J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8(1): 9 - 14 , 2017

Thymoquinone Protects Against Mercuric Chloride- Induced Hepatorenal Toxicity in Male Rats.

EL-Sawi, M. R.; M. A. Amer and Soad Muftah
Zoology Department, Mansoura University, Mansoura- EGYPT

ABSTRACT

The liver and kidney are imperative intentions for the toxicity of drugs, xenobiotics, thymoquinone (TQ) against mercuric chloride ($HgCl_2$) induced hepatorenal toxicity in rats. $HgCl_2$ at a dose of 0.02 mg/kg b w injected subcutaneously for 30 days, induced hepatotoxicity which confirmed in this study by increased activity ($P \leq 0.05$) of liver function enzymes; CPK, whereas, serum total protein content and Na^+-K^+-ATPase activity were decreased significantly. Herein, $HgCl_2$ induced also nephrotoxicity; as it induced noticeable signs of renal dysfunctions such as elevated levels ($P < 0.05$) of serum creatinine, blood urea nitrogen (BUN), uric acid as well as urine N-acetyl glucosaminidase (NAG) activity and total protein content. On the other hand there was a decline in the levels of urinary uric acid; creatinine and creatinine clearance. $HgCl_2$ induced also oxidative stress in both liver and kidney tissues where reduced glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activities decreased significantly; increased significantly. Additionally, TGF-β %, CD4% and CD8 % increased significantly in both liver and kidney tissues. In conclusion: Treatment of TQ concomitantly with $HgCl_2$ ameliorates effectively the significant changes of all the tested parameters.

Keywords: Thymoquinone, Mercuric chloride, Hepatorenal toxicity, Rats.

INTRODUCTION

Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immunological, dermatological, reproductive and developmental disorders (Risher and Amler, 2005). Its wide industry uses has related effects on human and animal biosystems have been well documented (WHO, 1991). Exposure to this biologically active chemical agent has been shown to be aggravated through contaminated water and food (Magos and Clarkson, 2006). Ghosh and Sil, (2008) stated that gastrointestinal tract, kidney and liver are targeted mainly by $HgCl_2$ form. In this respect, the biological toxicity of $HgCl_2$ has been explained by tracing the fate of various Hg forms in biological systems Gutierrez *et al.* (2006).

Initially, plasma ALT, AST, ALP activities were used to measure hepatotoxicity induced by $HgCl_2$-treatment in addition to SOD, CAT activities and GSH content of hepatic tissue (Bashandy and Al Wasel, 2011). Nephrotoxicity induced by $HgCl_2$ treatment was confirmed by measuring creatinine, BUN, creatinine clearance, urea, uric acid concentrations in plasma (Mahmoud *et al.,* 2014)).

Thymoquinone (the main compound of the essential oil of *Nigella sativa*) is 2-methyl-5-isopropyl-1,4-benzoquinone. TQ has an antioxidant effect as LPO in liposomes (Houghton *et al.,* 1995). TQ has potential scavenging power singlet oxygen molecules. The latter one is more common in plants. It is also a potential redundant of NO, LPO, MDA, myeloperoxidase (MPO) formations. In addition, TQ stimulates the molecular antioxidant systems such as GSH, GST, glutathione peroxidase (GPx), SOD and CAT (Darakhshan *et al.,* 2015).

TQ was found to increase mRNAs levels of some antioxidant enzymes such as GST (El-Sayed, 2011) reported that. Essawy *et al.* (2012) stated that *N. sativa,* where TQ is the main constituent, administration, significantly ameliorated alanine amino transferase and alkaline phosphatase disturbed by carbon tetra-chloride toxicity in rat. However, the hepato-protective activity of TQ observed in animal system is found to link with its antioxidant capacity and its up-regulation effects on GSH, GST, GPx, (Darakhshan, 2015). Cytoprotective effect of TQ against CCl_4-induced hepatic damage was communicate to be attributed to the antioxidant effects of thymoquinone (Hassanein *et al.*, 2016), they suggested that the protection was related to the ability of TQ to inhibit LPO.

The biochemical results obtained by Mahmoud *et al.* (2014) showed that GM-induced nephrotoxicity was associated with significant increases in blood urea, creatinine and serum TNF-α in rats and added that TQ attenuates such parameters of renal oxidative stress, inflammation and apoptosis.

Furthermore, Ragheb *et al.* (2009) concluded that TQ has protective effects on oxidative stress and nephropathy induced by doxorubicin (DOX) in rats. *Yaman and Balikci (2010)* also, confirmed the potency of *N sativa* to scavenge the free radicals induced by GM treatment and protect kidney against both biochemical and histological damages.

