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Therapeutic effects of ascorbic acid as natural antioxidants on some biochemical and some hormones measurements in intoxicated rats by both chlorpyrifose and glyphosate.

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Abstract
The objective of this study aimed analyzes potential cytotoxicity of the chlorpyrifose and/or glyphosate and protective effect of vitamin C. 110 male albino rats were treated with chlorpyrifose and glyphosate daily for 30 and 60 days. Hepatotoxicity was monitored by quantitative analysis of the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, Gamma-glutamyl transferase GGT, total protein, and albumin. The second aim of this study to investigate effect of used chlorpyrifose or glyphosate alone or together on lipid peroxidation (LPO) levels of animals as an index of antioxidant status and oxidative stress, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) respectively, Triiodothyronine (T3), thyroxin (T4) and thyroid-stimulating hormone (TSH) and Testosterone (TST) were measured and liver was collected for histopathological study. The results refer to a significant elevation of (MDA and NO) while a significant decrease in other parameters (CAT, GSH and SOD) and increase in liver functions (ASAT, ALAT, GGT and ALP). Also a significant decrease in total protein, albumin level. The hormones were recorded significant increase in T3 and T4 while decrease in TSH and TST in toxicated groups as compared to the control groups. Histopathology revealed vacuolar of hepatocytes with random hepatocyte necrosis and mononuclear cell infiltration. The administration of the vitamin C had beneficial and decrease side effects against the deleterious changes of chlorpyrifose or glyphosate alone or together. In conclusion results suggest a potential contribution of pesticide mixtures to the etiology of some body diseases while vitamin C has beneficial effects as it tends to dampen chlorpyrifose or glyphosate alone or together toxicity in rats.

Keywords: antioxidants chlorpyrifos, glyphosate, hormones, lipid peroxidation, and liver functions

Introduction
Agriculture is an important sector in the economy of Egypt, and therefore, use of pesticide and herbicide has increased during the recent years, due to high imports of pesticides and herbicides, consequently leading to many health hazard effects on experimental animals and humans such as disruption of reproductive function [1, 2].

Organophosphorus pesticides are among the most widely used pesticides, and extensive uses have profound impact on the environment and are hazardous to humans [3-5].

Chlorpyrifose (CPF) induced oxidative stress is mediated through several mechanisms such as increase production of reactive oxygen species (ROS), alteration of antioxidant defenses, and the effect of antioxidant enzyme function which resulted in cellular oxidative damages such as DNA damage, protein oxidation, and lipid peroxidation [6,7].

Exposure to sub-lethal concentrations of chlorpyrifos may cause biochemical and behavioral changes in animal models [8, 9] demonstrated that CPF affects the thyroid and adrenal gland homeostasis both in human and animal models and a decrease in testosterone (T) biosynthesis in testes of rats [10, 11].

Application of pesticides to fruit and vegetables can contribute to pesticide exposure to human through diet [12]. In developing countries, small-scale farmers are exposed to CPF during mixing, loading, and spraying using backpack sprayers resulting in adverse health effects [13]. Further, farmers in developing countries are at high risk to CPF exposure due to the use of backpack reservoirs for application, low knowledge on safety, and limited use of protective gear [14, 15] recorded that exposure rats for organophosphorus triggers slight increase in ALT and AST levels and significant increase in GGT level, which are marker enzymes in serum used in appraisal of hepatic damage.

However, toxicity of glyphosate on the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determinedin vitro study [16]. Furthermore, in 2015, the WHO International Agency for Research on Cancer reclassified glyphosate as "probably carcinogenic to humans" [17]. In addition, studies in the gonadal axis have shown that glyphosate interferes with the activity of aromatase, leading to changes in reproductive development in rats [18, 19]. Glyphosate is a herbicide with great toxicity, and is more commonly known as 'Roundup' and is formulated as isopropyl amine salt [20].

CPF-induced oxidative stress is mediated through several mechanisms such as increase production of reactive oxygen species (ROS), depletion of antioxidant defenses, and the impairment of antioxidant enzyme function which resulted in cellular oxidative damages such as DNA damage, protein oxidation, and lipid peroxidation [7, 21] recorded that CPF lead to reduced GSH levels and decreased activities of SOD, CAT, and GPx in rats which treated with CPF.

CPF is considered as an endocrine disrupter and significantly decreased biosynthesis of testosterone in rat Leydig cells [22, 23]

showed decreased concentrations of TSH in rats exposed to glyphosate with no variation in the levels of the thyroid hormones (THs) T3 and T4.

