavidin peroxidase was applied and incubated for 10 minutes at room temperature then rinsed with PSB.

One to two drops of diaminobenzidine (DAB) chromogen was added according to the manufacturer's instructions.

Then the sections were washed in distilled water and counterstained with hematoxylin. As a negative control, the specimens were processed in the absence of the primary antibodies. Positive staining was defined by visual identification of DAB brown pigmentation in the light microscopic examination (Bremner et al., 1994).

Statistical analysis:

Results were expressed as means ± standard error of the mean (SEM) and were analyzed for statistically significant difference using one-way analysis of variance (ANOVA) followed by the Dunnett's test post analysis test. p values < 0.05 were considered significant.

Graph Pad Prism ® was used for statistical calculations (Version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS:

Biochemical assays.

1-The effect of captopril and nigella sativa oil and their combination on MDA, NO, GSH and GSH-Px testicular levels in CCL4 - induced testicular toxicity in rats:

There were significant increase (P. value < 0.05) in the mean testicular levels of MDA and NO with significant decreased in GSH and GSH-Px in CCL4- induced testicular toxicity (+ ve control) rat group compared to normal (-ve control) rat group. The levels of these parameters were significantly improved in CCl4- induced testicular toxicity rats treated with captopril (group III), nigella sativa oil (group IV) and captopril with nigella sativa oil (group V), respectively compared to +ve control rats (group II) (table 1).

2- The effect of captopril and nigella sativa oil and their combination on serum FSH, LH and testosterone

hormonal levels in CCL4 induced testicular toxicity in rats:

There was a significant decrease (P. value < 0.05) in the mean serum FSH, LH, testosterone hormone levels in CCL4 induced testicular toxicity (+ ve control) rat group compared to normal (- ve control) rat group. The levels of these hormones were significantly increased in CCl4- induced testicular toxicity rats treated with captopril (group III), nigella sativa oil (group IV) and captopril with nigella sativa oil (group V), respectively compared to + ve control rats (group II) (table 2).

Table (1): The effect of captopril and nigella sativa oil and their combination on MDA, NO, GSH and GSH-Px testicular levels in CCL4 - induced testicular toxicity in rats.

Parameters Groups	NO (nmol/gm) Mean ± SE	MDA (nmol/gm) Mean ± SE	GSH (nmol/gm) Mean ± SE	GSH-Px (U/gm) Mean ± SE
G. I: normal rats injected with paraffin oil (negative control)	55 .8 ± 2.9	7.94 ± 0.53	19.6 ± 0.61	15.96 ± 1.03
G. II: CCl4 injected rats (positive control)	122.2±6.2o	16.65 ± 0.69 o	7.34±0.21 o	6.59 ± 0.42 o
G.III: captopril treated group in CCl4 - injected rats	78 .3 ± 5.7*	11.85 ± 0.77 *	13.58±0.76*	11.71 ± 0.39 *
G.IV: nigella sativa oil treated group in CCl4 - injected rats	77.1 ±4.6*	12.42 ± 0.24*	12.47 ± 0.64*	11.68 ± 0.27 *
G.V: combined captopril and nigella sativa oil treated group in CCl4 - injected rats	62.7 ± 4.9 *	8.48 ± 0.26*	16.53±0.27*	13.24 ± 0.69*

The results represent the mean ± S.E.of 8 rats.
o Significant differance compared to the normal group (negative control) (P <0.05).
*** Significant difference compared to the CCL4 - induced group (positive control) (P <0.05).**

Table (2): The effect of captopril and nigella sativa oil and their combination on FSH, LH and testosterone hormone levels on CCL4 - induced testicular toxicity in rats.

Groups	FSH (mIU/ml) Mean ± SE	LH (mIU/ml) Mean ± SE	Testosterone (ng/ml) Mean ± SE
G. I: normal rats injected with paraffin oil (negative control)	45.8 ± 2.9	2 .37 ± 0.95	5.28 ± 0.34
G. II: CCl4 injected rats (positive control)	17.1±1.01 o	1.23 ± 0.81o	2.21 ± 0.16o
G.III: captopril treated group in CCl4 - injected rats	38 .3 ± 2.1*	1 .6 ± 0.97*	3 .69 ± 0.27*
G.IV: nigella sativa oil treated group in CCl4 - injected rats	37.1 ±2.3*	1.7 ± 0.84*	3.82 ± 0.25*
G.V: combined captopril and nigella sativa oil treated group in CCl4 - injected rats	42.2 ± 2.6 *	2.2 ± 0.92*	4.93 ± 0.29 *

The results represent the mean ± S.E.of 8 rats.
o Significant differance compared to the normal group (negative control) (P <0.05).
*** Significant difference compared to the CCL4 - induced group (positive control) (P <0.05).**

Histolopathological Examination:

A-Light microscopic results:

Group I (negative control rats):

In H.&E. Stained sections, the parenchyma of the testis was formed of the seminiferous tubules and the interstitial tissue in between. Each seminiferous tubule was lined with stratified epithelium which was seen to be formed of germinal cells and supporting Sertoli cells. The germinal cells were stacked in the form of many layers from the basement membrane toward the lumen of the tubules. These layers were formed of spermatogenic cells, which are; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and mature sperms (Fig. 1).

