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Study the Effect of Vitamin C against Methomyl Toxicity on Hematological and Biochemical Parameters in Male Albino Rats

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ABSTRACT

Background: Methomyl (ME), a carbamate insecticide, is widely used in agriculture and public health programs to control various pests. However, its excessive use can lead to adverse health effects, including toxicity to the liver, kidneys, and hematopoietic system.

Objective: This study aimed to investigate the protective effects of vitamin C (Vit. C) against the toxic effects of methomyl (ME) in male albino rats.

Materials and Methods: Seventy-two male rats were divided into four groups: a control group, a methomyl-intoxicated group, a vitamin C-treated group, and a group co-treated with both vitamin C and methomyl. Each group was further subdivided into three subgroups based on treatment duration: 3 weeks, 6 weeks, and a 3-week recovery period after 6 weeks of treatment.

Results: Hematological analysis of the methomyl-intoxicated group revealed elevated white blood cell and platelet counts, along with decreased red blood cell count and hemoglobin content. Co-administration of vitamin C and methomyl mitigated these effects. Biochemically, the methomyl-treated group exhibited significant increases in hepatic enzyme activities (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), as well as elevated serum lipid profiles (total lipid, total cholesterol, and triglycerides). Additionally, renal markers, including urea and creatinine, were elevated in the methomyl-treated group. Conversely, serum total protein and albumin levels were significantly decreased. The recovery period and co-treatment with vitamin C partially ameliorated these biochemical alterations compared to the control group.

Conclusion: Vitamin C administration can alleviate the toxic effects induced by methomyl.

Keywords: Methomyl, Vit. C, Hematological Parameter, Biochemical Parameters.

INTRODUCTION

Methomyl, S-methyl (EZ)-N-(methylcarbamoyloxy) thioacetimidate (ME) used in agriculture as carbamates insecticides, paper backed treatment of fruits, commercial ornamentals and excluding flies in dairies and poultry houses**.** Also, it is used for controlling of insects at different stages of its developments to control spiders, ticks, and arthropods as it has a rapid effect on the target pests and has a short life in the environment **(1)**.

The World Health Organization (WHO) **(2)**, reports that ME considered as a severely hazardous compound (Class 1B). The interaction of methomyl (ME) with humans may lead to toxic effects categorized into three types based on the exposure route: (1) oral exposure, which is highly toxic; (2) inhalation exposure, which is moderately toxic; and (3) dermal exposure, considered slightly toxic **(3)**.

The mechanism of toxicity associated with pesticides involves the overproduction of reactive oxygen species (ROS), resulting in peroxidative damage to cellular components, including lipid membranes, proteins, and nucleic acids. Additionally, methomyl functions as an endocrine disruptor and is recognized for its potent genotoxic properties, which can cause both structural and numerical chromosomal aberrations in mammalian cells (4).

In mammals, methomyl undergoes metabolic conversion to form a mercapturic acid derivative (MAD), which is primarily eliminated by the liver and kidneys. However, the accumulation of MAD may lead to nephrotoxic conditions. The metabolism of MAD in the bloodstream results in the production of carbon dioxide, which can contribute to hypoxia and impair respiratory function. Furthermore, the accumulation of ME can lead to its hydrolysis, forming S-methyl-N-hydroxy thioacetimidate, which is also subsequently metabolized to carbon dioxide (5).

Moreover, ME initiates an inflammatory response in cardiac tissue through the phosphorylation of NFκB. Also, ME activates the inflammatory pathway in the heart through NFκB phosphorylation. Also, it causes cardiac toxicity, genotoxicity and teratogenicity, reproductive toxicity, and hormonal disruption (6).

Many antioxidants such as vitamin C, E, Zinc, and selenium, have been used to reduce the toxic effects of pesticides. Vitamin C can be taken through common dietary sources or in the form of supplements. Vitamin C is a water-soluble molecule that necessary for normal physiological functions occurs within the body. Vitamin C can be used effectively to reduce the induced genotoxic effects by suppressing oxidative stress (7).

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In the current study, vitamin C, have been selected to study its effect against ME oxidative stress.

Based on the previous information, we hypothesized that vitamin C can reduce ME-induced hematological and biochemical disturbance. So, the aims of the study were: (i) Evaluation the effect of ME on hematological parameters and some biochemical parameters (ii) Investigation the possible protective role of vitamin C against the methomyl toxicity.