The major natural antioxidants which are derived from the natural sources by dietary intake are vitamins A, C, E and carotenoids [24]. Accordingly, interest has recently grown in the role of the natural antioxidant as a strategy to prevent oxidative damage as a factor in the pathophysiology and histopathology of various health disorders [25]. Among antioxidants, ascorbic acid (vitamin C) affordable non-enzymatic antioxidant molecules that have been used to mitigate oxidative damage and readily scavenges physiological ROS as well as reactive nitrogen species ''RNS" [26].

Thus, the aim of the present study was to elucidate whether CPF and GLY have oxidative stress. Therefore, this work was designed to evaluate the responses of rat at biochemical level and histological of liver after exposure to sub-lethal concentrations of CPF or glyphosate.

Materials & Methods

Animals

All animal in this study were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

Wister albino averaged weights (190±10 g) (obtained from the Egyptian Holding Company for Biological Products and Vaccines, Egypt) were housed in stainless steel cages with water and food *ad libitum*, temperature of 22±2ºC, humidity around 56% and 12 hrs light-dark cycle.

Chemicals and reagent

Chlorpyrifos (CPF), (Pestban ® 48% EC) and Glyphosate (GLY) (roundup 48%) were obtained from Agrochem, Alwatneia Co., Alex., Egypt; CPF toxicity was induced by oral gavage tube of CPF (7.5, 15 mg/kg) daily for 60 consecutive days **[27]**. Glyphosate Toxicity was induced by oral gavage tube of GLY (500, 1000 mg/kg) daily for 60 consecutive days according to **[28]**. Vitamin C (Ascorbic acid) from Unipharma Company, Egypt, evaluated for its safety effects and a dose of (200 mg /Kg) for 60 days was selected according to [29].

Experimental design

110 male albino rats were randomly divided into 11 equal groups and labeled as groups 1,2,3,4,5,6,7,8,9,10 and 11 each group contain 10 rats, rats received all treatments daily via oral gavage tube along the period of the experiment, CPF has two doses: low dose (L) = 7.5 mg/kg and high dose (H) = 15 mg/kg, and also GLY has two doses : low dose(L)= 500 mg/kg and high dose (H) = 1000 mg/kg. Group (1): Control rats, Group (2): Vit C (200 mg/kg B.W), Group (3): CPF (L), Group (4): CPF (H), Group (5): CPF (H) +Vit C and Group (6): GLY (L), Group (7): GLY (H), Group (8): GLY (H) + Vit C Group (9): CPF (L) and GLY (L), Group (10): CPF (H) and GLY (H), Group (11): CPF (H), GLY (H) + Vit C. The animals will observe daily for sign of toxicity during the period of experiment. Five rats from each group was scarify on the 30 and 60 days.

Sample collection

The animals were sacrificed after the treatments. Blood samples were taken without anticoagulant for biochemical parameters. Livers was quickly removed, and immediately rinsed in saline ice. The parts from livers were homogenized and this homogenates were centrifuged at 8000 rpm for 30 min, and then the supernatants were used for the measurement of LPO and antioxidant enzyme activities (CAT, SOD, GSH and NO).

LPO level

Lipid peroxidation process is determined by the thiobarbituric acid (TBA) method which estimates the malondialdehyde formation (MDA) according to [30].

Antioxidants biomarker

The level of SOD activity was determined in hepatic homogenate according to the method described by **[31].** Catalase activity of the tissue homogenate was determined according to the method of [32]. The assay of GSH activity was determined according to the method of [33]. Nitric oxide level of the tissue homogenate was determined according to the method of **[34].**

Hormonal profile

Serum T3 and T4 were determined according to the method described by [35]. TSH was determined according to the method described by [36, 37]. Testosterone level were determined according to the method described by **[38]** using the electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Biochemical parameters

Serum ALAT, ASAT was determined according to the method of [39]. ALP was determined according to the method described by [40]. (γ-GT) was determined according to the method of [41]. Total protein was determined according to the method described by [42]. Albumin was determined according to the method of [43]. TBIL was determined according to the method of [44]. Blood glucose was determined according to the method of [45].

Statistical analysis

The statistical package for social sciences SPSS/PC computer program (version 19) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA). The data were expressed as mean ± S.E. Differences were considered statistically significant at ($P < 0.05$).

Results

Resulted data found in table (1) showed a significantly increase ($p < 0.05$) in MDA and NO level in CPF, GLY and in combination between them in low and high doses groups, as compared with control groups after 30 and 60 days. On the other hand, insignificant differences recorded in Vit C treated groups when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C observed a significant decrease ($p < 0.05$) when

compared with intoxicated groups after 30 and 60 days.