In toluidine blue stained sections, the spermatogonia lied next to the basement membrane. They were of two types; type A spermatogonia which were the predominant ones and appeared dome shaped with large oval pale nuclei and prominent nucleoli. Type B cells were more rounded in shape with rounded nucleui. The primary spermatocytes represented the next row of cells, present in more than one layer and were considered the largest among the surrounding cells. They were rounded cells, with large nuclei with deeply stained chromatin granules of uniform size, which were distributed through the nucleoplasm (Fig. 2).

The secondary spermatocytes were smaller. The early or round spermatids were stacked into several layers (3-6) of rounded cells that had lightly stained cytoplasm and rounded vesicular nuclei. Sperms may appear in some tubules with condensed, flattened, elongated nuclei and their tails were directed toward the lumen. The Sertoli cells rest on the basement membrane of the tubule and extends to the lumen. They are elongated or pyramidal in shape and partially enveloped the spermatogenic cells. Each cell exhibits ill defined outlines, lightly stained cytoplasm, irregular pale vesicular nucleus and prominent large nucleolus (Figs. 1,2).

Using PAS, strong positive reaction was observed in the basement membrane of the seminiferous tubules and the interstitial tissue. The germ cells except the spermatid show negative reaction (Fig.3).

Using masson trichrome stain, few collagen fibers were observed in the basement membrane of the seminiferous tubules and around the blood vessels (Fig. 4).

Group II (CCL4 - injected rats):

Carbon tetrachloride injected rats showed degenerative changes in the seminiferous tubules. In H.&E. stained sections, there are marked distortion of the seminiferous tubules with epithelial vacuolations. The spermatogenic cells appeared deeply stained with dense nuclei (Fig.5).

In toluidine blue stained semithin sections showed separation and sloughing of the germinal epithelium (Fig.6).

Using PAS reaction, corrugated PAS positive basement membrane surrounding many semineferous tubules with separation and loss in some areas (Fig.7).

Thickening of the basement membrane was observed in some tubules. With masson trichrome stain, marked increase in the amount of collagenous fibers was observed in the basement membrane of the seminiferous tubules and around the blood vessels compared to the control group (Fig.8).

Group III (Captopril treated group in CCL4 - injected rats):

In H.&E. stained sections, many of the somniferous tubules appeared with less vacuolation with less separation of the cells. The interstitial connective tissue showed congested thick wall blood vessels (Fig.9).

In toluidine blue stained semithin sections, some tubules appeared with vacuolated cells (Fig.10).

Using PAS reaction the basement membrane appeared continuously compared to group II, but there was irregularity of the membrane in some areas (Fig.11).

With masson trichrome, there were less collagen fibers compared to group II (Fig.12).

Group IV (Nigella sativa treated group in CCL4 - injected rats):

H.&E. stained sections showed the semineferous tubules with less vacuolation compared to group II (Fig.13).

In toluidine blue stained sections showed many tubules appeared normal with the normal appearance of the cells. Some tubules showed separation between the cells. The interstitial tissue had thickened blood vessels (Fig.14).

Using PAS reaction, less thickened basement membrane with few irregularity compared to group II. Thickened blood vessels were observed (Fig.15).

Masson trichrome stained sections showed less collagen fibers compared to group II (Fig.16).

Group V: (Combined captopril and nigella sativa oil treated group in CCl4 - injected rats):

In H.&E. stained sections, most of the seminiferous tubules were more or less similar to control group (Fig.17).

B-Immunohistochemical results:

Group I (negative control rats):

Caspase-3 stained sections showed apparently normal spermatogenic cells with negative caspase-3 reaction (Fig.18).

Brownish stain indicated non specific reaction of the secondary antibody. Androgen receptor stained sections showed that the immunoreactivity was localized in nuclei of cells, which were presumably sertoli cells (Fig.19).

Group II (CCL4 - injected rats):

Caspase-3 stained sections showed multiple apoptotic germinal cells (Fig.20).

Sections stained with androgen receptors showed a decrease in the reaction of androgen receptor in the nuclei of the cells (Fig.21).

Group III (Captopril treated in group CCL4 - injected rats):

Sections stained with caspase-3 showed few apoptotic cells in the tubules (Fig.22).

Many androgen receptor positive cells were observed (Fig.23).

Group IV (Nigella sativa treated group in CCL4 - injected rats): Caspase-3 stained sections showed less apoptotic cells compared to previous groups (Fig.24).

Androgen receptor stained sections showed more positively stained cells (Fig.25).

