Protective Role of Coenzyme Q10 against Paraquat Induced Hepatotoxicity in Male Rats

[1]Elsayed k. Abdel-Hady and [2]Gamal H. Abdel-Rahman

Current address: [1,2]Biology Department, Faculty of Science, Taif University, Taif, KSA
[1]Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt
[2]Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt
nabro62@yahoo.com

Abstract: Paraquat is highly toxic compound for humans and animals. It has been used widely in agriculture as herbicide. The present study was designed to investigate the potential protective effect of Coenzyme Q10 against the hepatotoxicity of paraquat in male rats. The experiment was carried out using 24 male albino rats. Four groups of animals were used in this study: control, Coenzyme Q10 (10 mg/kg), paraquat-treated (9 mg/kg b.w), paraquat along with Coenzyme Q10 for 4 weeks. Light microscopic observations revealed that the hepatic tissue of control and Coenzyme Q10 groups showed normal hepatocytes structure. Histopathological observations of paraquat treated group showed severe damage in liver tissue such as hepatocytes degeneration and necrosis, congestion of blood vessels and hemorrhage. Biochemical studies indicated that serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) levels were elevated in paraquat treated group. Administration of paraquat significantly increased hepatic malondialdehyde (MDA) levels. Superoxide dismutase (SOD) activity and Glutathione (GSH) content in the liver of the paraquat administered rats were significantly decreased as compared with control group. Paraquat treated rats showed negative immunoreactivity to Alfa Fetoprotein (AFP) in the cytoplasm of the liver cells. Coenzyme Q10 administration attenuated the damages induced by paraquat in the liver of rats. The results of the present study indicated that Coenzyme Q10 has protective effect against liver damage induced by paraquat.
[Elsayed k. Abdel-Hady and Gamal H. Abdel-Rahman. **Protective Role of Coenzyme Q10 against Paraquat Induced Hepatotoxicity in Male Rats**. *Life Sci J* 2013;10(1):1933-1940] (ISSN:1097-8135).
http://www.lifesciencesite.com. 277

Key words: Paraquat – Coenzyme Q10 - Liver- Histopathology - Rats.

1. Introduction

Paraquat (1,1-dimethyl 4,4-bipyridinium dichloride) is widely used as herbicide, although it is very toxic for humans and animals **(Tortorelli *et al.*, 1990)** . It is used in about 130 countries to control broad-leaved weeds in growing corn, fruit trees, vegetables, and also as a desiccant before harvesting **(Copping, 2002; Fernandez *et al.*, 2002)**. Paraquat is a very dangerous pollutant of the environment, as it readily binds to both clay and organic matter in soil and is very slowly biodegraded **(Watts, 1994; US EPA, 1997)**. This compound is not volatile, and its contamination resulted in a number of health effects in California (U.S.A) **(Madeley, 2002)**. Paraquat is distributed quickly by blood to reach all organs and tissues **(IPCS, 1984)**, including the main target organ in poisoning–the lungs **(Murray and Gibson 1974; Dey *et al.*, 1990)**. Although kidney, heart and central nervous system are affected, lung damage and pulmonary fibrosis are the most widespread injuries and are main causes of death when exposed to paraquat **(Mohammadi-Karakani *et al.*, 2006)**. Previous studies indicating that parental exposure to pesticides including paraquat may develop certain disorders such as Parkinson's disease (PD) and leukemia in offspring **(Dick, 2006; Monge, *et al.*, 2009)**.

It has been indicated that paraquat produces multi-system toxicity by damaging the cellular membrane lipids **(Bus *et al.*, 1976).** The mechanism of cellular damage caused by paraquat involves the P-450 reductase-dependent formation of reactive oxygen species and peroxidation of membrane lipids in pulmonary cells. Although most studies carried out on Paraquat dealt with lungs, few works deal with paraquat effects on the liver. Antioxidants prevent oxidative damage caused by reactive oxygen species (ROS) in biological structures. Interactive relations between antioxidants and toxins may alleviate the toxicity of hepatotoxic agent **(Murray *et al.*, 1988; Sies and Stahl, 1992; McPherson, 1994 ;)**.

Alpha-fetoprotein (AFP) is an onco-fetal glycoprotein normally synthesized during fetal life and repressed in adults. High levels of AFP are observed during adulthood only under certain conditions, such as the presence of some neoplasias (*e.g.* hepatocellular carcinoma, testicular carcinoma and lung cancer (**Soresi *et al.*, 2003**). (AFP) is a good marker for several possible disorders affecting gestation and hepatic aberrations. Chronic liver disease and cirrhosis identified and diagnosed