Statistical data in table (1) recorded a significant decrease ($p < 0.05$) in hepatic CAT, GSH and SOD activities in rats intoxicated with CPF, GLY and in combination between them in low and high doses groups when compared with control groups after 30 and 60 days.. On contrast insignificant differences with recorded in Vit C when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C observed a significant increase ($p < 0.05$) when compared with intoxicated groups after 30 and 60 days.

CPF, GLY and in combination between them in low and high doses induced hepatic damage as reflected by significantly ($p < 0.05$) elevated serum ALT, AST, GGT and ALP enzymes activities when compared to control group after 30 and 60 days. While, insignificant differences recorded in Vit C when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C revealed a significant decrease ($p < 0.05$) when compared with intoxicated groups after 30 and 60 days. As shown in table (2).

Data presented in table (2) recorded that a significant decrease ($p < 0.05$) in serum total protein and albumin level in rats intoxicated with CPF, GLY and in combination between them in low and high doses when compared with control groups after 30 and 60 days. On contrast insignificant differences with recorded in Vit C when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C observed a significant increase ($p < 0.05$) when compared with intoxicated groups after 30 and 60 days.

CPF, GLY and in combination between them in low and high doses induced damage as reflected by significantly ($p < 0.05$) elevated serum T3 and T4 enzymes activities when compared to control group after 30 and 60 days. On the other hand, insignificant differences with recorded in Vit C when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C observed a significant decrease ($p < 0.05$) in serum T3 and T4 when compared with intoxicated groups after 30 and 60 days. As shown in Table (3).

Table (3) recorded a significant decrease ($p < 0.05$) in serum TSH and TST level in rats intoxicated with CPF, GLY and in combination between them in low and high doses when compared with control groups after 30 and 60 days. On contrast insignificant differences with recorded in Vit C groups when compared to control groups. Rats treated with CPF (H) + Vit. C, GLY (H) +Vit.C and CPF (H) + GLY (H) + Vit.C observed a significant increase ($p < 0.05$) of TSH, TST when compared with intoxicated groups after 30 and 60 days.

Histopathological Examination

In control rat's livers, sections showed hepatic polygonal cells (HC) with round nucleus, normal central vein (CV) and blood sinusoids. In the CPF and GLY group, liver sinuses were contracted slightly. However, cells were expanded, liver cords broadened, and liver sinuses contracted extensively. Rats suffered greater hepatic damage with CPF and GLY. The liver slices became fuzzy and edematous with extremely loose cytoplasm. The animals treated with CPF or GLY with received vitamin C lead to improve of liver.