A study to see the protective effect of TQ against drug induced nephrotoxicity in rats was done by Chansoria *et al.* (2013). They found that TQ reduced the serum urea, creatinine and K^+ levels in drug induced renal toxicity and suggested that the nephroprotective effect of TQ was found to be significant.

The present study suggested that TQ can counteract exposure in liver and kidney tissues of rats and hence alleviating the altered biochemical features and ameliorating cellular damage. Therefore, the experiment was designed to reveal the potential beneficial effect of TQ against the hepatic toxicity which is accompanied with renal disorder due to $HgCl_2$ exposure.

MATERIALS AND METHODS

Chemicals

Thymoquinone was take from Sigma-Aldrich; ALT, AST, LDH kits from Spectrum, Cairo, Egypt. ALP, TP kits were purchased from Egyptian American Company for Laboratory Services, Egypt. Kit of N-acetyl-glucosaminidase from Biovision, California,

USA. Kits of GSH, MDA, CAT, SOD, NO, NOS, creatinine, urea, uric acid and CPK were purchased from Bio-Diagnostic Company, Giza, Egypt. The rest of the chemicals utilized were of analytical grade and were obtained from El-Gomhorea Company, Mansoura, Egypt. TGF-β, CD_4% and CD_8% were estimated using flow cytometry technique.

Materials

Animals

Twenty four adult male rats (*Rattus rattus*), weighing 100-120 gram, VACSERA center at Helwan city, Helwan, Egypt. Rats were fed on commercial chow and they had free access to water all the experimentation period. Randomly, rats were grouped in huge about 25 ± 2°C on a 12:12 h light/dark cycle. They are regrouped after a week of acclimatization as follow: Control group: Rats received no treatment, TQ group: Rats received daily dose of 10 mg/Kg b w (intraperitoneal route) for 30 days (as reported by Apaydın *et al.,* 2016), $HgCl_2$ group: Rats injected with daily dose of 0.02 mg $HgCl_2$/Kg b. wt. (subcutaneously) for 30 consecutive days (as reported by Fouda *et al.,* 2008) and TQ & $HgCl_2$ group: Rats received both TQ as in group 2 and $HgCl_2$ as in group 3.

Urine, Blood collection and serum preparation

24h urine samples were collected for each rat, filtered. At the end of the experiment, the animals were fasted overnight, after which they were anaesthetized and sacrificed to obtain blood samples. Blood samples were collected in centrifuge tubes, allowed to stand for 30 min at room temperature to clot, and then centrifuged for 10 minutes at 3000 rpm to separate the sera. The blood was obtained from all animals by cutting the lateral jugular veins with sharp razor. Urine and sera samples were kept at -20°C for the estimation of various biochemical parameters later on.

Liver and kidney tissue homogenates preparation

Sacrificed rats were immediately dissected, their livers and kidneys were excised, washed by cold normal for each. A volume of the homogenate was used for GSH estimation was collected and stored at – 20 ^{0}C for subsequent biochemical analyses.

Statistical analyses

All statistical analyses such as mean (M), standard error (SE) and least significant difference (LSD) between groups were calculated using the software SPSS version 16. The values at $p \leq 0.05$.

RESULTS

Table 1 showed that, the mean value of serum ALT, AST, ALP and CPK as well as tissue LDH activities were statistically increased in rats injected with $HgCl_2$; whereas TP content and Na^+-K^+-ATPase activity decreased with both control and TQ. Treatment of $HgCl_2$ simultaneously with TQ decreased significantly serum ALT, AST, ALP and CPK activities when compared with $HgCl_2$ group; whereas TP content and Na^+-K^+-ATPase activity were significantly increased (no significant changes were seen with respect to control group). Treatment of rats with TQ only has no significant effects on all tested parameters when compared with the control rats.

Table 1. Liver function parameters of control and different treated rat groups.

Groups Parameters	Control	TQ	$Hgel_2$	TQ+ $Hgel_2$
Serum				
ALT (U/L)	37.5±1.54	32.6±2.80	69.0±6.4^{ab}	44.9±2.92^{bc}
AST (U/L)	69.0±2.35	67.1±3.63	117.6±8.28^{ab}	81.6±4.55^{c}
ALP (U/L)	216.2±8.34	198.6±9.13	363.1±33.69^{ab}	270.6± 32.34^{bc}
TP (mg/dl)	6.6±0.09	6.74±0.08	4.41± 0.48^{ab}	5.79± 0.28^{bc}
CPK (U/L)	160.5±0.92	162.6±1.96	322.1±36.28^{ab}	212.1±9.25^{c}
Tissue				
LDH (U/mg)	806.7±9.27	805.4±4.03	1304.9±111.55^{ab}	895.0± 52.96^{c}
Na+-K+-ATPase (U/mg protein)	7.86±0.11	7.84±0.10	6.71±0.16^{ab}	7.62±0.14^{c}

Values are expressed as M± SE of 8 animals.