according to liver biopsy showed variations in AFP **(Gad, 2005).**

Coenzyme Q10 (CoQ10) is known as ubiquinone, a vitamin-like substance present in all cells in the membranes of endoplasmic reticulum, peroxisomes, lysosomes, and the inner membrane of mitochondria. In recent years, CoQ10 has gained considerable attention as a dietary supplement capable of influencing cellular bioenergetics and counteracting some of the damage caused by ROS **(Linnane *et al.,* 2002; Butler *et al.,* 2003; Rosenfeldt *et al.,* 2003; Zhou *et al.,* 2005)**. Previous studies demonstrated the protective effects of CoQ10 in various models of oxidative and inflammatory tissue damage **(Upaganlawar *et al.,* 2006; Sohet *et al.,* 2009 ; Spindler *et al.,* 2009)**, and therefore it protects the body against the deleterious effects of ROS. The enzyme has hence been therapeutically employed in many disorders for its cytoprotective and antioxidant properties **(Alleva *et al.,* 2001; Ruiz-Jimenez *et al.,* 2007)**. In clinical populations, CoQ10 has been used as a supplementary treatment for chronic diseases such as Chronic Heart Failure (CHF), muscular dystrophies, Parkinson's disease, cancer, and diabetes **(Keith *et al.,*1998; Shults *et al.,* 2002)**. The aim of the present study was to examine the protective effect of CoQ10 against herbicide paraquat-induced hepatotoxicity in male rats.

2. Materials and Methods

Animals and experimental design

Adult male albino rats were obtained from animal farm in Taif, Saudi Arabia. The animals were kept at standard housing facilities (24±1 ◦C, 45±5% humidity and 12 h light/dark cycle). They supplied with standard laboratory food and water *ad-libitum* and left to acclimatize for one week before the experiments. The animals were then divided into 4 groups, 6 animals each. Group I: animals of this group were served as control received saline solution orally. Group II: animals were orally given CoQ10 (10 mg/kg b.w.). Group III: animals were given Paraquat intraperitoneally at dose of (9 mg/kg b.w.) (1\10) LD_{50}. Group IV: animals were intraperitoneally injected with Paraquat at a dose of 9 mg / kg b.w. plus Coenzyme Q10 (10 mg/kg b.w.) orally daily. Time of the experiment was four weeks. All the treatments were done in compliance with the Guide for the Care and Use of Laboratory animals.

Histological and Histopathological Examinations

At the end of the experiment, animals were sacrificed under ether anaesthesia. Liver from animals was carefully separated and immediately fixed in 10% neutral buffered formalin (PH 7.2), dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 5µm thickness were prepared for histopathological examination. Sections were stained with haematoxylin and eosin (H&E) using the standard techniques **(Bancroft and Stevens, 1982)** and then were examined under light microscope.

Biochemical determinations

Blood samples were collected from the marginal ear vein of the rats, placed into plain Vacutainer silicone-coated tubes, and allowed to clot at room temperature. The blood samples were centrifuged at 3000 rpm for 15 minutes. Serum samples were frozen immediately at -20°C and stored until required for analysis.

Serum marker enzyme assays

AST, ALT, ALP levels were determined in the serum by routine colorimetric methods on a Roche modular autoanalyser (Roche modular autoanalyser, Tokyo, Japan).

Oxidative stress and antioxidant enzyme assays

For determination of oxidative stress and antioxidant enzymes in the liver, supernatant obtained after centrifugation of hepatic tissue homogenates was used. Reduced Glutathione was assayed as described by **Beutler *et al.*, (1963)**. Superoxide Dismutase activity was determined according to the method of **Nishikimi *et al.* (1972)**. Malondialdehyde, as an indicator of lipid peroxidation, was determined according to **Ohkawa *et al.* (1979)**.

Immunolocalization of AFP in rats hepatocytes

For immunolocalization by light microscope, the liver section were fixed in 99% ethanol and 1% acetic acid for 12-15 hours at 40◦C and rehydrated. The endogenous peroxidase activity was blocked with 0.05% hydrogen peroxidase in absolute methanol for 30 min. The slides were then washed 5 min in phosphate buffered saline (PBS) at pH 7.4 after each incubation step. The slides were incubated for 5 min in an ultra violet light at room temperature in order to block non specific background staining of excess stain. Primary antibody (Anti-rat. IGg) were applied for 10 min followed by biotinylated Goat anti polyvalent and incubated for 10 min as well. Slides were treated with streptovidin peroxidase and incubated for 10 min at room temperature, as a final step for AFP localization.

Statistical analysis

The results were tested by univariate Analysis of Variance (ANOVA) followed by Mann–Whitney Rank Sum Test. The values were considered significantly at $P<0.05$. The statistical analysis was performed using SPSS .

3. Results

Biochemical determinations

a-Liver functions

The serum ALT , AST and ALP levels were significantly elevated in paraquat treated group as compared with control group (Table1). Treatment with CoQ10 decreased remarkably the levels of serum ALT, AST and ALP in the liver of paraquat-treated rats, although the enzyme levels were still higher than in the control (Table1).

Table (1): Effect of Coenzyme Q10 on Paraquat- induced changes in the Liver functions of different groups. Values are mean ± SD, n = 6 animals.