MATERIALS AND METHODS

Methomyl (ME), with the chemical formula $C_5H_{10}N_2O_2S$, is a synthetic carbamate insecticide recognized by the CAS chemical name S-methyl N-(methylcarbamoyloxy) thioacetimidate [(1)]. Its CAS registry number is 16752-77-5. The commercial formulation of methomyl, marketed under the brand name 'Lannate' (Methomyl Lannate® 90% SP), contains S-methyl N-[methylcarbamoyloxy] thioacetimidate and was sourced from Agricultural College, Ain Shams University. It was purchased from Beta Chemicals Ltd., Beijing, China, as a pure white very small crystal powder. It was dissolved in saline solution and prepared freshly.

Vitamin C (L-Ascorbic acid) obtained as drops, each 1 ml (20 drops) contained 100 mg. It was purchased from universal pharmaceutical industries, Unipharma Company, Egypt.

All other chemicals were obtained from the local scientific distributors in Egypt.

Animals

Seventy-two healthy male albino rats were used in the present experiment, their weights ranged from 100-150 gm. They were obtained from National Research Center (NRC), Dokki, Cairo, Egypt. They were housed under similar condition in ventilated cage and adjusted temperature. The rats were left for a week as an adaptation period. The experimental rats were fed *ad libitum* with a standard diet and provided with free access to water according to the design of the experiment. They were kept under suitable laboratory conditions during the whole period of experimentation.

Ethical considerations:

All animal procedures of the present experiment were approved by the Institutional Animal Care and Use Committee (IACUC), (CUFS/S/PHY/49/15) of the Faculty of Science, Ain Shams University, Egypt. All the experimental procedures were carried out according to the "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Experimental design:

At the end of adaptation period seventy-two rats were divided randomly into four groups (18 rats in each group, each group was subdivided into three subgroups (6 rats in each subgroup).

First group (1): control rats, allowed to be fed on standard food and drink fresh tap water throughout the study. This group was subdivided into 3 subgroups:

- **Subgroup A:** Fresh tap water was given for rats every day orally for 3 successive weeks.
- **Subgroup B:** Fresh tap water was given for rats every day orally for 6 successive weeks.
- **Subgroup C**: Fresh tap water was given for rats every day orally for 9 successive weeks.

Second group: Rats received daily 8 mg/kg b.wt of methomyl orally via gastric tube. This group was subdivided into 3 subgroups:

- **Subgroup A:** Methomyl was given for rats every day orally for 3 successive weeks.
- **Subgroup B:** Methomyl was given for rats every day orally for 6 successive weeks.
- **Subgroup C**: Methomyl was given for rats every day orally for 6 successive weeks then the animals were left for 3 weeks without treatment.

Third group: Rats received daily 200 mg/kg b.wt of vitamin C orally via gastric tube. This group was subdivided into 3 subgroups:

- **Subgroup A:** Vitamin C was given for rats every day orally for 3 successive weeks.
- **Subgroup B:** Vitamin C was given for rats every day orally for 6 successive weeks.
- **Subgroup C**: Vitamin C was given for rats every day orally for 6 successive weeks then the animals were left for 3 weeks without treatment.

Fourth group: Rats received daily 200 mg/kg b.wt of Vit C orally an hour before oral administration of methomyl (8 mg/kg b.wt). This group was subdivided into 3 subgroups:

- **Subgroup A:** Vitamin C and methomyl were given for rats every day orally for 3 successive weeks.
- **Subgroup B:** Vitamin C and methomyl were given for rats every day orally for 6 successive weeks.
- **Subgroup C**: Vitamin C and methomyl were given for rats every day orally for 6 successive weeks then the animals were left for 3 weeks without treatment.