Group V: (Combined captopril and nigella sativa oil treated group in CCl4 - injected rats): Caspase -3 stained sections showed few positive apoptotic cells (Fig.26).

Many positively stained androgen receptor cells were observed (Fig.27).

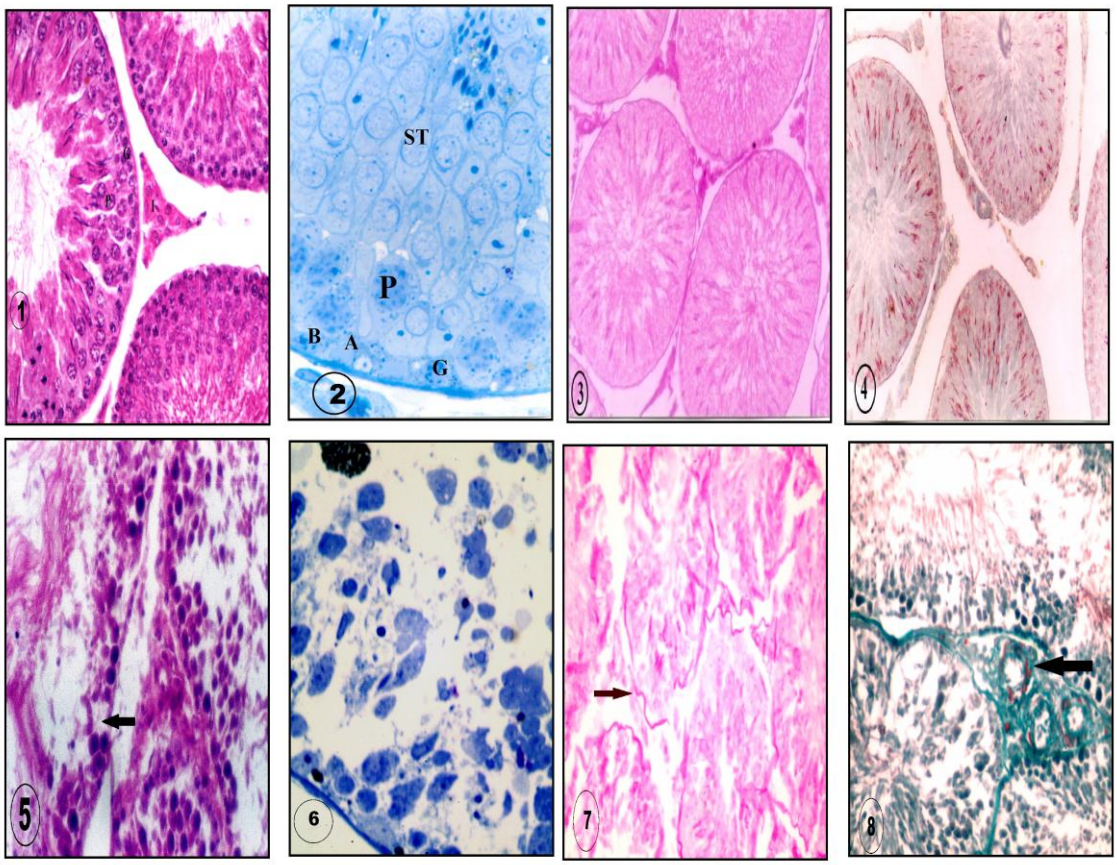

Fig.1:A photomicrograph of a section in the testis of group I showing the normal the semineferous tubules. (H&E, x400)

Fig.2: A photomicrograph of a semithin section of the testis of group I showing the spermatogonia (G). They are of two types; type (A) spermatogonia and type (B) cells. The early spermatids (ST) are stacked into several layers. (Toluidine blue x1000)

Fig.3: A photomicrograph of a section in the testis of group I showing PAS positive reaction in the basement membrane, spermatids and interstitial tissue. (PAS x400)

Fig.4:A photomicrograph of a section in the testis of group I showing few collagen fibers are observed in the basement membrane of the seminiferous tubules and around the blood vessels. (Masson trichrome x400).

Fig.5: A photomicrograph of a section in the testis of group II showing that are marked distortion of the tubules with epithelial vacuolation (arrow). The spermatogenic cells appear deeply stained with dense nuclei. (H&Ex 400)

Fig.6: A photomicrograph of a semithin section in the testis of group II showing separation and sloughing of the germinal epithelium. (Toluidine blue x1000)

Fig.7:A photomicrograph of a section in the testis of group II showing corrugated basement membrane (arrow) surrounding many semineferous tubules with separation and loss in some areas. (PASx400)

Fig. 8:A photomicrograph of a section in the testis of group II showing marked increase in the amount of collagenous fibers is observed in the basement membrane of the seminiferous tubules and around the blood vessels (arrow) compared to group I. (Masson trichrome x400)