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	54.87 ± 2.73	106.83 ±5.88	79.45 ± 2.01
Coenzyme Q10	57.9 ± 2.71	110.54 ± 5.48	81.12 ± 2.12
Paraquat	139.47 ± 1.98 *	187.12 ± 3.55 *	134.7 ± 1.95 *
Paraquat & Coenzyme Q10	112.52 ± 1.11 ☻	131.63 ± 2.21 ☻	111.81 ± 2.62 ☻

* Significant difference at $P<0.05$ compared with the control group.
☻ Significant difference at $P<0.05$ compared with the paraquat treated group.

b- Oxidative stress and antioxidant enzymes

Lipid peroxidation

Malondialdehyde (MDA) is the main oxidation product of peroxidized polyunsaturated fatty acids and represents an important index of lipid peroxidation. Administration of paraquat significantly increased (38.1%) hepatic MDA levels in rats as compared to the control group (Table 2). CoQ10 treatment resulted in a significant decrease (10.3%) in MDA as compared to the paraquat group (Table 2).

Glutathione (GSH)

GSH content in the liver of the paraquat administered rats was significantly decreased as compared to control group (Table 2). The GSH content decreased by 12.9% in the paraquat treated group. However, the GSH content was significantly increased (11.4%) in rats treated with paraquat along with CoQ10 as compared to the paraquat group (Table 2).

Superoxide dismutase (SOD)

Administration with paraquat resulted in decreased activity (31.0%) of the enzyme in the liver as compared to the control group (Table2). Administration of CoQ10 along with paraquat increased the activity (19.1%) of SOD as compared to the paraquat group (Table 2).

Table (2): Oxidative stress and antioxidant enzymes in the liver of different groups. Values are mean ± SE, n = 6 animals.

Groups	Parameters		
	Lipid peroxidation (nmoles MDA/mg protein)	Glutathione (µmole /g wet tissue)	Superoxide dismutase (SOD) (units/mg protein)
Control	4.2 ± 0.09	51.2 ± 0.23	2.58 ± 0.09
Co Q10	4.6 ± 0.15	53.2 ± 0.12	2.66 ± 0.11
Paraquat	5.8 ± 0.25*	44.6 ± 0.15*	1.78 ± 0.08*
%	38.1 higher than control	12.9 lower than control	31.0 lower than control
Paraquat & Co Q10	5.2 ± 0.21 ☻	49.7 ± 0.19 ☻	2.12 ± 0.18 ☻
%	10.3 lower than araquat	11.4 higher than araquat	19.1 higher than Paraquat

* Significant difference at $P<0.05$ compared with the control group.
☻ Significant difference at P<0.05 compared with the paraquat group.

Histopathological examinations

The microscopic examinations of sections of liver of control (Fig.1A). and CoQ10 treated rats (Fig.1B) showed the normal structure of the hepatic lobule. The hepatocytes appeared polyhydral in shape, bood sinusoid were situated between cords of hepatocytes The central vein is surrounded by the hepatocytes with eosinophilic cytoplasm and distinct nuclei.

The histopathological examination of the liver of paraquat-treated rats revealed remarkable changes versus control animals.

Fig.1C showed cellular infiltration, congested portal vein and a lot of degenerative cells in portal areas. (Fig.1D) revealed that there are interstitial haemorrhage and cytoplasmic vacuolation. (Fig.1E) exhibit that there is a severe hepatocyte necrosis. Treatment with CoQ10 ameliorated the paraquat-induced liver injuries and improved the histological liver appearance (Fig.1F).

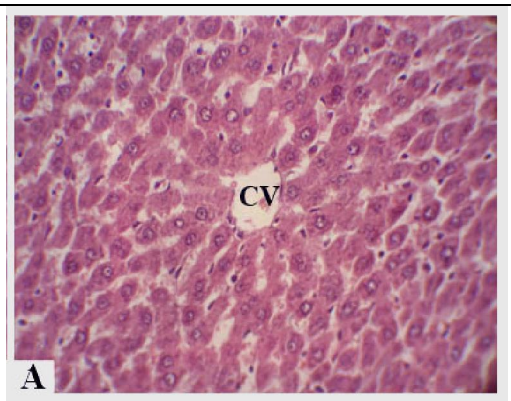

Fig. (1A): Photomicrograph of a section of the liver of control rats showing the normal structure of the hepatic lobules and the central vein (CV). (H & E, X 400)

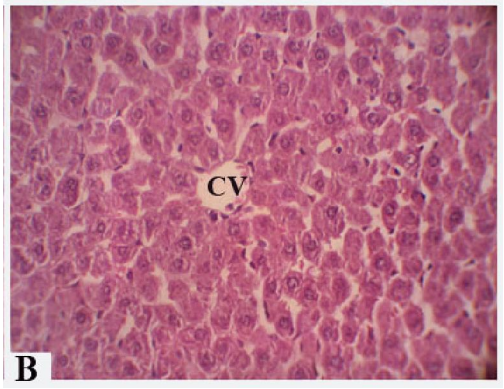

Fig. (1B): Photomicrograph of a section of the liver of rats treated with CoQ10 showing the normal structure of the hepatic lobule with a central vein (CV). (H & E, X 400)