At the conclusion of the experiment, the rats were fasted overnight and anesthetized using ether vapor. Following anesthesia, the animals were sacrificed, and blood samples were collected for biochemical analyses . The first portion of the blood samples was drawn into heparinized tubes containing ethylene-diamine tetra-acetic acid (EDTA) as an anticoagulant for hematological investigations. This included parameters such as white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin

concentration (MCHC), and platelet (PLT) count. The second portion of the blood samples was used for serum analysis. Blood was placed in clean centrifuge tubes and allowed to clot at room temperature for 2 minutes. The coagulated blood was then centrifuged at 3,000 rpm for 15 minutes. A portion of the clear serum supernatant was frozen at –20ºC for subsequent biochemical analysis. This serum was utilized to determine several biochemical parameters, including serum urea, creatinine, glucose, total protein, albumin, and lipid profile including total lipids, cholesterol, triglycerides (TG), along with liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP).

Hematological Measurements:
Red and white blood cell counts were performed using a hemocytometer according to **Dacie and Lewis** [8]. Hemoglobin concentration was also estimated following the protocol established by **Dacie and Lewis** [8]. The mean corpuscular volume (MCV), representing the average volume of individual red blood cells, and mean corpuscular hemoglobin (MCH), which reflects the average hemoglobin content per red cell, were measured, along with mean corpuscular hemoglobin concentration (MCHC).

Serum Biochemical Measurements:

Serum aspartate aminotransferase (AST) activity was assessed using a kinetic method with a kit sourced from RANDOX, measuring enzyme activity at 340 nm and expressing results in U/L according to Tietz's methodology. Similarly, serum alanine aminotransferase (ALT) activity was determined using kit number AL1200 from RANDOX, also measured at 340 nm and expressed as U/L as per International Federation of Clinical Chemistry standards. The activity of alkaline phosphatase (ALP) was measured using kinetic methodology with kit number AB542 from RANDOX, at 340 nm and expressed in U/L.

Serum glucose levels were evaluated. Total protein content in the serum was determined colorimetrically, where bromocresol green forms a colored complex with albumin, the intensity of which correlates with the albumin concentration in the sample.

Total lipids were colorimetrically quantified with a kit from Bioadwic, with readings taken at 525 nm. Serum cholesterol levels were determined using an "Enzymatic Colorimetric" method, employing kit number 10028 from Human Co. (Germany) and measured at 500 nm. This assay is based on enzymatic hydrolysis and oxidation to form the indicator quinoneimine from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. Serum triglycerides were estimated using the enzymatic colorimetric method with kit number TR 210 from RANDOX, measured at 500 nm and expressed as mg/dL serum.

The colorimetric assay for serum creatinine utilized a kit from RANDOX, measured at 492 nm and expressed as mg/dL serum. Serum urea levels were assessed using an enzymatic colorimetric method with a kit from Diamond Diagnostics.

Statistical analysis

Statistical analyses were performed using SPSS (Version 16). Data were presented as mean $\pm$ standard error (SE). Significant differences among groups were assessed using one-way analysis of variance (ANOVA), followed by post hoc testing with the Duncan test for pairwise comparisons, considering a p-value of < 0.05 as significant. Percentage differences (% D) were calculated using the following formula: [(Treated value – Control Value) / Control Value] x 100.

RESULTS

Table 1 and 2 in the present study shows the significant decrease in the RBCs counts and Hb value in methomyl treated group.

Table (1): Red blood corpuscles (RBCs) count, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) levels in the control and treated groups.