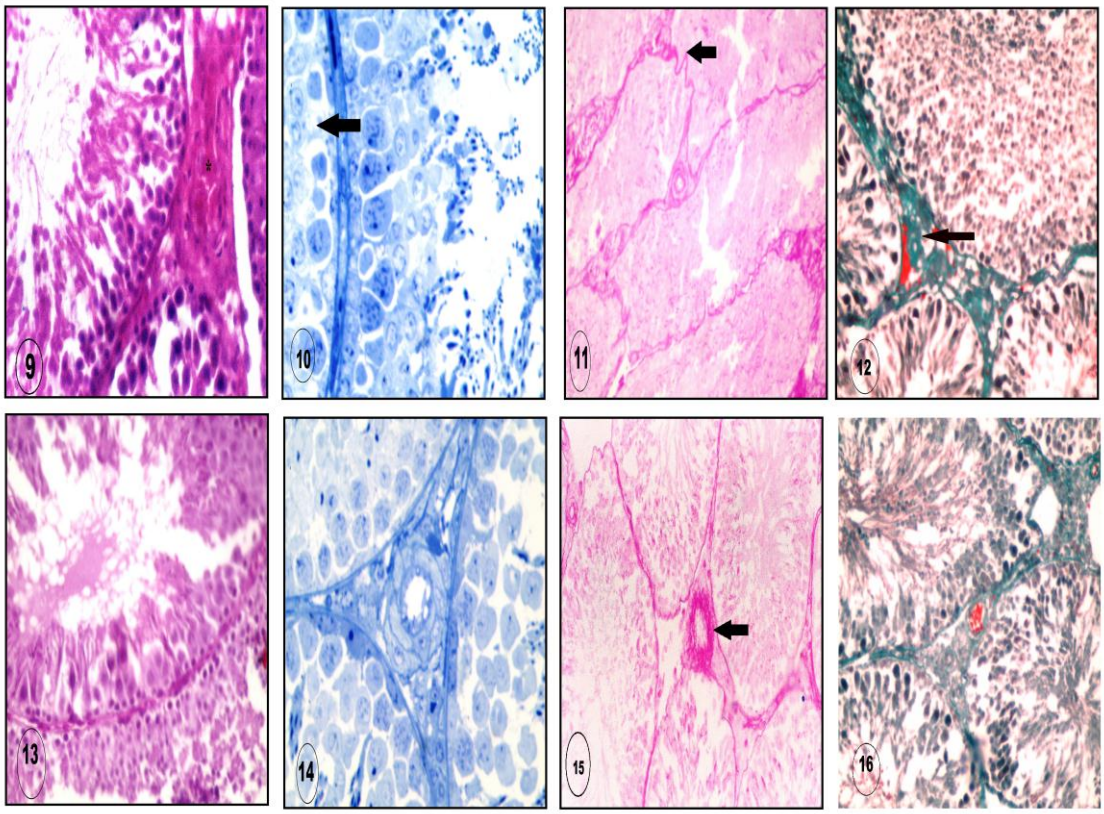

Fig.9: A photomicrograph of a section in the testis of group III showing the semineferous tubules appeared with less vacuolation with less separation of the cells. The interstitial connective tissue showed congested thick wall blood vessels. (H&E x400)

Fig.10: A photomicrograph of a semithinsection in the testis of group III showing some tubules appears with vacuolated cells (arrow). (Toluidine blue x1000)

Fig.11: A photomicrograph of a section in the testis of group III showing the basement membrane appears continuous with irregularity of the membrane in some areas (arrow). (PAS x400)

Fig.12: A photomicrograph of a section in the testis of group III showing there is less collagen fibers compared to group II with moderate collagen fibers around blood vessels (arrow). (Masson trichrome x400)

Fig.13: A photomicrograph of a section in the testis of group IV showing, the semineferous tubules with less vacuolation compared to group II. (H&E x400)

Fig.14: A photomicrograph of a semithin section in the testis of group IVshowing some tubules have spaces between the cells. The interstitial tissue had thickened blood vessels. (Toluidine blue x 1000)

Fig.15: A photomicrograph of a section in the testis of group IV showing less thickened basement membrane with few irregularities compared to group II. Thickened blood vessels were observed (arrow). (PASx400)

Fig.16: A photomicrograph of a section in the testis of group IVshowing less collagen fibers compared to group II. (Masson trichromex400)

