Original Article

The Potential Protective Role Of Vitamins C Against Adverse Effects Of Some Plant Growth Regulators In Albino Rats

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Abstract

Gibberellic acid (GA3) and Ethephon (ETP) are plant growth regulators that are largely used in agriculture to accelerate and control the production of many vegetables and fruits. This study aimed to evaluate the toxicity of GA3 and ETP and protective role of vitamin C on some immunological, hematological and liver function parameters in albino rats. Animals were divided into six groups (6 animals per group) as follows: G1, the control group, received saline solution (1ml/kg). G2 received vitamin C (200 mg/kg). G3 received GA3 (75 mg/kg). G4 was given ETP (150 mg/kg). G5 received vitamin C+GA3 with the same previous doses. G6 was given vitamin C+ETP with the same previous doses. All doses were given orally and once daily for six weeks. The results showed significant decrease in levels of total immunoglobulins (Igs), IgG, IgA, and IgM and increase in serum pro-inflammatory cytokines INF-γ, TNF-α, IL-1β and IL-6 compared with control group. Significant increase in total WBCs and lymphocytes and decrease in neutrophils were recorded, in addition to, significant decrease in RBCs and hemoglobin content compared with control group. Moreover, elevated levels of ALT, AST, ALP, GGT, bilirubin and AFP and decreased levels of total proteins and albumins were indicated in GA3 and ETP groups. Liver lipid peroxidation (MDA) and nitric oxide (NO) levels were significantly elevated and liver antioxidant enzymes SOD and CAT and GSH content were significantly decreased in GA3 and ETP groups. Administration of vitamin C with GA3 and ETP ameliorated most of the previous changes caused by GA3 and ETP administration. It could be concluded that both GA3 and ETP could induce alterations in liver functions, immunological, hematological and oxidative stress parameters in rats and vitamin C could ameliorate these changes because of its anti-inflammatory, antioxidant and free radicals scavenging properties.

Keywords: Plant growth regulator, Gibberellic acid, Ethephon, Oxidative stress, Vitamin C.

INTRODUCTION

Plant growth regulators (PGRs) are endogenous plant hormones that are used in agriculture to enhance and control the production of a wide variety of crops. They affect physiological processes of plants and extreme use of these chemicals would certainly be accompanied with many health hazards (Wani et al., 2017 and Hajam et al., 2018).

Gibberellic acid (GA) is a natural PGR that is widely used to increase the growth of many fruits and vegetables. GA was discovered as a metabolic byproduct of the fungus Gibberella fujikuroi (Riley, 1987). American Society of Agricultural Science considered GA as one of the six major classes of PGRs that are widely used in agricultural field in many countries (Husseiny et al., 2020). GA affects many mechanisms of plant growth including increasing cell division, promoting stem elongation, flowering, and fruit development. If GA or one of its metabolites is applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated (Hassan et al., 2019 and Bai et al., 2012). People can be exposed to GA3 by utilizing fresh fruits and vegetables and occupational exposure could occur through inhalation of the powder and skin contact giving an acute toxicity picture (Hosseinchi et al., 2013). Chronic consumption of GA3 may induce many hazardous effects more than those of insecticides. It could induce oxidative stress, tumors in different organs as hepatic, breast and lung carcinomas and chromosomal aberrations in lymphocytes of humans and mice (Martinez et al., 2007).

Usage of artificial fruit ripening has increased lately to accommodate increasing customer needs. Among different ripening agents, calcium carbide, acetylene, ethylene, propylene and ethephon are the widely used ripening agents today (Bhadoria et al., 2015). Ethephon (2-chloroethylphosphonic acid; Ethrel) is an ethylene-based PGR that is used in agriculture as an artificial ripening agent and organophosphorous insecticide and it has direct and indirect effects on agricultural and industrial workers, direct as through its inhalation during usage in agriculture and indirect through the diet (Fruits and vegetables) which is sprayed with it (Moustakime et al., 2018).

Ethephon (ETP) has broad-spectrum of activities as it is used to promote pre-harvest ripening of fruits, vegetables and cereals, flower induction, fruit coloration, and fruit abscission. Also, it is being extensively used to accelerate post-harvest ripening of bananas (Abeles et al., 2012). The ETP inhibits acetyl cholinesterase (AChE) enzyme activity, especially in mice, rats, dogs and humans. As a result of these changes, systemic disorders as well as liver impairment can be occurred (Daabo 2022 and Tousson et al., 2020). Ethylene, the metabolic product of ETP was found to cause tumors and necrosis in soft organs and reproductive impairment. Using ETP alone or combined with other pesticides have been reported to induce mutagenic, teratogenic, biochemical alterations and increase in all types of structural chromosomal aberrations in mice, rats, dogs and humans (Mokhtari et al., 2020 and Abd Eldaim et al., 2019).