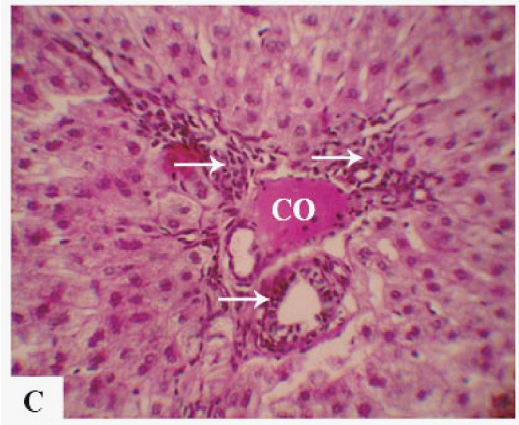

Fig. (1C): Photomicrograph of a section of the liver of Paraquat treated rats showing congested blood vessel (CO) and the lymphocytic infiltration (arrows). (H & E, X 400)

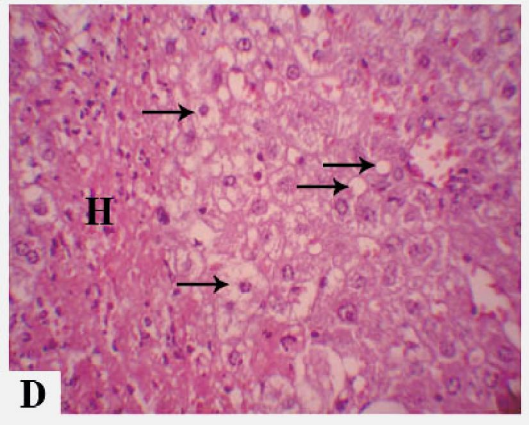

Fig. (1D): Photomicrograph of a section of the liver of Paraquat treated rats showing hemorrhage (H) and cytoplasmic vacuolation (arrows). (H & E, X 400)

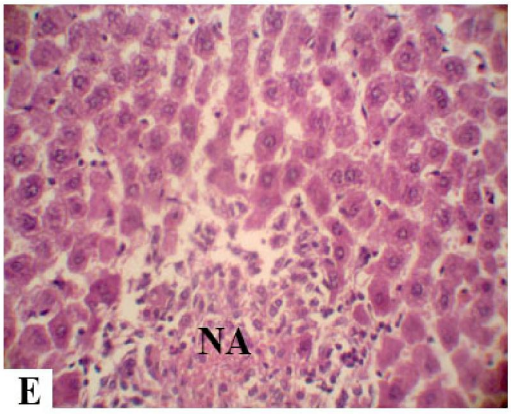

Fig. (1E): Photomicrograph of a section of the liver of Paraquat treated rats demonstrating necrotic area (NA). (H & E, X 400)

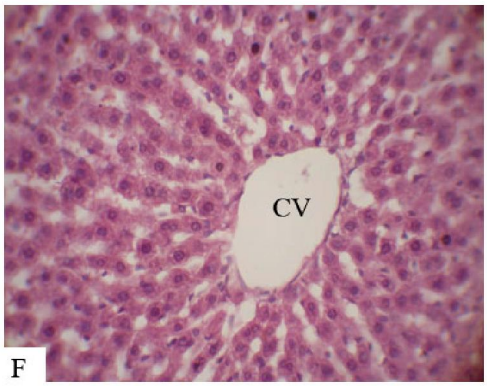

Fig. (1F): Photomicrograph of a section of the liver of Paraquat administrated rats along with CoQ10 showing that liver is more or less similar to the control (H & E, X 400)

Immunohistochemical observations

In control, Coenzyme Q10 and Paraquat treated groups, the immunoreactivity to alpha fetoprotein (AFP) was negative in the cytoplasm of the liver cells (Figs .2 A, B, C).

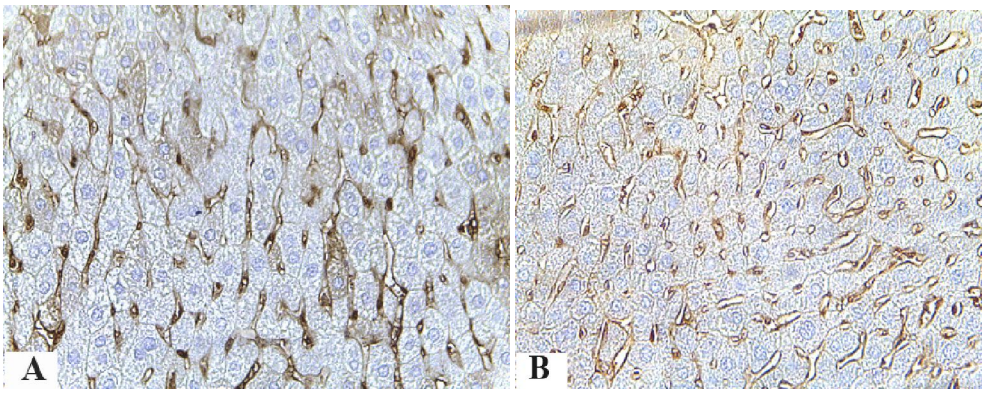

Fig. (2A): Photomicrographs of a section of the liver of control rats showing negative immunoreactivity to AFP. (Peroxidase – antiperoxidase method, X 400)