Results in table 2 showed that, the mean value of liver superoxide dismutase and glutathione reduced content were significantly reduced in rats injected with $HgCl_2$ when compared with both control and TQ groups; whereas MDA, NO contents and NOS activity were significantly increased. Treatment of $HgCl_2$ simultaneously with TQ increased significantly liver SOD and CAT activities when compared with $HgCl_2$ group; whereas MDA, NO contents and NOS activity were significantly decreased (no significant changes were seen with respect to control group). Treatment of rats with TQ only has no significant effects on liver SOD, CAT, NOS activities as well as MDA, GSH and NO contents.

Table 2. Hepatic oxidative stress parameters of control and different treated rat groups.

Groups Parameters	Control	Thymoquinone	$Hgcl_2$	Thymoquinone+ $Hgcl_2$
MDA (mmol/g)	78.13±2.27	68.97±3.38	106.49±3.62^{ab}	77.13±4.91^{c}
GSH (µmol/g)	24.47± 1.39	25.99±1.24	19.33± 1.25^{ab}	26.38±1.70^{c}
SOD (U/mg)	21.51±1.24	24.50±1.15	15.70±1.54^{ab}	21.48±1.21^{c}
CAT (mol/min/g)	1.07± 0.05	1.10±0.05	0.69±0.09^{ab}	1.05±0.06^{c}
NOS (Pmol/min/mg)	17.96±0.89	17.97±1.05	26.61±2.32^{ab}	20.90±2.59^{c}
NO (µmol/g tissue)	150.27±8.80	143.70± 4.46	195.61±8.41^{ab}	163.52±5.99^{c}

Values are expressed as M± SE of 8 animals.

Table 3 demonstrated the mean percentage of liver TGF-β, CD4 and CD8 were significantly elevated in rats injected with $HgCl_2$ when compared with both control and TQ groups. Treatment of $HgCl_2$ simultaneously with TQ increased significantly liver TGF-β, CD4 and CD8 percent in comparison with $HgCl_2$ group, but does not exhibit significant changes comparing to normal control group, referring to restoration of normal levels. no significant effects on liver TGF-β, CD4 and CD8 percent.

Table 3. Hepatic immune response markers of control and different treated rat groups.

Groups Parameters	Control	TQ	$Hgcl_2$	TQ+$Hgcl_2$
TGF-β %	31.61± 0.61	33.71± 1.42	55.38± 3.95^{ab}	40.46 ± 2.59^{ac}
CD4 %	27.1± 1.24	25.25±1.11	38.56± 3.21^{ab}	27.58±1.16^{c}
CD8 %	28.50± 3.05	25.94± 2.42	48.98± 2.8 ^{ab}	31.96± 1.27 ^{c}

Values are expressed as M± SE of 8 animals.

Results in table 4 showed that, the mean value of serum creatinine, urea, uric acid and BUN contents as well as urine TP and urine NAG activity were significantly increased in rats injected with $HgCl_2$ when compared with both control and TQ groups; whereas creatinine clearance, urine urea and urine uric acid contents as well as tissue Na^+-K^+-ATPase, LDH and ALP were significantly decreased. Treatment of $HgCl_2$ simultaneously with TQ decreased significantly as well as urine TP and urine NAG activity when compared with $HgCl_2$ group; whereas creatinine clearance, urine urea and urine uric acid contents as well as tissue Na^+-K^+-ATPase, LDH and ALP were significantly increased and many of them have restored normal levels but the others did not. Treatment of rats with TQ has no significant effects on all tested parameters.

Table 4. Kidney function markers of control and different treated rat groups.