GA3 and ETP could induce a pro-oxidant state and tissue damage by inducing apoptotic and necrotic events in liver. Also, GA3 and ETP could induce elevation in lipid peroxidation which is an index of oxidative stress (Andrew et al., 2018, Kaya et al., 2018). Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals which induce oxidative stress (Ozsoy et al., 2008). One of the most important vitamins for the body is vitamin C (ascorbic acid) which is a well-known cell protective natural antioxidant and cofactor for mediating countless enzymatic reactions that are accountable for numerous biological activities. Also, it has anti-inflammatory and anti-microbial actions. It has been extensively used to manage critically ill patients (Marik, 2018 and Mousavi et al., 2019). In addition, vitamin C enhances the immune system via several pathways, such as, augmenting the activity of lymphocytes and phagocytes, increasing interferon levels and scavenging reactive oxygen species (ROS) (Yaqinuddin et al., 2022).

The knowledge concerning the adverse effects of GA3 and ETP on the immune system is limited. In addition, little is known about the protective role of vitamin C against GA3 and ETP induced toxicity in mammals. Therefore, the current study was conducted to examine the possible modifying effects of vitamin C against immunological disturbance, changes in levels of pro-inflammatory and anti-inflammatory mediators, hematological alterations, liver toxicity and injury and oxidative stress markers induced by GA3 and ETP in male albino rats.

Materials and Methods

Experimental animals

Thirty-six adult male *Sprague Dawely* albino rats with average weight (150 - 160 g) and of 10-12 weeks age were included in the present study. Rats were purchased from the Experimental Animal Care Center, Faculty of Medicine, Minia University, Egypt. Animals were kept in a 12-h light/12-h dark cycle and housed in cages {(25°C), humidity (60%)} and offered water and laboratory food for 1 week before starting the experiment for acclimatization.

Experimental groups

Rats were divided into six groups (6 animals per group) as follows:

- Control group: Rats were gavaged daily with physiological saline (0.9% NaCl) for 6 consecutive weeks.
- Vitamin C group: Rats were daily gavaged with vitamin C (200 mg/kg) for 6 consecutive weeks.
- Gibberellic acid group: Rats were orally administered with Gibberellic acid (75 mg/kg) daily for 6 consecutive weeks.
- Ethephon group: Rats were orally administered with Ethephon (150 mg/kg) daily for 6 consecutive weeks.
- Vitamin C+ Gibberellic acid group: Rats were co-administered with vitamin C and Gibberellic acid orally for 6 consecutive weeks, with the same doses.
- Vitamin C+ Ethephon group: Rats were co-administered with vitamin C and Ethephon orally for 6 consecutive weeks, with the same doses.

Collection of blood samples

After 24 hours of the last dose, animals were anaesthetized by ethyl and were sacrificed and two blood samples were collected from each rat. One sample was left to clot for a half-hour, at 37˚C; and was centrifuged at 3000 rpm for 15min by refrigerated centrifuge, then, the serum was separated, and stored at -80℃ for biochemical and immunological analysis. The other blood sample was collected in heparinized tubes to determine hematological parameters. Liver was taken rapidly from each animal after dissection and was stored at -80℃ for measuring antioxidant enzymes.

Chemicals and Reagents

Ethephon (2-chloroethyl phosphonic acid) compound was obtained from Chema Industries, Alexandria, Egypt. Gibberellic acid was purchased from Sigma-Aldrich (USA). Sodium chloride was purchased from El- Nasr Co., Egypt.

Liver and the antioxidant enzymes kits were purchased from Bio Diagnostic chemical company. Serum rat TNF-α, INF-γ, IL-6, IL-1β, IL-10, AFP, total Ig, IgA, IgM and IgG ELISA kits were purchased from National High-Tech Enterprise with research called Elabscience and CUSABIO chemical company, USA.

Methods

Immunological studies

Serum concentrations of total Ig, IgM, IgA, IgG, IFN-γ, TNF-α, IL-1β, IL-6, and IL-10 were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Hematological studies

The heparinized blood samples were examined regarding count of red blood cells (RBCs), hemoglobin concentration (Hb), hematocrit value (PCV), white blood cells (WBCs), and differential count of (WBCs) according to standard methods using an Animal Blood Counter.

Liver functions assay

Estimation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were carried out according to the method of Reitman & Frankel (1957). Serum alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT) were carried out using the methods of (Belfield & Goldberg 1971; Persijn & van der Slik 1976) respectively. AFP was determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kits. Methods of (Gornal, *et al.*, 1949); (Doumas, *et al.*, 1971) and Walter & Gerade (1970) were used for estimation of serum total protein, albumin and total bilirubin respectively.