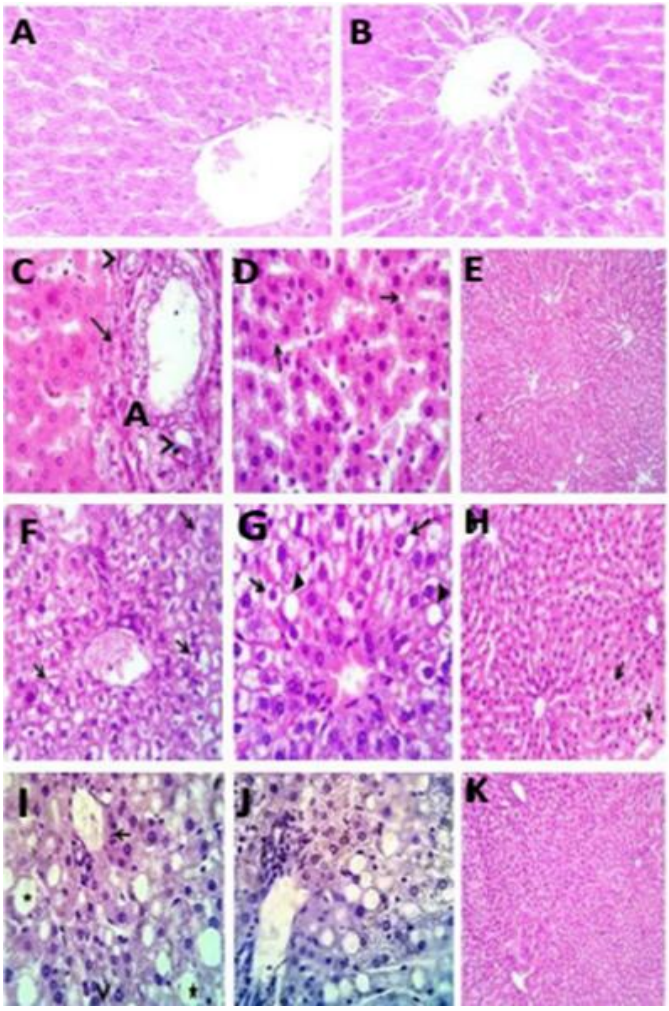

Fig 1

Normal liver. (B) Normal hepatocyte and hepatic sinusoids. (C) Disorder in the hepatic parenchyma appeared with minor interstitial leukocytic infiltration beside fibrosis in portal area (arrows). (D) Some of the hepatic cells showed round vacuoles, mostly microsteatosis (arrow). (E) Liver revealed apparently normal histo-morphological structures. (F) Centrilobular cloudy swelling and hydropic degeneration (open arrows) of some hepatocytes, congestion (star). (G) Showed hydropic degeneration and fatty change (arrow head) in a moderate number of hepatocytes and steatosis. (H) Showing vacuolar degeneration and fatty changes (arrows) in a few hepatocytes. (I) showed focal degenerative and necrotic changes (arrow head) in hepatocytes beside macro and macrosteatosis (stars). (j) Congestion of hepatic blood vessels, sinusoidal dilatation, kupffer cell hypertrophy and different degenerative changes. (k) Apparently normal hepatic parenchyma with minute mononuclear cell infiltration in the portal triads.

Table 1: malondialdehyde (MDA) (nmol/ml), SOD and CAT activity (U/L), GSH and NO level in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

	days		Groups											
			Control	**Vit C**	**CPF (L)**	**CPF (H)**	**CPF (H) +Vit C**	**GLY (L)**	**GLY (H)**	**GLY (H)+Vit C**	**CPF (L)+GLY (L)**	**CPF (H)+ GLY (H)**	**CPF (H) +GLY (H)+ Vit C**
MDA (nmol/ml)	30	Mean± S.E	182.5±1.6^{a}	177.1±1.0^{a}	239.4±2.4^{b,h}	249.2±1.5^{d}	211.5±1.0^{f}	243.4±1.5^{b}	260.2±2.3^{J}	216.0±1.0^{f}	277.1±1.3^{L}	297.0±2.1^{e}	238.7±1.2^{h}
	60	Mean± S.E	180.5±0.9^{a}	176.8±2.0^{a}	268.1±1.4^{c}	295.5±1.5^{e}	254.5±1.6^{g}	284.6±1.6^{i}	307.3±2.0^{k}	256.4±1.3^{g,J}	327.7±2.0^{m}	345.7±1.8^{n}	283.8±1.4^{i}
SOD (U/mg wet tissue)	30	Mean± S.E	46.8±1.2^{a}	44.1±1.0^{a}	32.8±1.7^{b}	25.7±1.6^{c,f}	31.0±2.0^{b,e}	29.9±2.7^{b,f}	23.1±2.7^{h,c,f}	33.0±1.4^{b}	15.2±2.3^{g,i,h}	10.1±1.5^{k}.	19.5±0.8^{d,h}
	60	Mean± S.E	47.8±1.2^{a}	44.7±1.3^{a}	25.5±1.5^{c,f}	18.5±1.6^{d,g,h}	27.5±1.9^{f,e}	20.3±2.2^{d,h}	15.5±2.1^{h,g,i}	21.0±2.6^{c,d,J}	11.0±1.7^{i,k}	7.3±1.2^{k}	16.8±1.3^{h,J}
CAT (U/mg wet tissue)	30	Mean± S.E	1.67±0.02^{a}	1.65±0.02^{a}	1.47±0.01^{b}	1.40±0.01^{d,g}	1.50±0.01^{b}	1.43±0.01^{d}	1.38±0.01^{g,h}	1.51±0.01^{b}	1.35±0.01^{f,h}	1.30±0.01^{J}	1.42±0.01^{d}
	60	Mean± S.E	1.69±0.02^{a}	1.63±0.03^{a}	1.26±0.01^{c}	1.22±0.01^{e}	1.34±0.01^{f}	1.20±0.01^{e}	1.17±0.01^{i}	1.29±0.01^{c,J}	1.10±0.01^{k}	1.05±0.02^{L}	1.19±0.01^{e,i}
GSH (mmol/gm wet tissue)	30	Mean± S.E	2.94±0.02^{a}	2.93±0.01^{a}	2.50±0.01^{b}	2.40±0.01^{d}	2.66±0.02^{f}	2.39±0.01^{d}	2.10±0.01^{J}	2.28±0.02^{L}	2.20±0.01^{m}	1.85±0.02^{e}	2.05±0.02^{h}
	60	Mean± S.E	2.91±0.02^{a}	2.92±0.03^{a}	2.01±0.02^{c,g}	1.81±0.01^{e}	2.04±0.02^{c,h}	1.91±0.02^{i}	1.59±0.02^{k}	1.99±0.02^{g}	1.74±0.01^{n}	1.45±0.01^{p}	1.79±0.01^{e}
NO (µmol/L)	30	Mean± S.E	40.4±1.3^{a}	40.7±0.8^{a}	57.8±1.1^{b}	69.7±0.9^{c}	53.3±1.1^{f}	60.5±1.0^{b}	73.2±1.2^{d,h}	60.3±1.0^{b}	70.8±1.2^{c,h}	83.7±1.3^{J}	70.1±1.4^{c,h}
	60	Mean± S.E	41.6±1.2^{a}	40.9±1.4^{a}	72.2±1.1^{c,d}	90.7±1.5^{e}	75.3±0.7^{d}	79.9±1.0^{g}	99.0±1.1^{i}	80.8±1.8^{g,J}	90.2±1.1^{e}	109.7±1.5^{k}	80.0±1.8^{g}