Groups Parameters	Control	TQ	$Hgcl_2$	TQ +$Hgcl_2$
Serum				
Creatinine (mg/dl)	0.45±0.01	0.45±0.01	0.73±0.07^{ab}	0.49± 0.02^{c}
Urea (mg/dl)	24.48± 0.55	23.97±0.79	62.20±7.09^{ab}	35.49±2.84^{bc}
Uric acid (mg/dl)	2.33± 0.04	2.37± 0.08	3.94± 0.14^{ab}	2.79± 0.28^{ac}
BUN (mg/dl)	11.38 ±0.24	11.15± 0.37	29.01 ±2.87 ^{ab}	16.54^{bc}± 1.32
Creatinine Clearance (ml/min/100 g)	0.53±0.01	0.52±0.01	0.20±0.02^{ab}	0.39± .03^{abc}
Tissue homogenate				
Na+-K+-ATPase (U/mg protein)	4.35±0.03	4.48±0.06	3.42±0.26^{ab}	4.13±0.10^{c}
ALP (U/mg)	230.7± 10.55	219.1 ±15.81	160.1 ± 14.6 ^{ab}	205.3 ±13.0 ^{ac}
LDH (U/mg)	42.84± 1.15	44.37±1.24	27.96± 2.65 ^{ab}	38.8± 0.78 ^{c}
Urine				
NAG (mU/ mg creatinine)	10.63± 0.88	10.43±0.33	29.05±3.68^{ab}	18.1±1.47^{abc}
Total protein (mg/dl)	0.58±0.04	0.62±0.03	2.40± 0.15^{ab}	1.33±0.14^{abc}
Creatinine (mg/dl)	84.72±0.98	84.93±1.52	61.40±5.42^{ab}	74.9±2.51^{abc}
Uric acid (mg/dl)	7.77±0.27	7.66±0.32	4.00±0.56^{ab}	6.93±0.53^{c}

Values are expressed as M± SE of 8 animals.

Results in table 5 showed that, the mean value of kidney SOD and CAT activities as well as GSH content were decreased significantly in rats injected with $HgCl_2$ whereas, MDA, NO contents and NOS activity were significantly increased when compared with both control and TQ. Treatment of $HgCl_2$ simultaneously with TQ increased significantly kidney SOD and CAT activities when compared with $HgCl_2$ group; whereas MDA, NO contents and NOS activity were significantly decreased. Treatment of control rats with TQ has no significant effects on kidney SOD, CAT and NOS activities as well as MDA, GSH and NO contents in comparison with the normal control values**.**

Table 5. Renal oxidative stress markers of control and different treated rat groups.

Parameters	Control	TQ	Hgcl2	TQ + $Hgcl_2$
MDA(mmol/g)	61.7± 2.26	57.29± 1.9	85.70±5.20^{ab}	67.95± 3.72^{c}
GSH (µmol/g)	0.66± 0.03	0.73±0.07	0.32±0.04^{ab}	0.75± 0.05^{c}
SOD (U/mg)	12.2± 0.32	13.53±0.51	8.10±0.96^{ab}	10.61±0.80^{bc}
CAT (mol/min/g)	32.5±1.06	33.44±0.97	22.21±2.02^{ab}	31.80±1.79^{c}
NOS(Pmol/min/mg)	10.6±0.42	10.19± 0.87	19.61± 0.73^{ab}	15.73±1.36^{abc}
NO (µmol/g tissue)	108.1±3.21	99.97±4.32	159.56±10.24^{ab}	106.85±21.46^{c}

Values are expressed as M± SE of 8 animals.

Results in table 6 showed that, the mean percentage of kidney TGF-β, CD4 and CD8 significantly increased in rats injected with $HgCl_2$ comparing to both control and TQ groups. Treatment of $HgCl_2$ simultaneously with TQ increased significantly kidney TGF-β, CD4 and CD8 percent when compared with $HgCl_2$ group. Rats treated with TQ showed insignificant changes in kidney TGF-β, CD4 and CD8 percent.

Table 6. Renal immune response markers of control and different treated rat groups.

Groups Parameters	Control	Thymoquinone	$Hgcl_2$	Thymoquinone +$Hgcl_2$
TGF-β %	33.88± 0.91	33.05± 0.64	54.76 ± 3.48^{ab}	45.11 ± 2.1^{abc}
CD4 %	22.85± 1.1	22.8±1.4	40.43 ± 2.59^{ab}	28.08 ± 3.27^{c}
CD8 %	29.520± 2.9	28.41± 2.51	55.21 ± 0.86^{ab}	40.48 ± 3.06^{abc}

Values are expressed as M± SE of 8 animals.