Oxidative stress and anti-oxidant markers measurements

About (0.5 g) of liver was homogenized in 5 ml of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA. The homogenate was centrifuged at 4000 r/min for 20 minutes, and the supernatant was prepared and stored at -80 °C until used for malondialdehyde (MDA) measurements according to the Ohkawa *et al*., (1979) method; nitric oxide (NO) according to the method of Montgomery and Dimock (1961); catalase according to the method of Fossati *et al.,* (1980); glutathione (GSH) by the method of Beutler (1963) and superoxide dismutase (SOD) by the method of Nishikimi *et al.,* (1972).

Statistical Analysis

The data obtained for each group was expressed as mean± Standard Error (SE) and compared using Statistical Processor System Support (SPSS, Chicago, IL) version 16.0. The comparison was made by one way ANOVA followed by post-hoc-Least Significant Difference analysis (LSD) for comparison between groups. P-value ≤0.05 was deemed to be significant.

Results

Immunoglobulin concentrations

The present study revealed that concentrations of serum total immunoglobulin were decreased significantly ($P \leq 0.01$) ($P \leq 0.001$) in GA3 and ETP groups respectively. Also, serum IgG, IgM and IgA were reduced significantly ($P \leq 0.001$) ($P \leq 0.01$) ($P \leq 0.001$) in rats intoxicated with GA3 or ETP when compared with control group. On the other hand, administration of vitamin C alone didn't show significant changes in immunoglobulin concentrations compared to control group, while, combining vitamin C with ETP significantly ($P \leq 0.05$) increased serum total Ig compared with ETP group. Levels of serum IgM, IgG and IgA were increased significantly in (GA3&Vit C) and (ETP& Vit C) groups ($P \leq 0.01$) ($P \leq 0.001$) ($P \leq 0.01$) compared with growth regulators groups (Table 1).

Table (1) Effects of Gibberellic acid, Ethephon alone or combined with vitamin C on immunoglobulin concentrations in male albino rats.

Groups Parameters	Control	Vitamin C	gibberellic acid	Ethephon	gibberellic acid & Vit C	Ethephon & Vit C
Total Ig (mg/ml)	5.35 ±0.34	5.27 ±0.29	3.91 ±0.49 **	3.77 ±0.46 ***	4.62 ±0.22	4.93 ±0.23 #
IgM (ng/ml)	61.81 ±2.16	58.83 ±2.73	42.04 ±5.45 ***	41.32 ±5.19 ***	55.82 ±1.85 ##	54.88 ±2.12 ##
IgG (ng/ml)	20.15 ±1.41	18.32 ±0.81	14.82 ±1.95 **	14.08 ±1.81 **	20.93 ±1.59 ###	24.40 ±1.76 *, ###
IgA (ng/ml)	48. 0 ±1.52	50.0 ±0.57	33.0 ±4.71 ***	29.67 ±1.20 ***	44.0 ±2.88 ##	41.67 ±2.18 ##

Values are expressed as means ± SE (n= 6). * P <0.05, ** P <0.01, *** P <0.001 vs control.
#P <0.05, ## P <0.01, ###P <0.001 vs growth promoter groups

Inflammatory cytokines

The present study showed a significant elevation in serum pro-inflammatory cytokines INF-γ, IL-1β, IL-6 ($P \leq 0.001$) and TNF-α ($P \leq 0.01$) in groups intoxicated with GA3 or ETP. Levels of IL-10 were also released in intoxicated groups more than that of the control group. Combining vitamin C with GA3 significantly decreased serum INF-γ ($P \leq 0.05$), TNF-α , Il-1β and Il-6 ($P \leq 0.01$), while, administration of vitamin C with ETP significantly reduced inflammatory cytokines INF-γ, TNF-α ($P \leq 0.01$) and Il-1β and Il-6 ($P \leq 0.001$) compared with growth regulator groups. IL-10 was increased significantly ($P \leq 0.05$) in GA3& Vit C and ETP& Vit C groups when compared with growth regulator groups.

Table (2) Effects of Gibberellic acid, Ethephon alone or combined with vitamin C on pro-inflammatory and anti-inflammatory cytokines in male albino rats.

Parameter Groups	Control	Vitamin C	gibberellic acid	Ethephon	gibberellic acid & Vit C	Ethephon & Vit C
INF-γ (pg/ml)	0.65 ±0.03	0.76 ±0.08	1.89 ±0.39 ***	2.03 ±0.41 ***	1.29 ±0.08 * #	1.26 ±0.09 * ##
TNF-α (pg/ml)	39.12 ±1.81	40.20 ±1.42	62.64 ±7.82 **	60.41 ±7.5 **	37.71 ±2.42 ##	39.96 ±2.73 ##
Il-1β (pg/ml)	59.74 ±5.65	58.45 ±2.45	89.73 ±5.64 ***	106.2 ±5.22 ***	69.98 ±5.64 ##	80.05 ±5.73 **, ###
IL- 6 (pg/ml)	55.92 ±1.85	54.88 ±2.12	74.46 ±5.78 ***	84.84 ±4.84 ***	61.24 ±1.72 ##	63.24 ±3.55 ###
IL –10 (pg/ml)	73.75 ±2.39	74.9 ±2.18	86.25 ±2.39	81.15 ±2.36	93.1 ±8.81 *	94.22 ±5.53 *

Values are expressed as means ± SE (n= 6). * P <0.05, ** P <0.01, *** P <0.001 vs control.
#P <0.05, ## P <0.01, ###P <0.001 vs growth promoter groups.