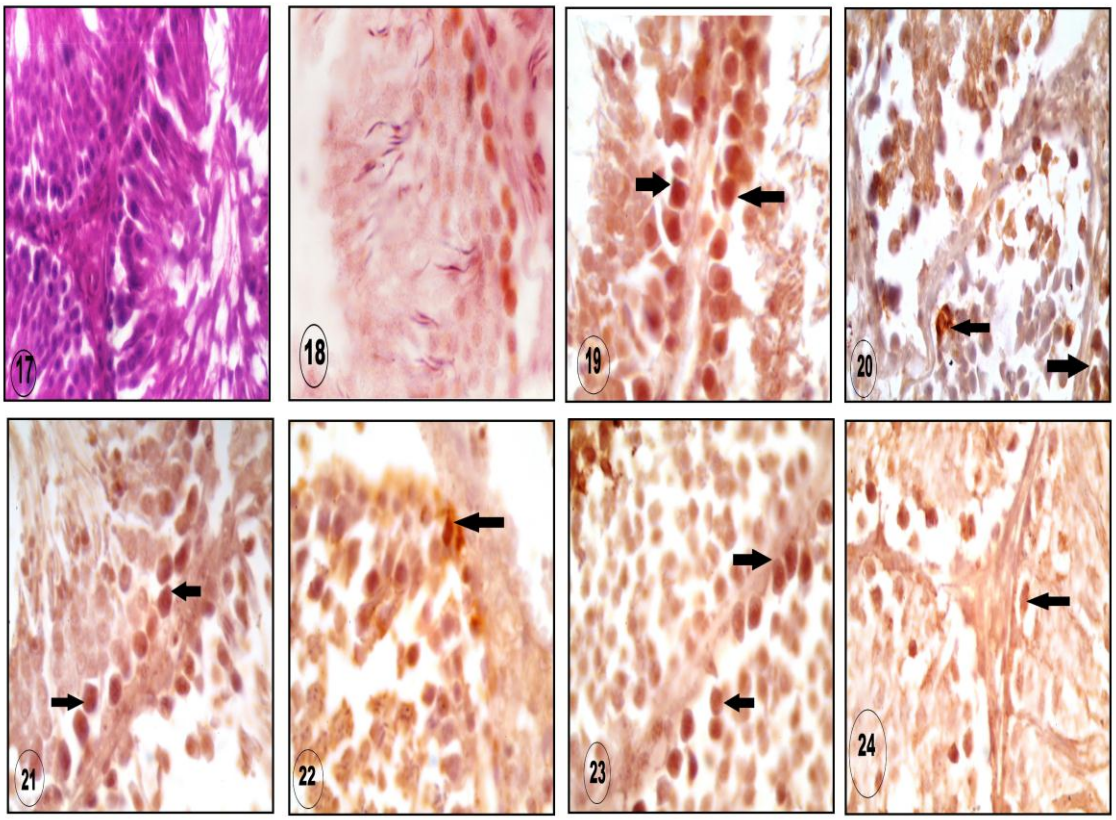

Fig.17: A photomicrograph of a section in the testis of group V showing that most of the seminiferous tubules were more or less similar to control group. (H&Ex400)

Fig.18: A photomicrograph of a section in the testis of group I showing apparently normal spermatogenic cells with negative caspase-3 reaction. (Caspase-3 x1000)

Fig.19: A photomicrograph of a section in the testis of group I showing that immunoreactivity for androgen receptor is localized in nuclei of cells, which were presumably sertoli cells. (Androgen receptorx1000)

Fig. 20: A photomicrograph of a section in the testis of group II showing multiple apoptotic germinal cells (arrow). (Caspase-3x1000)

Fig. 21: A photomicrograph of a section in the testis of group II showing a decrease in the positive reaction of androgen receptor in the nuclei of the cells (arrow). (Androgen receptorx1000)

Fig.22: A photomicrograph of a section in the testis of group III showing few apoptotic cells in the tubules (arrow). (Caspase-3x1000)

Fig.23: A photomicrograph of a section in the testis of group III showing many androgen receptor positive cells is observed (arrow). (Androgen receptor x1000)

Fig.24: A photomicrograph of a section in the testis of group IV showing less apoptotic cells compared to previous groups (arrow). (Caspase-3, x1000)

Fig.25: A photomicrograph of a section in the testis of group IV showing more positively stained cells compared to the previous groups (arrow). (Androgen receptor, x1000)

Fig.26: A photomicrograph of a section in the testis of group V showing few positive apoptotic cells (arrow). (Caspase-3, x1000)

Fig.27: A photomicrograph of a section in the testis of group V showing many positively stained androgen receptor cells (arrow). (Androgen receptor, x1000)

DISCUSSION

One of the important factors that causes male infertility is the exposure to numerous toxic substance that disturbs the oxidant/antioxidant balance of the male reproductive system (Türk et al., 2016).

CCL4 is used widely in the dry cleaning industry, filling fire extinguishers, the fumigation of grains, as an insecticide, propellants for aerosol cans and manufacturing refrigeration fluid (Sahreen et al., 2015 and Diab et al., 2018).

It has been used widely to induce oxidative stress in experimental animals. It causes free radical liberation in many tissues such as the liver, kidney, heart, lung, testis, brain and blood (Abdou et al., 2012, Pirinççioğlu et al., 2012 and Haghi et al., 2014).