DISCUSSION

In the present research, TQ was investigated as a protective agent against $HgCl_2$-induced hepatorenal toxicity in male rats. This toxicity was evidenced by the alteration in liver marker enzymes; which confirmed in this study by increased activity of serum AST, ALT, ALP and LDH enzymes, which are considered as good markers to circulation. The obtained results go parallel with the other findings reported by several authors (Rajesh and Latha, 2004; Bashandy and Al Wasel, 2011). Ravikumar *et al.* (2005) suggested that, rise in liver homogenate ALT activity is always due to damage of hepatocytes and is usually incorporated with rise in AST and ALP activities. They added that, ALT and AST are cytoplasmic aminotransferases that release extracellularly into the circulation upon hepatocytes damage. They are commonly used as biomarkers for measuring hepatocellular injury in both experimental and clinical investigations (Goorden *et al.,* 2013) and reflects the hepatocellular damage. TQ alleviated increased levels of serum enzymes activity and caused subsequent recovery toward normal levels, indicating plasma membrane stabilization as well as hepatic tissues repair. The present results are in accordance with some previous studies (Ravishah *et al.,* 2012). Treatment of $HgCl_2$ concomitant with TQ ameliorated serum ALT, AST, ALP and CPK activities when compared with their levels in $HgCl_2$-intoxicated animals; whereas TP content and Na^+-K^+-ATPase activities were significantly increased reaching them to the normal control levels. These results confirm the ameliorative effect of TQ and are compatible with the previous results (Darakhshan *et al.,* 2015). In addition, the study demonstrated that $HgCl_2$ exposure resulted in a highly significant depletion in hepatic and renal GSH level, SOD and CAT activities. In contrast to significant increase in MDA (lipid peroxidation index) NO and NOS levels. Truly, Hg^{2+} form has proven a tremendous containing -SH residue and is found to attach to small molecular weight peptides along with GSH, amino acids together with cysteine and SH-containing proteins (Perottoni *et al.,* 2004b); leading to a profound deterioration of the important metabolic procedures. Hg-brought on pathological components (Wiggers *et al.,* 2008).

As an antioxidant; glutathione by carrying or reacting with mercury, GSH forms glutathione-mercury complex that save cellular proteins from binding to mercury and preventing it from causing damage to both enzymes and tissues (Kromidas *et al.,* 1990) and decreases significantly the intracellular damage by preventing mercury from entering cells or tissues.

Co-administration of TQ increased significantly liver and kidney SOD and CAT activities when compared with $HgCl_2$ group; whereas MDA, NO contents and NOS activity were significantly decreased. This ameliorating effect of TQ may be due to it has potential scavenging power ROS, It is also a potential redundant of NO and TBARS formations. In addition, TQ stimulates the molecular antioxidant systems such as GSH content, SOD and CAT enzymes (Darakhshan *et al.,* 2015). Furthermore, the antioxidant effect of TQ, inhibit non-enzymatic lipid peroxidation in liposomes (Houghton *et al.,* 1995). This antioxidant property of TQ explains its protective action against hepatotoxicity (Nagi *et al.,* 1999). The findings of the present study go in parallel with the study of Essawy *et al.* (2012) and Umar *et al.* (2012). However, the hepato-protective activity of TQ observed in the animal system of the present study is found to link with its antioxidant capacity and its down-regulation effects on ALP, ALT and AST (Darakhshan, 2015).

In the present study, regarding renal function markers, the mean value of serum blood urea nitrogen contents well as urine TP and urine NAG activity were significantly increased in rats injected with $HgCl_2$; whereas creatinine clearance, urinary urea and uric acid contents as well as tissue Na^+-K^+-ATPase, LDH and ALP activities were significantly decreased. Treatment of $HgCl_2$ simultaneously with TQ decreased significantly serum creatinine, urea, uric acid and BUN contents as well as urine TP and urine NAG activity; whereas creatinine clearance, urinary urea and uric acid contents as well as tissue Na^+-K^+-ATPase, LDH and ALP activities were significantly increased when compared with $HgCl_2$ intoxicated group.

The present conclusion has been confirmed earlier results obtained by Badary *et al.* (1997) who stated that TQ alleviates the nephrotoxicity induced by cisplatin and enhances its antitumor activity. The authors related this protective action of TQ to its antioxidant property. The present findings, are comparable with early findings of El Daly (1998), Abul-Nasr *et al*. (2001) and Budancamanak *et al.* (2006) who investigated the protective effects of TQ on the renal injury in collagen-induced arthritis and concluded that TQ treatment was able to reduce significantly blood urea, serum NO and creatinine. TQ was significantly seen to lower blood urea, serum creatinine, triglycerides and total cholesterol (Sayed-Ahmed and Nagi, 2007 and Ragheb *et al.,* 2009). They suggested that the nephro-protective activity of *N. sativa* acts in the kidney as a potent scavenger of free radicals that prevent the toxic effects and alteration of the biochemical parameters. Similarly, Yaman and Balikci (2010), Yildiz *et al.* (2010), Hadjzadeh *et al.* (2012), Saleem *et al.* (2012) and Chansoria *et al.* (2013) have got the same results and conclusions.