Each value represented means of 5 records ± S.E

a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.

CPF: chlorpyrifos. GLY: glyphosate, Vit. C: Vitamin C, H: High dose, L: Low dose.

Table 2: Serum ALAT, ASAT, ALP and GGT activity (U/L), TP and ALB level in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

	days		Groups										
			Control	**Vit C**	**CPF (L)**	**CPF (H)**	**CPF (H) +Vit C**	**GLY (L)**	**GLY (H)**	**GLY (H)+ Vit C**	**CPF (L) +GLY (L)**	**CPF (H) + GLY (H)**	**CPF (H) + GLY (H)+ Vit C**
ALAT (U/L)	30	Mean± S.E	43.37±1.9^{a}	41.02±1.7^{a}	61.26±1.5^{b}	70.68±0.8^{d}	60.19±2.1^{b}	59.84±2.9^{b}	74.19±1.2^{c,d}	59.21±1.9^{b}	76.10±1.7^{c}	79.40±0.7^{c,f}	68.55±1.1^{d}
	60	Mean± S.E	45.18±1.0^{a}	41.68±1.9^{a}	76.76±1.2^{c}	84.4±1.5^{e}	76.08±1.3^{d}	82.52±1.2^{e,f}	90.98±1.0^{g}	70.94±1.0^{d}	92.34±1.2^{g}	111.48±2.2^{h}	75.34±1.4^{c}
ASAT (U/L)	30	Mean± S.E	90.1±0.89^{a}	84.27±1.4^{a}	116.76±1.5^{b}	122.68±1.8^{d}	104.08±1.7^{g}	117.23±1.4^{b}	125.80±2.2^{d}	107.42±2.0^{g}	135.44±1.3^{f}	143.12±1.3^{c,e}	124.92±1.6^{d}
	60	Mean± S.E	92.22±1.8^{a}	84.94±1.5^{a}	139.07±1.4^{c,f}	140.64±1.2^{c,e}	118.22±1.5^{b}	143.59±2.1^{e}	159.22±0.8^{h}	123.96±1.1^{d}	167.00±1.4^{i}	187.70±2.2^{j}	143.40±0.9^{e}
ALP (U/L)	30	Mean± S.E	66.73±1.8^{a}	61.46±1.8^{a}	87.75±0.8^{b}	93.37±2.0^{d}	80.38±0.8^{g}	93.11±1.9^{d}	103.63±1.4^{e}	87.78±1.1^{b}	117.76±0.9^{j}	131.54±1.2^{k}	99.20±0.9^{i}
	60	Mean± S.E	64.42±1.2^{a}	59.68±1.2^{a}	107.68±2.1^{c,e}	108.64±1.8^{c,f}	87.74±1.2^{b}	112.90±1.8^{f}	122.29±1.2^{h}	97.10±1.3^{d,i}	128.43±1.6^{k}	146.38±1.7^{L}	109.44±1.8^{c,f}
GGT (U/L)	30	Mean± S.E	1.61±.02^{a}	1.57±0.01^{a}	3.39±0.02^{b}	3.85±0.02^{d}	2.87±0.02^{f}	3.55±0.02^{h}	4.14±0.02^{J}	3.16±0.02^{L}	5.31±0.02^{n}	6.57±0.02^{q}	5.15±0.02^{i}
	60	Mean± S.E	1.60±.02^{a}	1.58±0.02^{a}	4.96±0.01^{c}	5.63±0.02^{e}	4.27±0.01^{g}	5.16±0.02^{i}	5.87±0.02^{k}	4.85±0.02^{m}	6.00±0.02^{p}	7.00±0.01^{R}	5.57±0.03^{S}
TP (g/dl)	30	Mean± S.E	6.73±0.1^{a}	6.63±0.1^{a}	5.62±0.03^{b}	5.39±0.03^{d}	5.66±0.02^{b}	5.47±0.02^{d}	5.24±0.01^{c,e}	5.61±0.02^{b}	5.21±0.01^{c}	5.14±0.01^{c,e}	5.59±0.01^{b,f}
	60	Mean± S.E	6.81±0.1^{a}	6.57±0.04^{a}	5.28±0.02^{c}	5.20±0.02^{c,e}	5.49±.03^{d,f}	5.13±0.01^{e,g}	5.08±0.01^{g}	5.48±0.02^{d,f}	4.95±0.01^{h}	4.68±0.06^{i}	5.26±0.01^{c}
ALB (g/dl)	30	Mean± S.E	4.57±.02^{a}	4.56±0.01^{a}	3.52±0.02^{b}	3.44±0.01^{d}	4.08±0.01^{g}	3.42±0.01^{d}	3.37±0.01^{i}	4.00±0.02^{k}	3.25±0.01^{c,f}	3.12±0.03^{J}	3.79±0.02^{L}
	60	Mean± S.E	4.58±003^{a}	4.60±0.02^{a}	3.27±0.01^{c}	3.21±0.01^{e,f}	3.86±0.01^{h}	3.20±0.01^{e}	3.09±0.01^{J}	3.77±0.02^{L}	3.01±0.03^{m}	2.78±0.02^{n}	3.59±0.02^{p}