Hematological changes

Results presented in table (3) revealed that orally intoxication with GA3 and ETP showed a significant ($P \leq 0.01$) decrease in total erythrocytes count associated with a significant decrease in hemoglobin concentrations ($P \leq 0.01$), ($P \leq 0.05$) respectively when compared with control group, while, levels of PCV, MCH, MCV and MCHC weren't changed. Injection of vitamin C at the same time in combination with GA3 or ETP significantly ($P \leq 0.05$) increased total erythrocyte counts, while, hemoglobin concentrations were elevated significantly ($P \leq 0.001$) in GA3& Vit C group. Total white blood cells count was increased significantly ($P \leq 0.001$) due to GA3 and ETP intoxication. Also, lymphocytes were significantly ($P \leq 0.01$), ($P \leq 0.05$) increased in GA3 and ETP groups, while, neutrophils percentage was decreased significantly ($P \leq 0.01$), ($P \leq 0.05$) in both intoxicated groups. Non-significant changes in monocytes, acidophils and basophils percentages were observed. Administration of vitamin C in combination with GA3 or ETP significantly modulated the increase in WBCS ($P \leq 0.001$), ($P \leq 0.01$), reduced lymphocytes percentage ($P \leq 0.05$), ($P \leq 0.001$), and increased neutrophils percentage ($P \leq 0.001$).

Table (3) Effects of Gibberellic acid, Ethephon alone or combined with vitamin C on hematological parameters in male albino rats.

Groups Parameter	Control	Vitamin C	gibberellic acid	Ethephon	gibberellic acid & Vit C	Ethephon & Vit C
RBCs ($x10^6$ µl)	7.60 ± 0.3	7.94 ±0.12	6.06 ±0.34 **	6.37 ±018 **	6.96 ±0.34 #	7.32 ±0.72 #
Hb (g/dl)	13.54 ±0.96;/	15.13±0.27	10.51 ±0.59**	10.91 ±0.57 *	14.31 ±0.37 ###	12.1 ±1.09
PCV %	44.76 ±3.35	49.45 ±0.71	40.19 ±1.72	45.93 ±0.78	45.97 ±1.93	47.37 ±2.29
MCV (fl)	58.76 ±2.62	62.33 ±1.36	64.96 ±2.95	69.16 ±1.81	60.34 ±1.92	69.53 ±2.61
MCH (pg)	17.8 ±0.95	19.08 ±0.56	17.51 ±1.41	16.31 ±0.98	18.82 ±0.74	17.61 ±1.18
MCHC (g/dl)	30.47 ±2.84	30.63 ±0.89	26.69 ±1.11	23.57 ±1.95	31.35 ±1.95	29.86 ±3.30
WBCs ($x10^3$ µl)	6.71 ±0.34	6.48 ± 0.19	9.57 ±0.52***	9.04 ±0.23 ***	7.75 ±0.51*, ###	7.30 ±0.15 ##
Lymphocyte %	42.83±2.41	40.01 ±0.27	59.24±2.23 **	54.87±5.80*	47.65 ±4.34 #	41.166±1.91# ##
Neutrophile %	49.29±3.07	50.29 ± 1.67	32.94±3.04 **	37.97±6.20 *	45.52 ±4.56 ###	52.18 ±2.47 ###
Acidophile %	0.82 ±0.24	1.5 ±0.3	0.81±0.34	0.73±0.40	1.12 ±0.33	0.55 ±0.27
Basophile %	0.14+0.11	0.3 ±0.1	0.06 + 0.06	0.28±0.19	0.17± 0.1	0

Monocyte %	6.22±1.11	7.48 ±1.37	6.57±0.85	6.54±0.28	5.6 ±0.55	5.25 ±0.48

Values are expressed as means ± SE (n= 6). * P <0.05, ** P <0.01, *** P <0.001 vs control.
#P <0.05, ## P <0.01, ###P <0.001 vs growth promoter groups.