Concerning the immune system, the main function of CD4 cells is the regulation of immune responses by recognizing the presence of antigens and toxins. The activated CD4 lymphocytes produce then, cytokines which increase the effectiveness of CD8 lymphocytes (Hidayati and Habib, 2015). The authors also, reported that *N. sativa* oil has an antiimmuonotoxic effect. In the present study, the percentages of CD4+ and CD8+ cells as well as TGF-β of both liver and kidney were increased significantly in $HgCl_2$ group in comparison with control group while, they decreased in $HgCl_2$ concomitantly treated with TQ rats comparing with $HgCl_2$. The elevated CD4+ and CD8+ percentage may be relate to the abnormal immune status due to $HgCl_2$ toxicity. Our finding go parallel with that of Xiaojing Hu *et al*. (2009) and Kiely et al. (1996) who registered significant increases in both CD4 and CD8 in brown Norway rats as a result of induction of T-cell autoimmune syndrome due to $HgCl_2$ treatment. Their data provide an evidence that CD8 act in proinflammatory capacity this may be due to increased percentage of TGF-β which may increase the release of collagen.

In Conclusion: The results of the present study obtained, herein, demonstrated a clear hepatorenal-protective action by thymoquinone in this experimental model against $HgCl_2$-induced hepatorenal toxicity. The overall hepatorenal-protective effect of TQ is probably due to a counteracting of free radicals due to its antioxidant potential and its scavenging capacity of free radicals as well as enhancing antioxidant and upregulation of immune system. Thus, this study suggests co-treatment of TQ along with $HgCl_2$-intoxication will definitely be helpful to solve the problem associated with hepatorenal-toxicity.

REFERENCES

Abul-Nasr S. M., El-Shafey M. D. M. and Osfor M. M. H. (2001): Amelioration by *Nigella sativa* of methotrexate induced toxicity in male albino rats: a biochemical, haematological and histological study. *Scintia Agri Bohemica.*, 32, 123–160.

Al-Gharably N. M., Badary O. A., Nagi M. N., Al-Sawaf H. A., Al-Rikabi A. C. and Al-Bekairi A. M. (1997): Protective effect of thymoquinone against carbon tetrachloride-induced hepatotoxicity in mice. *Res Commun Pharmacol Toxicol.*, 2, 41–50.

Apaydın F. G., Baş H., Kalender S. and Kalender Y. (2016): Subacute effects of low dose lead nitrate and mercury chlorideexposure on kidney of rats. *Environ Toxicol Pharmacol*., 41, 219-24.

Badary O. A., Nagi M. N., Al Shabanah O. A., Al Shawaf H. A., Al Sohaibani M. O. and Al Bekairi A. M. (1997): Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. *Can J Physiol Pharmacol*., 75, 1356–1361.

Barcellos-Hoff M. H. and Dix T. A. (1996): Redox-mediated activation of latent transforming growth factor-beta 1. *Mol. Endocrinol*., 10, 9, 1077–83.

Bashandy S. and Al Wasel S. (2011): Carbon tetrachloride-induced hepatotoxicity and nephrotoxicity in rats: Protective role of vitamin C. *JPT*., 6, 30, 283-292.

Budancamanak M., Kanter M., Demirel A., Ocakci A., Uysal H. and Karakaya C., (2006): Protective effects of thymoquinone and methotrexate on the renal injury in collagen induced arthritis. *Arch Toxicol.,* 80, 768-76.

Chansoria A., Trivedi M., Salman M. and Dixit R. (2013): Evaluation of protective effect of thymoquinone against anti-tubercular drug induced nephrohepatic toxicity in rats. *IJRS.*, 2, I-1, 1-4.

Clarkson T. W. and Magos L. (2006): The toxicology of Hg and its chemical compounds. *Crit Rev Toxicol*., 36, 609–662.

Darakhshan S., Bidmeshki P. A., Hosseinzadeh C. A. and Sisakhtnezhad S. (2015): Thymoquinone and its therapeutic potentials. *JPR*., 95-96, 138-158.

El-Daly E. S. (1998): Protective effect of cysteine and vitamin E. *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. *J Pharm Bel*., 53, 87–95.

El-Sayed W. M. (2011): Upregulation of chemoprotective enzymes and glutathione by *Nigella sativa* and thymoquinone in CCl4-intoxicated rats. *Int J Toxicol.,* 30, 707-714.

Essawy A. E., Abdel-Moneim A. M., Khayyat L. I. and Elzergy A. A. (2012): *Nigella sativa* seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. *JAPS*., 2, 21-25.

Forouzanfar F., Bazzaz B. S. F. and Hosseinzadeh H. (2014): Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. *Iran J Basic Med Sci.,* 17, 12, 929–938.

Fouda A. M., Daba M. H., Dahab G. M. and Sharaf El-Din O. A. (2008): Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin Pharmacol Toxicol.,* 103, 2, 109-18.

Ghosh A. and Sil P. C. (2008): A protein from Cajanus indicus Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. *Biol. Pharm. Bull*., 31, 9, 1651-1658.