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C +Methomyl	%D	p-value
RBCs 1st 3 weeks	4.51 ^{a}± 0.182 (6)	4.88^{a} ± 0.241 (6)	8.2	3.67^{b} ±0.180 (6)	-18.63	4.37 ^{a} ± 0.167 (6)	-3.10	0.002
RBCs 2nd 3weeks	5.00^{a}± 0.123 (6)	5.17 ^{a}±0 .311 (6)	3.4	3.67 ^{b} ± 0.105 (6)	-26.6	4.79 ^{a}± 0.073 (6)	-4.2	<0.001
RBCs 3rd 3 weeks	4.63^{a}± 0.174 (6)	4.98 ^{a} ±0.177 (6)	7.56	4.13 ^{b}± 0.143 (6)	-10.8	4.77 ^{a} ±0.123 (6)	3.02	0.007
Hb 1st 3 weeks	11.75^{a}± 0.704 (6)	11.83^{a} ± 0.477 (6)	0.68	9.00^{b} ±0.321 (6)	-23.4	9.95^{b} ± 0.491 (6)	-15.31	0.002
Hb 2nd 3 weeks	13.00^{a}± 0.365 (6)	12.66^{a}±0 .494 (6)	-2.61	8.68 ^{b} ± 0.698 (6)	-33.23	11.70^{a} ±0 .823 (6)	-10.00	<0.001
Hb 3rd 3 weeks	12.83 ^{a}± 0.477 (6)	13.16^{a} ±0.307 (6)	2.57	11.66^{a}± 0.760 (6)	-9.11	12.33^{a} ±0.558 (6)	-3.89	0.270
MCV 1st 3 weeks	86.88 ^{a}± 2.94 (6)	90.90 ^{a} ± 1.11 (6)	4.63	93.45 ^{a} ± 4.04 (6)	7.56	84.20 ^{a} ± 3.56 (6)	-3.08	0.191
MCV 2nd 3 weeks	87.02 ^{a}± 3.34 (6)	90.11 ^{a}± 2.72 (6)	3.55	94.55 ^{a} ± 5.24 (6)	8.65	88.36 ^{a} ± 2.48 (6)	1.54	0.495
MCV 3rd 3 weeks	89.58 ^{a}± 2.11 (6)	88.18 ^{a}±2.27 (6)	-1.56	95.46 ^{a}± 4.01 (6)	6.56	89.86 ^{a} ± 4.03 (6)	0.31	0.420
MCH 1st 3 weeks	27.66 ^{a}± 0.803 (6)	27.50 ^{a} ± 1.38 (6)	-0.578	27.83 ^{a} ± 2.039 (6)	0.614	27.50 ^{a} ± 0.764 (6)	-0.578	0.998
MCH 2nd 3 weeks	29.01 ^{a}± 0.925 (6)	29.31 ^{a}±0.893 (6)	1.034	28.80 ^{a} ± 1.59 (6)	-0.723	29.25 ^{a} ± 0.704 (6)	0.827	0.986
MCH 3rd 3 weeks	29.23 ^{a}± 0.378 (6)	28.71^{a} ± 0.485 (6)	-1.778	27.46 ^{a}± 1.25 (6)	-6.055	28.88 ^{a} ±0.999 (6)	-1.197	0.506
MCHC 1st 3 weeks	32.96^{a}± 0.347 (6)	31.73^{a} ± 0.420 (6)	-3.73	32.38 ^{a} ± 0.431 (6)	-1.75	32.45 ^{a} ± 0.360 (6)	-1.54	0.206
MCHC 2nd 3weeks	32.30^{a}± 0.161 (6)	32.18 ^{a}± 0.332 (6)	-0.37	32.45 ^{a} ± 0.319 (6)	0.46	32.10 ^{a} ± 0.418 (6)	-0.62	0.879
MCHC 3rd 3 weeks	32.38 ^{a}± 0.294 (6)	32.91^{a} ±0.490 (6)	1.64	33.0 ^{a}± 0.577 (6)	1.91	32.66 ^{a} ±0.211 (6)	0.86	0.729

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.

Red blood corpuscles (RBCs) count, Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

Table (2): White blood cells (WBCs) and platelets (PLTs) counts in the control and treated groups.

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C +Methomyl	%D	p-value
WBCs 1st 3 weeks	6.56[a]± 0.549 (6)	6.06[a] ± 0.574 (6)	-7.62	10.16 [b] ±1.12 (6)	54.87	7.56[a] ±0 .581 (6)	15.24	0.005
WBCs 2nd 3weeks	7.06[a]± 0.776 (6)	6.62[a]± 0.414 (6)	-6.23	11.8 [b] ± 1.32 (6)	67.13	7.56[a] ± 0.563 (6)	7.08	0.001
WBCs 3rd 3 weeks	6.4[a]± 0.469 (6)	6.03 [ab]± 0.899 (6)	-5.78	8.03[b]± 0.505 (6)	25.46	7.25[ab]±0.514 (6)	13.28	0.119
PLTs 1st 3 weeks	181.67 [a]± 6.54 (6)	198.0[a] ±10.31 (6)	8.99	271.5 [b] ± 24.63 (6)	49.5	198.5 [a]±9.49 (6)	9.26	0.001
PLTs 2nd 3weeks	188.67 [a]±8.19 (6)	219.16 [a]± 13.76 (6)	16.16	308.67 [b]±32.46 (6)	63.60	197.83[a]±5.99 (6)	4.86	0.001
PLTs 3rd 3 weeks	185.33[a]±7.56 (6)	206.17 [ab]±3.61 (6)	11.24	223.0 [b]± 6.29 (6)	20.32	193.33[a]±1.67 (6)	4.32	0.016

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.
White blood cells (WBCs) and platelets (PLTs).