This present study was designed to evaluate the possible protective effect and the underlying mechanism of single and combined administration of captopril and nigella sativa oil in CCL4 induced testicular toxicity including biochemical and histopathological investigations in the testicular tissue of male rats.

In the present work, administration of CCL4 in a dose of 1 ml/kg b.w. on alternate days for 8 weeks induced testicular toxicity in rats. The toxic effect may explained by the high affinity of the testis to CCL4.

The testes contain cytochrome P450 which activates the conversion of CCl4 to toxic metabolites. The initial step in the testicular tissue injury by CCl4 is its cytochrome P450-mediated formation of trichloromethyl radical (CCl3) and trichloromethylperoxyl (CCl3OO) radicals. The overproduction of these free radicals initiates membrane lipid and protein oxidation, eventually leading to various testicular damages (Noureen et al., 2017).

In addition, free radicals cause a decreased in testicular GSH contents and alteration of reproductive hormones, oxidative DNA damages, genetic mutation, DNA strand breakage and chromosomal

variations (Jia, et al., 2002 and Khan and Ahmed, 2009).

Several antioxidants have been used to prevent oxidative stress in testicular tissue (Manjrekar et al., 2008; Khan and Ahmed, 2009; Soliman and Fahmy, 2011 and Khan, 2012), corrected the disturbances in reproductive hormone levels (Khan and Ahmed, 2009 and Khan, 2012), improved the abnormalities in sperm (Abdou et al., 2012), the reduction in sperm count, motility and DNA fragmentation in testicular tissue (Khan, 2012).

Furthermore, they ameliorated the testicular histopathological lesions induced by CCl4 in rats (Manjrekar et al., 2008 and Khan and Ahmed, 2009).

In this study the protective effect of nigella sativa oil and captopril against CCl4 - induced testicular toxicity in male rats was evaluated. Nigella sativa seeds contain about 30% fixed oil, and 0.4–0.45% volatile oil.

The fixed oil is composed mainly of unsaturated fatty acids. Thymoquinone is the major active component of the volatile oil (Abdel-Zahar et al., 2011).

Additionally, the protective effect of captopril against CCL4 - induced testicular toxicity due to its ability to suppress oxidative stress damage because it contains active sulfhydryl group and shares other structural features with cysteine which is the principal substrate for glutathione. In addition, captopril was able to increase the glutathione peroxidase and glutathione reductase activities in various tissues (Chen et al., 2019).

The result of the present study revealed a significant increase in the level of NO and MDA with a significant reduction in the GSH and GSH-Px levels in testicular tissue in CCL4 - induced rats.

These results are in agreement with the previously published data that reported that CCL4 induced testicular oxidative stress in experimental studies (Khan and Ahmed, 2009; Kahn et al., 2012; Shah and Khan, 2017; Rahmouni et al., 2018).

Rats pretreated with the single or combined administration of nigella sativa oil or capopril resulted in a decrsease in the elevated level of MDA and NO and improved the antioxidant enzyme; GSH and GSH-Px. The reduction of GSH and GSH-Px activity during CCl4 in testicular tissue may be explained by the overproduction of free radicals leads to enhanced lipid peroxidation or inactivation of the anti-oxidative mechanism (Khan et al., 2012).

The present study was in accordance with other previous studies that indicated nigella sativa seed oil and or thymoquinone were found to suppress oxidative stress and improved antioxidant status induced by CCL4 in liver, kidney and blood (Meral and Kanter, 2003; Kanter et al., 2008; El-Sayed, 2011; Al-Seeni et al., 2016).

In addition, Captopril and other angiotensin converting enzyme inhibitors were found to suppress lipid perioxidation, oxidative stress and improved CCL4 - induced liver toxicity (El- Katib and Mansour, 2001 and Mansour et al., 2011).

Moreover, Chen and his colleagues (2019) demonstrated the ability of captopril to prevent oxidative stress damage via increasing glutathione peroxidase and glutathione reductase activities in the tissues. Besides, Ambreen and his coworkers (2011) revealed that pretreatment of rats with lisinopril, an angiotensin-converting enzyme inhibitor, for 2 weeks before CCL4 adminstration protected against hepato - carcinogenesis induced by diethyl-nitrosoamines and helped by carbon tetrachloride.

The results of the present study showed a significant decrease in the serum level of FSH, LH and testosterone hormones in CCL4 induced rats.

This result is in agreement with the previously published data that indicated CCl4 cause reduction of reproductive hormonal levels in male rats (Soliman and Fahmy, 2011; Pirinççioğlu et al., 2012; Khan, 2012 and Rahmouni et al., 2018).

The reduction of serum levels of these hormones can be probably explained by CCL4-induced oxidative stress damage in testicular tissue with subsequent degeneration of germinal cells.