Goorden S. M., Buffart T. E., Bakker A. and Buijs M. M. (2013): Liver disorders in adults: ALT and AST. *Ned Tijdschr Geneeskd,* 157, A6443.

Gutierrez L. L. P., Mazzotti N. G., Araújo A. S. R., Klipel R. B., Fernandes T. R. G., Llesuy S. F. and Belló-Klein A. (2006): Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. Braz. *J. Med. Biol. Res.,* 39, 767-772.

Hadjzadeh M. A., Keshavarzi Z., Yazdi T. S. A., Ghasem S. M., Rajaei Z. and Khajavi R. A. (2012): Effect of alcoholic extract of *Nigella sativa* on cisplatin-induced toxicity in rat. *Iran J Kidney Dis.,* 6, 2, 99–104.

Hidayati T. and Habib I. (2015): Antiimmunotoxic of Black Cumin Seed Oil (*Nigella sativa* Oil) in DMBA (Dimethylbenzantracene)-Induced Mice. *Int. J. Pharm. Med. Biol. Sci.,* 4, 3, 171-174.

Houghton P., Zarka R., Heras B. and Hoult J. (1995): Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Medica*., 61, 33-36.

Hu X., Gu Y., Wang Y., Yun X. and Xu C. (2009): Increased CD4+ and CD8+ effector memory T cells in patients with aplastic anemia. *Haematologica*, 94, 428-429

Kiely P. D. W. and O'Brien D. O. (1996): Anti-CD8 treatment reduces the severity of inflammatory arthritis, but not vasculitis in mercuric chloride-induced autoimmunity. *Clin Exp Immunol.,* 106, 280-285.

Kromidas L., Trombetta L.D. and Jamall I. S. (1990): The protective effects of glutathione against methylmercury cytotoxicity. *Toxicol let*., 51, 6780.

Mahmoud A. M., Ahmed O. M. and Galaly S. R. (2014): Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. *Excli J.,* 13, 98–110.

Nagi M. N., Alam K., Badary O. A., Al-Shabanah O. A., Al-Sawaf H. A. and Al-Bekairi A. M. (1999): Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem Mol Biol Int.,* 47, 153-159.

Perottoni J., Rodrigues O. E. D., Paixao M. W., Zeni G., Lobato L. P., Braga A. L., Rocha J. B. T. and Emanuelli T. (2004b): Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic Hg and organoselenium compounds. *Food Chem. Toxicol.*, 42, 17–28.

Ragheb A., Attia A., Eldin W. S., Elbarbry F., Gazarin S. and Shoker A. (2009): The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review. *Saudi J Kidney Dis Transpl.,* 20, 741-52.

Rajesh M. and Latha M. (2004): Perliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulatio. *J. Ethnopharmacol.*, 91, 99-104.

Ravikumar V., Shivashangari K. and Devaki T. (2005): Hepatoprotective activity of Tridax procumbens against dgalactosamine/ lipopolysaccharide-induced hepatitis in rats. *J. Ethnopharmacol.,* 101, 55-60.

Ravishah S., Manjula S. N., Mruthunjaya K., Krishnanand P., Pramod C. K. N., Madhu R. M., Sweety J. and Basirian M. (2012): Hepatoprotective activity of roots of Lawsonia inermis against paracetamol and antitubercular drugs induced hepatotoxicity in rats. *Int. J. Pharm*., 2, 2, 306–316

Risher J. F. and Amler S. N. (2005): Hg exposure: evaluation and intervention, the inappropriate use of chelating agents in diagnosis and treatment of putative Hg poisoning. *Neurotoxicol.*, 26, 4, 691–699.

Saleem U., Ahmad B., Rehman K., Mahmood S., Alam M. and Erum A. (2012): Nephro-protective effect of vitamin C and *Nigella sativa* oil on gentamicin associated nephrotoxicity in rabbits. *Pak J Pharm Sci.*, 25, 4, 727–730.

Umar S., Zargan J., Umar K., Ahmad S., Katiyar C. and Khan H. (2012): Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *Chem Biol Interact*., 197, 1, 40–46.

Uz E., Bayrak O., Uz E., Kaya A., Bayrak R., Uz B., Turgut F., Bavbek N., Kanbay M. and Akcay A. (2008): *Nigella sativa* oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. *Am J Nephrol*., 28, 3, 517–522.

WHO (1991). World Health Organization. Inorganic Hg, *Environmental Health Criteria*, No 118, WHO, Geneva.

Wiggers G. A., Peçanha F. M., Briones A. M., Pérez-Girón J. V., Miguel M., Vassallo D. V., Cachofeiro V., Alonso M. J. and Salaices M. (2008): Low Hg concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. Am. J. Physiol. Heart Circ. Physiol., 295, 1033–1043.