Each value represented means of 5 records ± S.E.

a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change.

CPF: chlorpyrifos. GLY: glyphosate, Vit. C: Vitamin C, H: High dose, L: Low dose.

Table 3: Serum triiodothyronine (T3), thyroxin (T4), thyroid-stimulating hormone (TSH) and Testosterone (TST) level (µg/ml) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

	days		Groups										
			Control	**Vit C**	**CPF (L)**	**CPF (H)**	**CPF (H) +Vit C**	**GLY (L)**	**GLY (H)**	**GLY (H)+ Vit C**	**CPF (L) +GLY (L)**	**CPF (H) + GLY (H)**	**CPF (H) + GLY (H)+ Vit C**
T3 (µg/ml)	30	Mean± S.E	0.91±0.01^{a}	0.90±0.01^{a}	1.22±0.01^{b}	1.33±0.01^{d}	1.15±0.02^{f}	1.29±0.01^{g}	1.39±0.01^{h}	1.27±0.01^{g}	1.43±0.01^{J}	1.59±0.01^{e}	1.44±0.03^{J}
	60	Mean± S.E	0.94±0.01^{a}	0.89±0.01^{a}	1.51±0.01^{c}	1.61±0.01^{e}	1.20±0.01^{b}	1.60±0.02e	1.72±0.02^{i}	1.31±0.01^{d,g}	1.81±0.01^{k}	2.13±0.02^{L}	1.48±0.03^{c}
T4 (µg/ml)	30	Mean± S.E	3.51±0.01^{a}	3.53±0.02^{a}	3.93±0.01^{b}	4.32±0.01^{d}	3.86±0.01^{f}	4.15±0.01^{h}	4.53±0.01^{e}	3.94±0.01^{b}	4.55±0.01^{e}	4.78±0.03^{k}	4.16±0.01^{h}
	60	Mean± S.E	3.52±0.01^{a}	3.50±0.01^{a}	4.46±0.02^{c}	4.57±0.02^{e}	4.01±0.01^{g}	4.41±0.03^{i}	4.72±0.01^{J}	4.16±0.01^{h}	4.79±0.01^{k}	5.15±0.02^{L}	4.37±0.01^{i}
TSH (µg/ml)	30	Mean± S.E	10.24±0.02^{a}	10.25±0.01^{a}	8.22±0.01^{b}	7.89±0.01^{d}	8.28±0.02^{f}	8.69±0.01^{g}	7.58±0.01^{i}	8.17±0.02^{J}	7.96±0.02^{h}	7.30±0.01^{L}	8.09±0.01^{n}
	60	Mean± S.E	10.22±0.02^{a}	10.234±0.01^{a}	7.53±0.01^{c}	7.21±0.01^{e}	7.90±0.02^{d}	7.96±0.02^{h}	7.22±0.01^{e}	7.67±0.02^{k}	7.19±0.01^{e}	6.25±0.01^{m}	7.76±0.03^{p}
TST. (µg/ml)	30	Mean± S.E	3.57±0.02^{a}	3.55±0.02^{a}	2.76±0.01^{b}	2.54±0.01^{d}	3.07±0.02^{f}	2.68±0.01^{h}	2.45±0.01^{e}	3.03±0.02 ^{J}	2.40±0.01^{i}	2.36±0.01^{i}	3.11±0.01^{f}
	60	Mean± S.E	3.59±0.01^{a}	3.54±0.01^{a}	2.59±0.01^{c}	2.47±0.01^{e}	2.91±0.01^{g}	2.44±0.01^{e}	2.39±0.01^{i}	2.82±0.02^{k}	2.16±0.01^{L}	1.96±0.01^{m}	2.80±0.02^{b,k}