Fig. (2B): Photomicrographs of a section of the liver of Coenzyme Q10 treated rats showing negative immunoreactivity to AFP. (Peroxidase – antiperoxidase method, X 400)

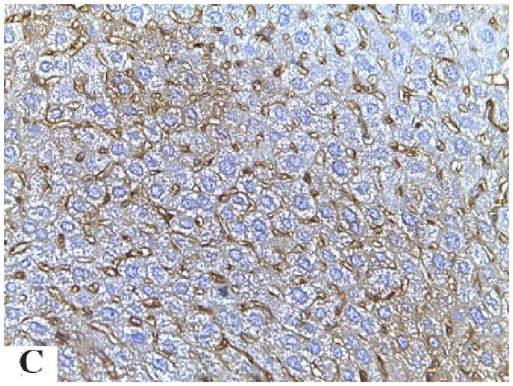

Fig. (2C): Photomicrograph of a section of the liver of Paraquat treated rats illustrating negative immunoreactivity to AFP. (Peroxidase – antiperoxidase method, X 400)

4. Discussion

The liver is one of the major organs in the body responsible for the removal of toxins and poisons. Paraquat is a very toxic herbicide and a dangerous pollutant of the environment. The toxic effects of paraquat were reported in both man and animals **(Clark *et al.*,1966 ; Smith,1985)**. Such toxicity is related to the ability of this herbicide to initiate lipid peroxidation which leads to degeneration of the biological membranes and hence cell death **(Damian *et al.*, 1991; Ali *et al.*, 2000 and Venkatesan, 2000)**. Previous investigators reported that as a result of changes in membranes lipid peroxidation leads to a reduction in membrane microviscosity **(Salmona *et al.*, 1992)**, and also signs of toxicity in the form of haemorrhagic and chronic inflammatory cells in different types of man or animal tissues were reported **(Borchard, 1974)**. Although paraquat has been proved to be safe when used by some investigators **(Smith, 1985)**, the present histological and biochemical findings demonstrated poisoning effects on the hepatic tissues of rats treated with paraquat. In the present study, the histopathological examination of the liver of paraquat-treated rats revealed remarkable changes relative to control animals. These changes included congestion in blood vessels, lymphocytic infiltration, haemorrhage, cytoplasmic vacuolation and severe hepatocyte necrosis. Similar pathological changes were reported by **Cagen and Gibson (1977) and Burk *et al.*(1980)** after paraquat treatment. In the present work, many hepatocytes of paraquat treated animals showed signs of cell necrosis. These observations are consistent with previous investigators results who indicated that metalaxyl induces apoptosis in hepatocytes of mice **(Sakr and Abel-Samie ,2008)**. Results of the present study are also in accordance with previous histopathologicallogical studies which showing degenerative changes in the liver of animals treated with paraquat **(Dixon, 1980; Summers, 1980; Laham *et al.*, 1984 and Abo-Shafy *et al.*,1997)**. ALP comprises a group of enzymes that are present in mammalian cell membranes, and An increase in the activity of ALP in serum may reflect pathologic changes in the liver as well as other organs including the lungs and kidneys **(Fernandez and Kidney 2007)**. Paraquat- induced suppression in serum ALT , AST and ALP of rats liver in the present study. The serum level of both ALP and ALT is used as a marker of the cell membrane integrity. This finding is in agreement with previous reports indicating paraquat exposure results in a significant increase in the ALP and ALT levels mouse embryonic stem cell model and also in vivo studies **(Perla *et al.*, 2008 ; Mohamed *et al.*, 2005)**. Elevation of these two enzymes confirms that paraquat exposure resulted in injuries which reflected in the liver. The injurious effects of paraquat may resulted from its generation of reactive oxygen species (ROS) that causes oxidative stress of various organs. In agreement with our results, several authors

confirmed that oxidative stress, increased lipid peroxidation, depletion of antioxidant defenses and increased production of proinflammatory mediators are implicated in the pathogenesis of herbicides-induced acute hepatic injury **(Fouchecourt and Riviere 1995; Nakatani *et al.,* 2000; Sakr 2007)**. The present work demonstrated that CoQ10 treatment provided a significant protective effect in rats exposed to paraquat as indicated by improvement of the disturbed biochemical parameters and amelioration of hepatic tissue damage observed by histopathological and immunohistochemical examinations. It has been also reported that environmental stressors (such as metals, particulate matter, and pesticides) can induce apoptotic cell death **(Ayed-Boussema *et al.,* 2008 Gong *et al.,* 2009 and Kobayashi *et al.,*2009)**. These findings were also previously reported in mice **(Sakr and Abel-Samie , 2008)**.

It has been reported that AFP is a tumor marker for hepatocellular carcinoma **(Abelev, 1974 ; Ruoslahti and Seppsala, 1979)**, but it is also detected in the serum of patients with non-malignant diseases of the liver including acute and chronic hepatitis and liver cirrhosis **(Endo *et al.,*1975; Sakamoto *et al.,*1975 and Theise *et al.,*1995)**. In relation to AFP pathological significance, serum AFP is useful as a tumor marker in patients with liver cancer **(Gitlin *et al.,* 1972 and Etarinov, 1966)**. In the present study, immunohistochemical study using AFP showed negative reaction when the liver exposed to paraquat for four weeks and at dose (9 mg/kg b.w.). This indicated that paraquat is not carcinogenic agent in the liver for short time exposure. Research must be continued to investigate that paraquat can cause liver cancer when rats exposed to it for long term or not.