Yaman I. and Balikci E. (2010): Protective effects of Nigella sativa against gentamicin-induced nephrotoxicity in rats. *Exp Toxicol Pathol*., 62, 2, 83–190.

Yildiz F., Coban S., Terzi A., Savas M., Bitiren M., Celik H. and Aksoy N. (2010): Protective effects of *Nigella sativa* against ischemia-reperfusion injury of kidneys. *Ren Fail.,* 32, 1, 126–131.

Yu Q. and Stamenkovic I. (2000): Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev.,* 14, 2 163–76.

التاثيرات الواقية للثيموكينون للسمية الكبدية- الكلوية المحدثه بواسطة الزئبق فى ذكور الجرذان

ممدوح رشاد فرج الصاوى ، ماهر عامر على عامر و سعاد مفتاح عون الله الشبو

قسم علم الحيوان ـ جامعة المنصورة

يعد الكبد والكلية بمثابة أهداف مؤكدة لحدوث التسمم بالعقاقير , والمضادات الحيوية الغريبه (الدخيله) , والاجهاد التأكسدى. ولقد كان الهدف من الدراسة الحالية هو تقييم دور الثيموكينون TQ فى مقاومة التسمم الكبدى الكلوى لدى الجرذان. ولقد تم جلب اثنان وثلاثون جرذا من الذكور (Rattusrattus) , تزن من 100–120جم, وذلك من مركز VACSERA بمدينة حلوان , فى مصر. ولقد تغذت الجرذان على طعام وماء وذلك بحسب المراد . وتم تجميع الجرذان بصورة عشوائية فى أقفاص بلاستيكيةضخمة (ثمانية جرذان فى كل قفص) عند درجة حراره 25±2 مئوية فى دائرة (حلقة) ضوئية/مظلمة ارتفاعها 12:12 (رطوبة). وتم تجميعهم بعد أسبوع من التأقلم كما يلى :مجموعة الكنترول: حيث لم يتلقى أى علاج , مجموعة TQ :حيث تلقت الجرذان جرعه يوميه مكونه من 10مجم/كجم من وزن الجسم . (التجويف البريتونى) لمدة 30 يوما متواصلة, مجموعة كلوريد الزئبق : تلقت الجرذان جرعه يوميه من 0.02 مجم/كلوريد الزئبق/كجم من وزن الجسم (تحت الجلد) لمدة 30 يوما متواصله ومجموعة الثيموكينون وكلوريد الزئبق: حيث تلقت الجرذان كلا من الثيموكينون كما فى المجموعة 2 وكلوريد الزئبق كما فى المجموعة 3. فكلوريد الزئبق بوزن 0.02 مجم/كجم من وزن الجسم .؛ بحقنه تحت الجلد لمدة 30 يوما متواصله , قد حفز التسمم الكبدى , والذى تأكد حدوثه فى هذه الدراسهمن خلال المستويات المتزايدة من انزيمات الكبد,ألانين أمينوترانسفيريز (ناقلة أمين ألانين) ,أسبارتات أمينو ترانسفيريز , الفوسفاتازالقلوى , اللاكتيت دى هيدروجينيزوالكرياتين فوسفوكينيز , بينما البروتين الكلى فى المصل ونشاط Na+–K+–ATPase قد انخفض بشكل كبير. ولقد حفز كلوريد الزئبق حدوث التسمم الكبدى ؛ كما أنه حفز علامات وأعراض الخلل الوظيفى الكلوى مثل ارتفاع مستويات الكرياتينين المصل , ونتروجين يوريا الدم , وحمض البوليك , ونشاط ان–أسيتيل جلوكوزامينيداز فى فى البروتينات الكلية والبول. ولقد انخفضت مستويات حمض البوليك البولى؛ والكرياتينين وتصفية الكرياتينين بشكل كبير. كما أن كلوريد الزئبق أيضا, قد حفز الإجهاد التأكسدى فى كل من أنسجة الكبد والكلية؛ وخفض من نسبة ومحتوى الجلوتاثيون , وأنشطة سوبر اكسيد ديسميوتيز وأنشطة الكتاليز انخفضت بصورة كبيرة؛ بينما ارتفعت نسبة ونشاط المالونديالديهايد وأكسيد النيتريك وأيضا نشاط سنيثاز اكسيد النيتريك بشكل كبير . ولقد زاد TGF-β% ،CD4% ،CD8% بصورة كبيرة فى أنسجة كلا من الكبد والكلية. وفى الختام فإن العلاج بالثيموكينون بشكل مصاحب مع كلوريد الزئبق يحسن بشكل فعال من التغيرات الكبيره لكل المعاملات التى تم اختبارها.