Each value represented means of 5 records ± S.E*.*

a,b,c,d.e means comparison between all groups which the groups have the same letter mean there is no significance difference and which have different letter mean there is a significance change*.*

CPF: chlorpyrifos. GLY: glyphosate, Vit. C: Vitamin C, H: High dose, L: Low dose.

Discussion

Lipid peroxidation (LPO) refers to the oxidative degradation of lipids. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters, and cholesterol itself. Their formation occurs in both enzymatic and non-enzymatic reactions involving reactive oxygen species, which are responsible for toxic effects in the body via damage to various tissues [46]. Lipid peroxide has acute toxicity and can be produced by oxygen radicals like the hydroxyl radical or nitrite oxide [47].

The present results of increased TBARS in liver following CPF and glyphosate exposure, indicate an increased oxygen free-radical generation by these chemicals and increase of NO. on the other hand CAT, SOD and GSH are decreased when treated with CPF and GLY, due to utilization of this antioxidant in the metabolism of CPF and glyphosate through lowering the level of GSH, led to an oxidative imbalance and induced oxidative processes, resulting in increased cell death [46, 48] reported that vitamins C has ameliorative effect against Chlorpyrifos and glyphosate which causes an increase in lipid peroxidation and a decrease in the activity of glutathione peroxidase, superoxide dismutase and catalase. Vitamin C administration attenuated the morphological damages induced by paraquat (herbicide) in the liver and kidney of experimental animals. Our results suggest an antitoxic effect of vitamin C against paraquat [49].

The daily oral administration of chlorpyrifos and glyphosate led to increase in ALT, AST, ALP and GGT activity and decrease in TP and ALB, which are marker enzymes in serum used in appraisal of hepatic damage (50). Therefore, elevated levels of ALT, AST, and GGT indicate liver damage as a result of CPF and GLY exposure [51, 52].

Results indicated that the administration of chlorpyrifos and glyphosate have necrotic effect on vital organs like liver, thus causing the leakage of enzymes into blood. ALT is the marker of liver function, increase in the activity of which indicates hepatocellular degeneration and AST, being organ specific, marks the degenerative changes occurring in chlorpyrifos and glyphosate exposed animals. A similar increase in AST and ALT was observed in goats [53] and rats [54-56] exposed to chlorpyrifos and glyphosate. Synthesis of plasma protein is one of the main functions of the liver [57]. Showed that rats exposure to glyphosate demonstrated a significant decrease in the levels of total protein in the male rats indicating a possible liver damage [58]. This agrees with [59] which recorded that the organic phosphorus pesticides decrease TP and Alb in rats exposed to this toxin.

Data obtained in this study clearly demonstrate the ability of glyphosate to impair liver enzymes. Alteration of basic liver functions was monitored by the detection of elevation of AST, ALT and ALP activities [60]. The results of the present study were similar to [16, 61, 46].

[62] showed that pretreatment with vitamin C has an ameliorating effect on these clinical chemistry parameters, suggesting it has a protective effect on the damage induced by CPF on organs producing these enzymes(ALAT, ASAT, ALP).

[63] The mitigation of the hypoalbuminemia by vitamin C may be due to protection of the liver from oxidative damage provoked by co-exposure to CPF and lead, apparently due to its antioxidant effect.