Oxidative stress and antioxidant markers

Table (4) showed that GA3 or ETP administration resulted in disturbance in oxidative stress parameters in which levels of MDA in the liver homogenate and NO were significantly ($P \leq 0.001$) increased, while the levels of antioxidant enzymes, CAT and SOD and GSH content were significantly ($P \leq 0.001$) reduced in GA3 or ETP groups when compared with control group. The antioxidant activity of vitamin C significantly ($P \leq 0.001$) had the ability to reduce oxidative stress markers by reducing levels of MDA and NO. GSH concentration was increased significantly ($P \leq 0.001$), ($P \leq 0.01$) in GA3& Vit C and ETP& VitC compared with GA3 or ETP groups. Catalase activities were also increased significantly ($P \leq 0.001$) in intoxicated groups treated with vitamin C. SOD concentration was increased significantly ($P \leq 0.001$) in GA3& Vit C group when compared with GA3 group and this increase was non-significant ($P > 0.05$) in ETP& Vit C group compared with ETP group.

Table (4) Effects of Gibberellic acid, Ethephon alone or combined with vitamin C on oxidative stress and anti-oxidant parameters in male albino rats.

Parameter Groups	MDA (nmol/g. liver)	NO (µmol/g. liver)	GSH (mg/ g. liver)	Catalase (U/g. liver)	SOD (U/g. liver)
Control	5.97 ±0.18	74.84 ±2.10	120.86 ±2.37	2.19 ±0.17	9.92 ±0.14
Vitamin C	6.24 ±0.24	81.32. ±5.81	111.45 ±0.06	1.97 ±0.02	10.58 ±0.51
gibberellic acid	14.59 ±0.94 ***	127.4 ±9.38 ***	34.59 ±1.66 ***	0.82 ±0.24 ***	5.05 ±0.01 ***
Ethephon	11.44 ±0.32 ***	114.41 ±6.28 ***	63.61 ±2.37 ***	0.96 ±0.03 ***	6.45 ±0.29 ***
gibberellic acid & Vit C	9.24 ±0.25 *** ###	95.48 ±4.83 *, ###	95.48 ±4.83 **, ###	1.65 ±0.05 **, ###	8.71 ±1.01 ###
Ethephon & Vit C	7.71 ±0.69 * ###	79.25 ±1.65 ###	93.45 ±5.55 ** ##	1.78 ±0.16 *, ###	7.35 ±0.45 ***

Values are expressed as means ± SE (n= 6). * P <0.05, ** P <0.01, *** P <0.001 vs control.
P <0.01, ###P <0.001 vs growth promoter groups.

Liver function parameters

Groups administered GA3 or ETP showed a significant increase ($P \leq 0.001$) in serum ALT, AST, ALP, GGT, bilirubin and AFP when compared to control group, on the other hand serum albumin concentration decreased significantly ($P \leq 0.001$) in GA3 and ETP groups, while, total protein levels decreased significantly ($P \leq 0.05$) in GA3 group only compared to control group. Treatment with vitamin C combined with GA3 or ETP significantly reduced the elevation in ALT ($P \leq 0.001$) ($P \leq 0.05$); AST and ALP ($P \leq 0.001$); GGT ($P \leq 0.01$); total bilirubin ($P \leq 0.001$) ($P \leq 0.05$) and AFP ($P \leq 0.01$) $P \leq 0.05$) respectively compared with GA3 or ETP groups. Also, administration of vitamin C with GA3 or ETP significantly ($P \leq 0.001$) ($P \leq 0.01$) increased albumin levels (Table 5).

Table (5) Effects of Gibberellic acid, Ethephon alone or combined with vitamin C on liver function parameters in male albino rats.

Groups Parameter	Control	Vitamin C	gibberellic acid	Ethephon	gibberellic acid & Vit C	Ethephon & Vit C
ALT (U/ml)	49.56 ±10.63	46.33 ±3.58	103.7 ±4.54 ***	89.63 ±10.81 ***	67.94 ±4.41 *, ###	71.37 ±3.52 *, #
AST (U/ml)	46.86 ±3.57	57.77 ±3.03	98.33 ±5.72 ***	97.32 ±3.84 ***	65.66 ±1.31 ***, ###	66.83 ±2.65 ***, ###
ALP (IU/L)	75.62 ±7.36	73.95 ±4.36	139.28 ±9.17 ***	130.36 ±7.34 ***	93.50± 4.66 *, ###	95.25 ±4.99 *, ###
GGT (U/L)	165.60 ± 8.53	167.26 ±7.03	389.20±61.01 ***	371.15 ±22.61 ***	258.6 ±6.83 ##	221.33 ±8.76 ##
Total bilirubin (mg/dl)	0.47 ±0.21	0.29 ±0.02	1.60 ±0.28 ***	1.39 ±0.23 ***	0.79± 0.05 ###	0.86 ±0.06 #
ALF (pg/ml)	22.07 ± 1.41	23.05 ± 1.59	41.76 ± 5.21 ***	40.25 ±4.98 ***	30.38 ±0.9 ##	29.32 ± 0.64 #
Total protein (g/dl)	9.03 ±1.21	8.77 ±0.28	7.23 ±0.31 *	7.55 ±0.23	8.50 ±0.48	8.85 ±0.24

Albumin (g/dl)	5.42 ±0.63	6.04 ±0.11	3.19 ± 0.07 ***	3.18 ±0.08 ***	4.97 ±0.45 ###	4.36 ±0.41 *, ##

Values are expressed as means ± SE (n= 6). * P <0.05. *** P <0.001 vs control.
#P <0.05, ## P <0.01, ###P <0.001 vs growth promoter groups.