In conclusion, results of the present study indicated that Coenzyme Q10 has protective effect against hepatoxicity induced by paraquat and this is mediated by its antioxidant activities.

Corresponding author:
Elsayed k. Abdel-Hady ,
Biology Department, Faculty of Science, Taif University, Taif, KSA
E-mail: nabro62@yahoo.com

References

1. **Abelev, G. I. (1974):** α-Fetoprotein as a Marker of Embryo-Specific Differentiations in Normal and Tumor Tissues. Transplant. Rev., 20: 3– 37.
2. **Abo-Shafy, A.; Sakr, S.; El-Saadany, M. and Abdel Samei, H. (1997):** Histological and Histochemical effects of the herbicide, paraquat on the liver of albino rats. Egypt. J. Appl. Sci., 10(7): 901–920.
3. **Ali, S.; Diwakar, G. and Pawa, S. (2000):** Paraquat induces different pulmonary biochemical responses in Wistar rats and Swiss mice. Chem. Biol. Interact., 125(2): 79–91.
4. **Alleva, R.; Tomasetti, M.; Battino, M.; Curatola, G.; Littarru, G.P. and Folkers, K. (2001):** The roles of coenzyme-Q10 and vitamin E on peroxidation of human low density sub-fractions. Proceedings of the National Academy of Sciences of United States of America 92: 9388–9391.
5. **Ayed-Boussema, I.; Bouaziz, C.; Rjiba, K.; Valenti, K.; Laporte, F. et al. (2008):** The mycotoxin Zearalenone induces apoptosis in human hepatocytes (HepG2) via p53-dependent mitochondrial signaling pathway. Toxicol *In Vitro* 22: 1671–1680.
6. **Bancroft, J.D. and Stevens, A. (1982):** Theory and practice of histologic technique. 2nd.
7. **Beutler, E.; Duron, O. and Kelly, M. B. (1963):** Improved methods for the determination of glutathione. J. Lab. Clin. Med., 61: 882–88.
8. **Borchard, F. (1974):** Ultrastructural and microscopical findings in 3 cases of paraquat poisoning with prolonged lethal course. Pneumonologie. 150–185.
9. **Burk, R. F.; Lawrence, R. A. and Lane, J. M. (1980):** Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. J Clin Invest., 65: 1024–31.
10. **Bus, J.S.; Aust, S.D. and Gibson, J.E. (1976):** Paraquat toxicity proposed mechanism of action involving lipid peroxidation. Envir Health Perspect., 16: 139–146.
11. **Butler, M.G.; Dasouki, M.; Bittel, D.; Hunter, S.; Naini, A. and DiMauro, S. (2003):** Coenzyme Q10 levels in Prader-Willi syndrome: comparison with obese and non-obese subjects. Am J Med Genet., 119(2); 168–171.
12. **Cagen, S. Z. and Gibson, J. E. (1977):** Liver damage following paraquat in selenium-deficient and diethyl maleate pretreated mice. Toxicol Appl Pharmacol., 40: 193–200.
13. **Clark, D.G.; Mcelligott, T.F. and Westonhurst, E. (1966):** The toxicity of paraquat. Br. J. Ind. Med., 23: 126–132.
14. **Copping, L.G. (2002):** Post Emergent Herbicides. In: Agrow Reports. PJB Publications, UK, p. 42.
15. **Damian, F.; Frank, B.; Winfried, H.; Hartmut, M.; Sebastian, E.; Herbert, K.; Conrad, A.; Julius, M.; Hans, W. and Klaus, W. (1991):** Failure of radiotherapy to resolve fatal lung damage due to paraquat poisoning. Chest., 100: 1146–1165.
16. **Dey, M.S.; Breeze, R.G.; Hayton, W.L., *et al.* (1990):** Paraquat pharmacokinetics using a