The current study investigated the effects of pesticide exposure on T3, T4 and TSH hormone level of rats. The function of both of these hormones is to stimulate the metabolism. The disturbances in the production of these hormones can impair metabolism and can lead to several developmental disorders and diseases. and producing hormonal disorders [64]. The exposure to pesticides has also been shown to enhance the chances for thyroid cancer [65]. The level of TSH was decreased dramatically while T3 hormone level was slightly increased in rats exposed to pesticides when compared to control group. Thus pesticides have significant but opposing effects on the levels of TSH and T3 hormone. However, the results of This variation in the results may be due to changes in environmental factors, differences in immunity of the selected population and differences in the use of pesticides. The mechanism by which CPF induced increase in secretion of thyroid hormones especially T3 and T4 is not clear.

Previous studies suggested that insecticides may decrease iodine binding proteins [66]. Oxidative stress is one of the possible mechanisms resulted from organophosphate toxicity, including CPF. [67] reported that CPF may have properties to induce oxidative stress indicated by enhancement of MDA production, decrease in GSH content, GST and CAT activities in rat tissues. Thus the stimulate effect of CPF on thyroid function observed in the present work could be attributed to deficient iodide trapping or oxidative stress of CPF.

The obtained data showed a significant increase in serumT3, T4, and decrease in TSH and TST these results agree with [68, 22] which recorded that rats treated with CPF revealed a significant decrease in TSH. And disagree with [69] which reported that chlorpyrifos treated rats showed a significant decreased T3 and T4 and increased TSH hormone levels [70] found that there was a significant increase in T4 in serum rats exposed to CPF of female rats in contrast to male rats which had no significant changes.

Reduced TST in rats due to that chlorpyrifos displayed an anti-androgen activity which was manifested as inhibition of testosterone stimulated increase in the weight of accessory sex organs [71, 18]. The results also are in agreement with [72] found that the mean values of serum testosterone levels in CPF treated group showed significant decreases as compared with those of the control group. These results are consistent with [10, 73].

The vitamin C having protected the thyroid acinar from oxidative damage may have aided in restoring thyroid hormones. Apart from its antioxidant properties, some other nonantioxidant activity of vitamin C may have complemented the restoration of thyroidal function. Vitamin C has been demonstrated to aid the synthesis of paraoxonase, an important esterase that aids in the detoxification of OPs [74].

The antioxidant vitamin C treats trauma resulting from too much thyroid hormone production, likely by preventing oxidative stress to tissue and there is some evidence to suggest that the administration of vitamins with anti-oxidant properties in patients with hyperthyroidism can decrease the severity of clinical symptoms [75]. Supplemented vitamin C had improved levels of the thyroid hormones T4, T3 and TSH. Participants

also showed an improvement in the malabsorption issues they were having from gastrointestinal problems [76].

Histopathological changes in liver tissues of the rat treated with CPF and GLY showed mild diffused granular degeneration, necrosis and vacuolar degeneration, bile duct proliferation and necrosis of hepatocytes. These changes indicated that the degeneration of liver increased with the time of exposure. This can also be corroborated with time dependent increase in the liver function enzymes. Histopathological changes observed in liver indicated time-related degeneration of liver upon exposure to CPF and GLY for 30 and 60 days. This fact was also depicted by the increasing AST and ALT level, which are the markers of liver function. It is reported that chlorpyrifos laid oxidative stress and tissue damage in the liver [77]. Vitamin C prevents histological damage induced in liver tissues. These results may be attributed to the ability of vitamin C to scavenge free radicals resulted from CPF and GLY stress which are agree with [78, 79].

Conclusion

These results concluded that vitamin C supplementation has hepato-protective effect. Vitamin C can directly and rapidly scavenge free radicals and/or inhibit their formation, additionally; it can act by upregulation endogenous antioxidant defenses. Vitamin C protects the DNA of the cells from damage caused by free radicals; it combats the effects of pesticides. It also, fights off these pollutants by stimulating enzymes in the liver that detoxify the body. It can act by regulation of endocrine hormones disrupter. Staying on top of oxidative stress is a necessity in our increasingly toxic world. Taking care to avoid those toxins as much as possible and to enrich our diets with life-giving antioxidants is a wise step to take in our endless quest for wellness. Results of the present study clearly indicate that prior feeding of antioxidant vitamin C combats oxidative stress induced by CPF and GLY in rats. Thus, sufficient dietary intake of vitamin C by individuals who regularly come in contact with these pesticides is beneficial in combating the adverse effects of CPF and GLY.

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