Discussion

Plant growth regulators (PGRs) are not nutrients, but chemicals that promote and influence growth, development and differentiation of cells and tissues of the plant to increase plant size, production and plant availability all the year. Despite the beneficial effects of agrochemical compounds, the extreme application of PGRs is related to many depraved effects on health (Rahm *et al.,* 2017). Vitamin C clearly has benefits in supporting function of both innate and acquired immunity. In addition to its role as an antioxidant and anti-inflammatory agent, vitamin C also has roles in several aspects of immunity and is of some importance to immune cells (Carr & Maggini, 2017).

In the present study, the significant decrease in levels of Immunoglobulins (Igs), IgG, IgA, and IgM after administration of GA3 and ETP (Table 1) may be attributed to the toxic effect of these agents. A previous study showed that ETP could induce developmental immunotoxicity on male offspring in mice (Wang *et al.,* 2017). However, the phosphonic acid derivative, trichlorphon was reported to be immune-toxic that induced dose-dependent suppressive effects on lymphocyte proliferation and myeloid cell respiratory burst activities in carp fish *in vitro* (Siwicki, et al 1990). In addition, other organophosphates such as malathion, parathion, diazinon and dimethoate were previously reported to induce immune-toxic effects (Abou Zeid *et al.,* 2018).

Normal levels of immunoglobulins are important for humoral immunity and proper immune response. (IgG) is the most common type of (Igs) and has a powerful ability to bind to bacteria and toxins, and thus, it takes on an important role in the biological defense system. While, (IgM) is mainly produced by B cells in the primary immune response and has a key role in the initial immune system defense for protecting the body and (IgA) is mainly present in secretions to prevent bacterial invasion from a mucous membrane (Yaqinuddin *et al*., 2022). So, the current results indicate that GA3 and ETP could weaken the body's defense system and impair production of antibodies. In the present study, vitamin C ameliorated the decreased levels of (Igs). Although antioxidant protection has been suggested, the role of vitamin C within the function of B cells is less clear (Hong *et al.,* 2016). *In vitro* studies have indicated that incubation of vitamin C with lymphocytes promotes proliferation resulting in enhanced antibody generation, and also provides resistance to various cell death stimuli (Molina *et al*., 2014, Huijskens *et al.*, 2014]. Furthermore, early studies in guinea pigs showed enhanced mitotic activity of isolated peripheral blood lymphocytes following intraperitoneal vitamin C treatment, and enhanced humoral antibody levels during immunization (Feigen *et al.,* 1982). Although one human intervention study has reported positive associations between antibody levels and vitamin C supplementation (Heuser and Vojdani, 1997), another has not (Anderson *et al.,* 1980).

In addition to alteration in immunoglobulin levels, GA3 and ETP induced inflammatory process through excessive production of pro-inflammatory mediators as there was significant elevation in serum pro-inflammatory cytokines INF-γ, TNF- α, IL-1β and IL-6 in groups intoxicated with GA3 and ETP compared with control group (Table 2). This finding is in agreement with the results of a recent study that indicated that ETP increased inflammatory response in the experimental Japanese quail (El-Sebai *et al.,* 2003) and this action was attributed to the link between inflammation and oxidative stress. Results of this study could indicate that both GA3 and ETP can induce inflammatory response in rats. Several studies showed that excessive and up-regulation of cytokines production and increased levels of pro-inflammatory cytokines from neutrophils in the liver were associated with liver cell damage and liver injury (Altuntaş *et al.,* 2015, Jayakumar *et al.,* 2006, Khan *et al*., 2011, Khan *et al.,* 2019). Pro-inflammatory cytokines might induce the formation of ROS which could trigger an inflammatory response through the activation of transcription factor NFκB. NF-κB then translocates into the nucleus where it activates a variety of inflammatory genes such as inducible nitric oxide synthase (iNOS), COX-2, IL-1β, IL-6, IL-8, and TNF-α. IL-1β and TNF-α could activate NF-κB forming a vicious cycle leading eventually to cell death. In addition, TNF- α, and IL-6 are two key cytokines involved in tissue damage during liver injury, although it has been suggested that TNF-α is the central mediator regulating other subsequent events. TNF-α is able to induce chemokines, macrophage chemotactic protein-1 and vascular cell adhesion molecule- 1, which are the key to hyper inflammation and consequent liver damage (Jaeschke, 2000).