subcutaneous toxic low dose in rat. Fundam. Appl. Toxicol., 14: 208–216.
17. **Dick, F.D. (2006):** Parkinson's disease and pesticide exposures, Br. Med. Bull., 79: 219–231.
18. **Dixon, R. (1980):** In Casarett and Doull,s Toxicology "2nd ed. John, D. (ed.) Macmillan publishing Co. New York. P.332.
19. **Endo, Y.; Kanai, K.; Mitamura, K.; Lino, S. and Suzuki, H. (1975):** Clinical significance of Alpha- Fetoprotein in hepatitis and liver cirrhosis. Ann. NY. Acad. Sci., 259: 234–238.
20. **Etarinov, Y.S. (1966):** Content of embryo-a specific alpha-globlin in fetal and neonatal sera and sera from adult humans with primary carcinoma of the liver. Fed Proc Transl Suppl., 25: 344–346.
21. **Fernandez, I.; Thomas, E.; Anhoni, J.M. and Rengam, S. (2002):** Poisaned and Silernced: A Study on Women Spraying Pesticides on Plantations. Tenaganita and Pesticide Action Network (PAN) Asia and the Pacific Malaysia.
22. **Fernandez, N. J. and Kidney, B. A. (2007):** Alkaline phosphatase: beyond the liver. Vet. Clin. Pathol., 36: 223–233.
23. **Fouchecourt, M. and Riviere, J. (1995):** Activities of cytochrome P450-dependent monooxyganases and antioxidant enzymes in different organs of Norway rats (*Rattus norvegicus*) inhabiting reference and contaminated sites. Chemosphere., 31: 4375 - 4386.
24. **Gad, A. (2005):** Ethnic difference in validity of a-fetoprotein. Asia–Pac. J. Clin.Oncol., 1: 64–70.
25. **Gitlin, D.; Perricelli, A. And Gitlin, G.M. (1972):** Synthesis of alpha- fetoprotein by liver yolk sac and Gastrointestinal tract of human conceptus. Cancer Res., 32: 979–982.
26. **Gong, Y.; Wu, J.; Huang, Y.; Shen, S. and Han, X. (2009):** Nonylphenol induces apoptosis in rat testicular Sertoli cells via endoplasmic reticulum stress. Toxicol. Lett., 186: 84–95.
27. **IPCS, (1984):** Environmental Health Criteria. In: Document No. 39-Paraquat and Diquat. International Programme on Chemical Safety. World Health Organization, Geneva.
28. **Keith, M.; Geranmayegan, A.; Sole, M.J.; Kurian, R.; Robinson, A.; Omran, A.S. and Jeejeebhoy, K.N. (1998):** Increased oxidative stress in patients with congestive heart failure. J. Am. Coll. Cardiol., 31(6): 1352–1356.
29. **Kobayashi, D.; Ahmed, S.; Ishida, M.; Kasai, S. and Kikuchi, H. (2009):** Calcium/calmodulin signaling elicits release of cytochrome c during 2,3,7,8-tetrachlorodibenzop- dioxin-induced apoptosis in the human lymphoblastic T-cell line, L-MAT. Toxicology 258: 25–32.
30. **Laham, S.; Long, G. and Broxop, B. (1984):** Subacute oral toxicity of tri-n-butylphosphate in the Sprague Dawley rat. J. Appl. Toxicol., 4(3): 150–154.
31. **Linnane, A.W.; Kopsidas, G.; Zhang, C.; Yarovaya, N.; Kovalenko, S.; Papakostopoulos, P.; Eastwood, H.; Graves, S. and Richardson, M. (2002):** Cellular redox activity of coenzyme Q10: effect of CoQ10 supplementation on human skeletal muscle. Free Radic Res., 36(4): 445–453.
32. **Madeley, J. (2002):** Paraquat – Syngenta's controversial herbicide. Berne Declaration, PAN UK. In: Swedish Society for Nature Conservation.PAN Asia and the Pacific, Foro Emaus.
33. **McPherson, A. (1994):** Selenium vitamin E and biological oxidation. In: Cole DJ, Garnsworthy PJ editors. Recent advances in animal nutrition. Oxford: Butterworth and Heinemann's; p. 3–30.
34. **Mohamed, A. F.; Ali Hasan, A. G.; Hamamy, M. I. and Abdel-Sattar, E. (2005):** Antioxidant and hepatoprotective effects of Eucalyptus maculate, Med. Sci. Monit., 11: 426–431.
35. **Mohammadi-Karakani, A.; Ghazi-Khansari, M. and Sotoudeh, M. (2006):** Lisinopril ameliorates paraquat-induced lung fibrosis. Clin. Chim. Acta., 367: 170–174.
36. **Monge, P.; Wesseling, C.; Guardado, J.; Lundberg, I.; Ahlbom,A.; Cantor, K.P.; Weiderpass, E. and Partanen, T. (2009):** Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica, Scand. J. Work. Environ. Health., 33: 293–303.
37. **Murray, R. K.; Granner, D. K.; Mayes, P. A. and Rodwell, V. W. (1988):** Harper's biochemistry (a Lange medical book), international ed. Appleton and Lang Lange Medical Publication; p. 574–576.
38. **Murray, R.E. and Gibson, J.E. (1974):** Paraquat disposition in rats, guinea pigs and monkeys. Toxicol. Appl. Pharmacol., 27: 283.
39. **Nakatani, T.; Tawaramato, M.; Kennedy, D.; Kojima, A. and Yuasa, I., (2000):** Apoptosis induced by chelation of interacellular zinc is associated with depletion of cellular reduced glutathione level in rat hepatocytes. Chem. Biol. Interact., 125: 51–163.
40. **Nishikimi, M.; Roa, N.A. and Yogi, K. (1972):** Measurement of superoxide dismutase. Biochem. Bioph. Res. Common., 46: 849–854.
41. **Ohkawa, H.; Ohishi, N. and Yagi, K. (1979):** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351–358.