In addition, the CCl4 toxic effects may affect the suprachiasmatic hypothalamic nucleus leading to the failure of anterior pituitary gland to secrete FSH and LH and resulting in testicular dysfunction (Khan et al., 2011).

In this study the single or combined pretreatment rats with captopril and nigella sativa oil of the CCl4- induced increased the serum levels of FSH, LH and testosterone.

These results can explained by captopril and nigella sativa oil ameliorated the CCl4 toxic effects on testicular tissue by preventing the excessive generation of free radicals with the subsequent improvement of the serum level of the reproductive hormones in rats.

Previous studies have indicated that several antioxidants ameliorated the testicular oxidative stress and the impairment in antioxidant status and improved the reduction in the reproductive homones level induced by CCl4 in male rats (Khan and Ahmed 2009; Cemek et al., 2010; Shah and Khan, 2017 and Rahmouni et al., 2018).

In harmony with the results of the present work, the previous studies that proved the protective effect of thymoquinone, the active component of volatile oil of nigella sativa seeds in different animal models of testicular oxidative stress induced by various chemical such as lead and methotrexate in adult rat testes (Gökçe et al., 2010; Mabrouk and Ben Cheikh, 2015; Mabrouk and Ben Cheikh, 2016).

Besides, Fouad and Jresat (2013) mentioned that captopril increased serum testosterone, suppressed lipid peroxidation, restored the depleted reduced glutathione, decreased the elevations of nitric oxide in testicular tissue resulted from cadmium administration and reduced the cadmium-induced expression of caspase-3 in testicular tissue by immunohistochemical analysis.

Finally, the obtained biochemical results were supported by the histopathogical part of this study.

It revealed that CCL4 induced degenerative changes in the testes in the form of distortion of the seminiferous tubules with vacuolation and sloughing of the germinal cells.

Corrugation in the basement membrane surrounding many semineferous tubules with separation and loss in some areas.

Thickening of the basement membrane was observed in some tubules. In addition, marked increase in the amount of collagenous fibers was observed in the basement membrane of the seminiferous tubules and around the blood vessels compared to the control rat group.

These results were in accordance with the previously published studies (Horn et al., 2006; Khan and Ahmed, 2009; khan 2012; Yüce et al., 2014; Sahreen et al., 2015 and Türk et al., 2016).

The pathological changes seen in the testis due to administration of CCL4 can be explained by the abundant affinity of the testicular tissues to CCl4.

Free radicals that are liberated from CCl4 bind to polyunsaturated fatty acid of sperm cell membrane to generate alkoxy and peroxy radicals that, in turn, alter sperm concentration, change hormonal levels, reduces enzyme activity and induction of testicular injury or necrosis (Ogeturk, et al., 2005).

Besides, necrosis of spermatocytes and/ or spermatids and seminiferous tubules degeneration (Guo, et., 2005 and Horn, et al., 2006).

Apoptosis is an indicator of DNA damage in the cells, including testicular germ cells, and an increase in free radicals results in increased apoptosis in testicular germ cell (Maheshwari et al., 2009).

Androgen is essential for male fertility and integrity of sexual functions. It exerts its actions via androgen receptors present in testicular cells. The expression of androgen receptor (AR) essential for spermatogenesis in the seminiferous tubules because androgen played a vital role in the initiation of spermatogenesis (Wang et al., 2009).

Some previous studies reported the presence of AR in germ cells, while others indicated that AR expressed only in Sertoli cells and interstitial area (Choong et al., 1996 and Abd El-Meseeh et al., 2016).

Mutational defects of AR that accompanying testicular cell dysfunction, which lead to defects in spermatogenesis. Consequently, quantitative decrease of the AR gene or protein expression and qualitative chromosomal defect in AR could be one of the causes of male sterility (Mou and Gui, 2016).

The immunohistochemical findings of the present study showed multiple apoptotic germinal cells associated with decreased in androgen receptor expression in CCL4-induced testicular toxicity in rats. It has been reported that CCl4 treated male rats induces testicular DNA damage and subsequent testicular apoptosis (Abdou et al., 2012; Khan, 2012; Yüce et al., 2014; Sahreen et al., 2015; Türk et al., 2016 and

Rahmouni et al., 2019), that is in harmony with our findings.

The increased testicular apoptosis can be explained by CCL4 induced lipid peroxidation and reduced antioxidant enzymes activity that may possibly cause testicular histopathological abnormalities and increase in testicular apoptosis with subsequent decrease in the androgen receptor expression.

In the present study the single and combined pretreatment with captopril and nigella sativa oil of CCL4 - induced rats resulted in improvement of the testicular histopathological changes and decreased the apoptoic changes in male rats with an improvement of androgen receptor expression in testicular tissue. These results can explained by the antioxidant effect of captopril and nigella sativa oil that suppress testicular oxidative stress and apoptosis with subsequent correction of the biochemical and histopathological changes induced by CCL4 in testicicular tissues in rats.