Biochemical parameters

(1) Glucose level

As recorded in **Table 3**, the serum level of glucose exhibited a non-significant difference in ME group and all treated groups in the three periods of treatment as compared to the control group.

Table (3): Glucose levels in the control and treated groups.

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C +Methomyl	%D	p-value
Glucose 1st 3 weeks	**97.66 [a]± 4.25 (6)**	**96.33 [a] ± 3.67 (6)**	**-1.36**	**103.00 [a] ±7.52 (6)**	**5.46**	**103.00 [a] ±6.12 (6)**	**5.46**	**0.760**
Glucose 2nd 3 weeks	**107.66 [a]±3.86 (6)**	**99.00 [a]± 6.51 (6)**	**-8.04**	**103.16 [a] ±8.07 (6)**	**-4.18**	**106.50 [a] ±3.85 (6)**	**-1.08**	**0.726**
Glucose 3rd 3 weeks	**93.33 [a]±4.52 (6)**	**95.8 [a] ± 5.85 (6)**	**2.6**	**100.16[a]±6.97 (6)**	**7.31**	**100.66[a]±5.17 (6)**	**7.8**	**0.386**

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.

(2) Liver function

As shown in **Table 4**, The ME- intoxicated group revealed a significant increase in the activities of ALT, AST and ALP enzymes as compared to the control group. While the results showed the recovering of ALT and AST activities by the combination between ME and Vit. C in all periods. Whereas the ALP enzyme activity returned to the normal activity in the ME and Vit. C treated group in the self-recovery period only, comparing with control group.

Table (4): Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels and the activity of alkaline phosphatase (ALP) in control and treated groups.

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C+Methomyl	%D	p-value
AST 1st 3 weeks	33.33 ^{a}± 0.881 (6)	35.50 ^{a} ±0.763 (6)	6.60	51.83^{b}± 2.761 (6)	55.6	36.66^{a} ± 0.666 (6)	10	<0.001
AST 2nd 3 weeks	34.16 ^{a}± 0.654 (6)	34.66^{a}±0.666 (6)	1.4	57.00^{b} ± 3.750 (6)	66.8	35.16^{a} ± 1.424 (6)	2.9	<0.001
AST 3rd 3 weeks	33.33 ^{a}± 1.358 (6)	33.83^{a}±0.703 (6)	1.50	47.50^{b} ± 1.727 (6)	42.51	36.00^{a} ± 1.032 (6)	8.01	<0.001
ALT 1st 3 weeks	32.33^{a} ± 0.843 (6)	35.33$^{a\ b}$±0.666 (6)	9.28	54.83^{c} ± 2.286 (6)	69.59	36.83^{b} ± 0.601 (6)	13.91	<0.001
ALT 2nd 3 weeks	32.50 ^{a}± 0.562 (6)	35.17^{a}±0.872 (6)	8.21	60.00^{b} ± 3.066 (6)	84.61	37.34^{a} ± 0.882 (6)	14.89	<0.001
ALT 3rd 3 weeks	34.16 ^{a}± 0.909 (6)	34.33^{a}±0.988 (6)	0.49	47.67^{b} ± 2.431 (6)	39.55	35.33^{a} ± 0.667 (6)	3.42	<0.001
ALP 1st 3 weeks	36.50^{a}±1.408 (6)	39.66^{a}±1.145 (6)	8.65	68.83^{c} ± 3.439 (6)	88.5	50.16^{b} ± 1.166 (6)	37.42	<0.001
ALP 2nd 3 weeks	36.66 ^{a}± 0.802 (6)	39.33^{a}±0.421 (6)	7.2	73.16^{c} ± 3.718 (6)	99.5	51.83^{b}± 1.137 (6)	39.55	<0.001
ALP 3rd 3 weeks	33.33 ^{a}± 1.201 (6)	36.17^{a}±0.601 (6)	8.52	48.83^{b}± 1.075 (6)	46.5	36.00^{a} ± 1.861 (6)	8.01	<0.001

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes. aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the activity of alkaline phosphatase (ALP).