42. **Perla, V.; Perrin, N.A. and Greenlee, A.R. (2008):** Paraquat toxicity in a mouse embryonic stem cell model. Toxicol. In Vitro 22: 515–524.
43. **Rosenfeldt, F.; Hilton, D.; Pepe, S. and Krum, H. (2003):** Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. Biofactors., 18(1–4): 91–100.
44. **Ruiz-Jimenez, J.; Priego-Capote, F.; Mata, J.M.; Quesada, J.M. and Luque de Castro, M.D. (2007):** Determination of the ubiquinol-10 and ubiquinone-10 (coenzyme-Q10) in human serum by lipid chromatography tandem mass spectrometry to evaluate the oxidative stress. Journal of Chromatography A., 1175: 242–248.
45. **Ruoslahti, E. and Seppsala, M. (1979):** Alpha-Fetoprotein in cancer and fetal development. Adv. Cancer Res., 29: 346–375.
46. **Sakamoto, S.; Yachi, A. and Anzai, T. (1975):** AFP- producing cells in hepatitis and in liver cirrhosis. Ann. NY. Acad. Sci., 259: 253–258.
47. **Sakr, S.A. (2007):** Ameliorative effect of ginger (Zingiber officinale) on mancozeb fungicide induced liver injury in albino rats. Australian J. Basic Appl. Sci., 1(14): 650-656.
48. **Sakr, S. A. and Abel-Samie, H. A. (2008):** Apoptosis related protein bax In liver of metalaxyl fungicide –Treated mice: The effect of Antox. Ozean. J. Appl. Sci., 1: 17–27.
49. **Salmona, M.; Donnini, M.; Perin, L.; Diomede, G.; Romano, M.; Marini, M.; Tacconi, M. I. and Luisetti, M. (1992):** A novel pharmacological approach for paraquat poisoning in rat and A549 cell line using Ambroxol, a lung surfactant synthesis inducer.
50. **Shults, C.W.; Oakes, D.; Kieburtz, K.; Beal, M.F.; Haas, R.; Plumb, S.; Juncos, J.L.; Nutt, J.; Shoulson, I.; Carter, J. *et al.* (2002):** Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. Arch Neurol., 59(10): 1541–1550.
51. **Sies, H. and Stahl, W. (1992):** Antioxidant functions of vitamins: vitamin C and E b-carotene and other carotenoids. Ann NY Acad Sci., 669: 7–21.
52. **Smith, L. L. (1985)**: Paraquat toxicity. Phil. Trans. Roy. Soc. London. 311: 647–652.
53. **Sohet, F.M.; Neyrinck, A.M.; Pachikian, B.D.; de Backer, F.C.; Bindels, L.B.; Niklowitz, P.; Menke, T.; Cani, P.D. and Delzenne, N.M. (2009):** Coenzyme Q10 supplementation lowers hepatic oxidative stress and inflammation associated with diet-induced obesity in mice. Biochem. Pharmacol., 78: 1391–1400.
54. **Spindler, M.; Beal, M.F. and Henchcliffe, C. (2009):** Coenzyme Q10 effects in neurodegenerative disease. Neuropsychiatr. Dis. Treat., 5: 597–610.
55. **Soresi M.; Magliarisi C.; Campagna P.; Leto G.; Bonfissuto G. and Riili A: (2003):** Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Anticancer Res. 2003;23:1747-53
56. **Summers, L. (1980):** " The Bipyridinium Herbicides" (A.P. Autor, ed.) P.2 Academic press, New York.
57. **Theise, N.D.; Fiel, I. M.; Hytiroglou, P.; Ferrell, L.; Schwartz, M.; Miller, C. and Thung, S. N. (1995):** Macroregenerative nodules in cirrhosis are not associated with elevated serum or stainable tissue alpha- fetoprotein . Liver 15: 30–34 .
58. **Tortorelli, M.C.; Hernandez, D.A.; Vazquez, G.R. and Salibian, A. (1990):** Effects of paraquat on mortality and cardiorespiratory function of catfish fry Plecostomus commersoni. Arch Environ. Contam. Toxicol., 19: 523–529.
59. **Upaganlawar, A.; Farswan, M.; Rathod, S. and Balaraman, R. (2006):** Modification of biochemical parameters of gentamicin nephrotoxicity by coenzyme Q10 and green tea in rats. Indian J. Exp. Biol., 44: 416–418.
60. **US EPA, (1997):** Paraquat dichloride. Registration eligibility decision (RED). US Environmental Protection Agency, Washington, D.C., EPA 738-F-96-018. US National Library of Medicine, 1995. Hazardous Substances Databank. Bethesda, MD, pp. 10–19.
61. **Venkatesan, N. (2000):** Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci., 66(2): 21–28.
62. **Watts, M.A. (1994):** Poisons in Paradise: Pesticides in the Pacific. Greenpeace New Zealand and Pesticide Action Network Asia and the Pacific, Auckland.
63. **Zhou, S.; Zhang, Y.; Davie, A.; Marshall-Gradisnik, S.; Hu, H.; Wang, J. and Brushett, D. (2005):** Muscle and plasma coenzyme Q10 concentration, aerobic power and exercise economy of healthy men in response to four weeks of supplementation. J Sports Med Phys Fitness. 45(3): 337.

2/2/2013