In agreement with these results several antioxidants have reported to ameliorate the histopatholoical changes induced by CCL4 adminstration (Yüce et al., 2014; Türk et al., 2016 and Rahmouni et al., 2019).

Ahmed and his coworkers (2018) showed finding similar to the results of the present work that royal jelly has a protective effect against cadmium - induced testicular toxicity by improving testicular oxidative stress, reproductive hormone levels. Furthermore, it ameliorated the histopathological changes in the testes and increased the expression of androgen receptor in testicular tissue due to its antioxidant effect.

CONCLUSION:

The results of the present study demonstrate the possible protective effect of the single and combined treatment with captopril and nigella sativa oil against CCl4 - induced testicular toxicity. This effect can be attributed to their antioxidant and anti-apoptotic effects.

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الملخص العربى

التأثير الوقائي للكابتوبريل وزيت حبة البركة والاستخدام المشترك لهما المضاد للتسمم المحدث برابع كلوريد الكربون على الخصيتين في ذكورالجرذان

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الملخص العربي :

يؤثر رابع كلوريد الكربون على الوظيفة التناسلية للذكور. هدفت هذه الدراسة إلى توضيح الدور الواقى المحتمل وطريقة العمل للكابتوبريل وزيت حبة البركة المضاد للتسمم المحدث برابع كلوريد الكربون على الخصيتين في ذكورالجرذان عند تناولهما بمفردهما ومجتمعين.أجريت هذه الدراسة على أربعين من ذكور الجرذان البيضاء البالغة تم تقسيمهم إلى خمسة مجموعات متساوية (كل منها ٨ جرذان). المجموعة الأولى هى جرذان المجموعة الضابطة. المجموعة الثانية هى الجرذان التى تم حقنها داخل البريتون برابع كلوريد الكربون المخفف بزيت البارفين يوم بعد يوم لمدة ٨ أسابيع بجرعة ١ميلى / كيلو جرام من وزن الجسم لمدة ٨ أسابيع. المجموعة الثالثة هى الجرذان التى تم اعطائها الكابتوبريل بجرعة ١٠٠ مجم / كجم يوميا. المجموعة الرابعة هى الجرذان التى تم اعطائها زيت حبة البركة بجرعة من ٤ مل / كجم يوميا بالفم. المجموعة الخامسة هى الجرذان التى تم اعطائها كلا من الكابتوبريل وزيت حبة البركة يوميا معا بنفس الجرعة المذكورة. تم اعطاء الادويه بالفم يوميا فى المجموعات الثالثه والرابعه والخامسه قبل رابع كلوريد الكربون باسبوعين وبالتزامن معه بنفس الجرعة المذكورة سابقا لمدة ٨ أسابيع. تم ذبح الفئران بعد ٢٤ساعة من اخر جرعة فى نهاية العشر أسابيع. تم اجراء الفحوصات الكيميائية لقياس مستويات أكسيد النيتريك والمالون ألدهيد والإنزيمات المضادة للأكسدة وهما الجلوتاثيون المختزل و الجلوتاثيون بيروكسيديز في نسيج الخصية وقياس مستوى هرمونات الخصوبه وهم الهرمون المنبة لتكوين الحويصلة والهرمون المنبه للجسم الاصفر والتستوستيرون فى المصل .وكذلك تم عمل الفحص الهستوباثولوجي بالميكروسكوب الضوئى والهستوكيميائية المناعية.

وقد أظهرت هذه الدراسة أن حقن الجرذان برابع كلوريد الكربون أدى إلى حدوث زيادة ذو دلالة احصائية فى مستويات أكسيد النيتريك والمالونالدهيد مع حدوث نقص فى مستويات الجلوتاثيون المختزل و الجلوتاثيون بيروكسيديز في نسيج الخصية. وانخفاض في مستوى هرمونات الخصوبه بالمصل. وأظهرت الفحوصات الهستوباثولوجية وجود تغيرات هستوباثولوجية فى الخصية فى المجموعة التى تم حقنها برابع كلوريد الكربون فى صورة وجود العديد من التغيرات الانحلاليه وزيادة موت الخلايا المبرمج الخلايا وانخفاض في مستقبلات الاندروجين . وقد اظهرت الدراسه ان معالجة الجرذان بالكابتوبريل أو زيت حبة البركة معا أو منفردين أظهر تحسن ذو دلالة احصائية فى القياسات الكيميائية وأيضا فى التغيرات الهستوباثولوجية والهستوكيميائية المناعية .

خلصت هذه الدراسة الى أن الكابتوبريل وزيت حبة البركة يمكن إستخدامهما كعلاج فى الوقاية من التسمم المحدث برابع كلوريد الكربون على الخصية وهذا التأثير نتيجة لدورهما فى كمضادات للاكسده وتقليل موت الخلايا المبرمج فى أنسجة الخصية.