IL-10 is an anti- inflammatory cytokine that has been reported to down-regulate TNF-α, as well as, other cytokines production, by suppressing their gene expression in an autocrine-like feedback loop (Oberholzer *et al.,* 2002). In this study, we observed that both GA3 and ETP induced non-significant increase in the level of IL-10 which was significantly increased in response to vitamin C administration, indicating that the inhibition of Pro-inflammatory cytokines formation observed with vitamin C supplementation is not independent of the activation of the feedback loop of IL-10. The present data indicate that vitamin C has anti- inflammatory effects.

Through the production of ROS, the inflammatory process may deplete stores of antioxidants, including vitamin C; so, its usage in treating inflammation associated with diseases has been considered. The literature indicates that the anti-inflammatory properties and antioxidant capacity of vitamin C can be attributed to their ability to modulate the DNA binding activity of nuclear factor kappa B. Vitamin C can reduce the plasma levels of the inflammatory mediators via down regulation of hepatic mRNA expression. Also, vitamin C restores the activity and the content of the antioxidants (El-Taukhy *et al.,* 2006).The anti- inflammatory effect of vitamin C was noticed in this study through its effect to decrease pro-inflammatory cytokines INF-γ, TNF- α, IL-1β and IL-6 and increase the anti-inflammatory cytokine IL-10.

Exposure to chemical pollutants could cause damage to the hematological profile and hematopoietic system in man and animals (Ambali *et al*., 2011). It was reported that GA3 induced positive influences on hematological parameters of rats, poultry, pigs and calves represented in an increase in circulating WBCs and PCV values (Abd-Elhamid *et al*. 1994). Also, GA3 induced significantly higher levels of WBCs; PCV; and lymphocytes and lower levels of RBCs in laying hens (Askar and IsmaeIl., 2012), in addition, it was reported that ETP decreased levels of Hb and hematocrit and number of peripheral blood lymphocytes in mice due to its toxic effect on lymphoid organs (Abou-Zeid *et al.,* 2018).

In the present study, administration of GA3 or ETP showed a significant decrease in RBCs associated with a decrease in Hb compared with control group. In addition, significant increase in WBCs and lymphocytes and significant decrease in neutrophils were recorded. Administration of vitamin C ameliorated all these changes (Table 3). The alteration in the numbers of total leukocytes, lymphocytes and neutrophils indicates that both GA3 and ETP could cause immunological disturbance.

Leukocytosis and lymphocytosis in rats treated with GA3 or ETP may be due to the inflammatory response induced as defense mechanism that induces production. Neutropenia in GA3 or ETP groups may be attributed to consumption of neutrophils during inflammatory response. The decreased count of RBCs may be because of the toxic effect of GA3 or ETP that may cause the failure to supply the circulation with cells from hematopoietic tissues. Also, it could be attributed to the possible destructive effect of GA3 and ETP on RBCs membranes. It was mentioned that the increased destruction of RBCs directly increases the catabolism of Hb (Ekanem *et al*., 2015, Salem *et al.,* 2018). The decreased levels of Hb after GA3 or ETP administration indicate that these growth regulators can cause anemia that may be induced by diminishing RBCs survival or interfering with several enzymatic steps in the heme pathway or even by increase in RBCs fragility (Ambali *et al*., 2010).

The protective role of vitamin C on the toxic effects of GA3 and ETP on hematological parameters noticed in this study may be attributed to its antioxidant and anti-inflammatory properties (Yaqinuddin *et al*., 2022). The antioxidant effect of vitamin C may have improved the integrity of the RBCs compromised by GA3 and ETP induced oxidative stress (Ambali *et al.,* 2010). Furthermore, Vitamin C could reduce elevated count of leukocytes and lymphocytes in the present study apparently due to its anti-inflammatory properties (Yaqinuddin *et al*., 2022). .

Lipid peroxidation is associated with a wide variety of toxicological effects, including decreased membrane fluidity and function, impaired mitochondrial and Golgi apparatus functions, and inhibition of enzymes. (MDA) is an end product of lipid peroxidation and is a frequently measured index of these processes. The NO is an important pathological mediator that is released during the inflammatory process by the iNOS and its induction is facilitated by the various molecular signaling mechanisms such as NFκB and has been implicated hepatitis (Abraldes *et al*., 2007). In the present study, GA3 and ETP intoxication could induce pro-oxidant and inflammatory events in the rats as there was significant increase in liver MDA and No levels in GA3 and ETP groups compared with control group. In agreement with the current results, several studies observed an enhancement in these parameters of adult rats after GA3 and ETP administration. These results suggest that both GA3 and ETP exposure could probably induce oxidative stress and a pro-oxidant event in rat liver (Tousson *et al.,* 2020).

SOD and CAT are the primary enzymes against ROS-mediated toxicity in all living organisms. Also, GSH is considered as major intracellular redox buffer that participate in the cellular defense system against oxidative stress by scavenging free radicals, peroxides, lipid peroxides and reactive oxygen intermediates (Ozsoy *et al.,* 2008). Results of the present study are In agreement with previous studies that observed an inhibition in liver GSH, SOD and CAT activities after GA3 and ETP administration. The decreasing effect of these agents on SOD and CAT activities could be due to the increase in ROS production as revealed by the high MDA levels after GA3 and ETP treatment or it might be due also to the increased generation of hydrogen peroxide, which in turn led to the inhibition of the activities of these enzymes (Daabo, 2022, Tousson *et al.,* 2020).

Administration of vitamin C with GA3 and ETP in this study, significantly ameliorated the changes induced by GA3 and ETP and caused decrease in MDA and No levels and an increase in antioxidant enzyme activities such as (CAT), (SOD), and increased (GSH) content in liver of rats. These effects could be because vitamin C has a well-known antioxidant protective role by either reducing or preventing oxidative stress. It has been shown to efficiently scavenge free oxygen radicals, including superoxide, hydrogen peroxide, hypochlorite and singlet oxygen. Ascorbic acid also contributes to

the redox mechanism by salvaging other antioxidants such as vitamin E, urate, and β-carotene from its oxidized form (Yaqinuddin *et al.,* 2022).

In the current work, administration of GA3 and ETP showed a significant elevation in serum ALT, AST, ALP, GGT and bilirubin when compared with the control group (Table 5). These findings are similar to previous studies that proved that administration of ETP to rats induced liver toxicity (Tousson *et al.,* 2020). Also, the current results agree with another study that demonstrated that ALT and AST significantly increased after calcium carbide, one of the most abundant plant growth regulators, administration to rats (Igbinaduwa *et al.,* 2016). Conversely, El-Okazy (2008) studied the effects of GA3 and ETP in combination on mice and demonstrated a dose-dependent decrease in AST levels. On the other hand, Andrew *et al.* (2018) reported that; no remarkable change in the levels of AST and ALT after rats fed on diets containing the growth regulator calcium carbide ripened mango fruits.

Increasing levels of ALT, AST, ALP, GGT and bilirubin in plasma serve as reliable indices of assessment of damage to the parenchymatous cells of the liver, and an indication of the disturbance and dysfunctions in liver functional enzymes (Tousson *et al.,* 2020). Thus, the observed significant increases in these parameters in the present study are pointers to GA3 and ETP-induced hepatotoxicity and lesions in liver tissues. These findings were consistent with those of previous study (Husseiny *et al.,* 2020) that showed mild piece-meal necrosis of hepatocytes on histopathological examination of liver in rats after administering GA3. In the present study, hepatotoxic and carcinogenic effect of GA3 and ETP was indicated by the elevated levels of AFP in groups intoxicated with these agents as AFP is one of several tumor markers that are found in the blood in high levels when a person has certain cancers and is found mainly in liver cancer. Previous studies found that GA3 significantly increased the incidence of hepatocellular carcinoma occurred in toads liver by a carcinogenic control. Also, it was reported that GA3 induced a significant increase in Swiss Albino mice body weights and tumors were induced in 18% of the males and 36% of the females and were located in the skin of the axillary region, breast, and lung (El-Mofty and Sakr, 1988, El-Mofty *et al.,* 1994)

Findings of the current study showed that administration of GA3 or ETP induced significant decrease in total protein and albumin compared with control group which is indicating of poor and impaired liver functions. Protein is important for structure, function, repair and tissue building and the reduction in total protein indicates increased proteolysis. A toxicant stimulating change in tissue protein leads to reduction in synthesis which may result in shifting in nitrogen metabolism. The decrease in the level of total protein and albumin in GA3 or ETP-treated rats might be due to changes in protein synthesis and/or metabolism (Daabo *et al.,* 2022). Administration of vitamin C with GA3 or ETP induced significant modulation on changes induced by GA3 or ETP in all the previous hepatic parameters which could be attributed to the anti-oxidant and anti-inflammatory properties of vitamin C.

Conclusion

Findings of the present study demonstrated that prolonged administration of GA3 or ETP induced immune-toxicity, disturbed liver functions, provoked inflammation and oxidative stress and deranged different hematological parameters. Administration of vitamin C reduced the adverse effects of GA3 or ETP toxicity in rats via its immune-modulatory, anti-inflammatory, and anti-oxidant activity.

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