(3) Lipid profile

ME treatment raised the total lipids, cholesterol, and triglyceride levels **(Table 5)**, combination between Vit. C and ME was found to inhibit the effects of ME poisoning on the metabolism of lipids.

Table (5): Total lipid, triglycerides, and total cholesterol levels in control and treated groups.

Groups / Parameter	Control	Vit C	% D	methomyl	%D	Vit C +Methomyl	%D	p-value
Total lipid 1st 3 weeks	250.6^{a}± 14.16 (6)	272.5^{a} ± 17.17 (6)	8.73	541.1^{c} ± 13.05 (6)	115.9	447.1^{b} ± 10.3 (6)	78.41	<0.001
Total lipid 2nd 3 weeks	268.1^{a}± 23.46 (6)	311.0 ^{ab}± 27.5 (6)	16.04	553.5 ^{c} ± 13.26	106.4	363.8 ^{b}± 26.9 (6)	35.69	<0.001
Total lipid 3rd 3 weeks	267.1^{a}± 19.3 (6)	372.8 ^{b}± 9.97 (6)	39.57	425.2^{c} ± 13.16 (6)	59.19	393.5 ^{bc} ± 8.03 (6)	47.32	<0.001
Cholesterol 1st 3 week	110.8 ^{a} ± 3.66 (6)	120.2 ^{a}± 1.85 (6)	8.48	136.8 ^{c} ± 1.99 (6)	23.47	125.5 ^{b} ± 1.94 (6)	13.27	<0.001
Cholesterol 2nd 3 weeks	110.0 ^{a} ± 3.922 (6)	119.83 ^{a}± 1.79 (6)	8.94	139.83 ^{c} ± 1.92	27.12	127.16 ^{b} ± 2.28 (6)	15.6	<0.001
Cholesterol 3rd 3 weeks	109.33 ^{a}± 3.8 (6)	117.83 ^{a}± 1.85 (6)	7.77	134.33^{c} ± 2.108	22.86	120.6 ^{b} ± 2.47 (6)	10.3	<0.001
Triglycerides 1st 3 weeks	122.00 ^{a}± 2.50 (6)	127.33 ^{a}± 2.57 (6)	4.37	149.83 ^{c}± 3.44 (6)	22.81	139.33 ^{b} ± 2.81 (6)	14.2	<0.001
Triglycerides 2nd 3 weeks	123.83 ^{a}± 2.30 (6)	128.0 ^{a}± 2.28 (6)	3.37	155.0 ^{c} ± 3.39 (6)	25.17	139.66 ^{b}± 2.97 (6)	12.78	<0.001
Triglycerides 3rd 3 weeks	123.66 ^{a}± 1.62 (6)	126.50 ^{a}± 5.83 (6)	2.29	148.4 ^{c} ± 3.21 (6)	20.00	137.0 ^{b}± 5.877 (6)	10.79	<0.001

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.

(4) Total protein and albumin level

Significant hypoproteinemia and hypoalbuminemia in ME-intoxicated rats were detected in Table 6.

Table (6): Total protein and albumin levels in control and treated groups.

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C +Methomyl	%D	p-value
Total protein 1st 3week	7.33^{a}± 0.088 (6)	7.567^{a} ±0.141 (6)	3.13	5.68^{c} ± 0.070 (6)	-22.5	6.85^{b} ± 0.171 (6)	-6.5	<0.001
Total protein 2nd3week	7.32^{a}± 0.070 (6)	7.38^{a}± 0.154 (6)	0.87	5.47^{c} ± 0.126 (6)	-25.3	6.80^{b}± 0.151 (6)	-7.1	<0.001
Total protein 3rd 3week	7.45^{a}± 0.0428 (6)	7.57^{a}± 0.23 (6)	1.4	6.23^{b} ± 0.236 (6)	-16.3	7.37^{a} ± 0.191 (6)	-1.2	<0.001
Albumin 1st 3 weeks	4.27^{a}± 0.239 (6)	4.39^{a}±0.202 (6)	3.05	3.19^{b} ± 0.150 (6)	-25.4	4.21^{a} ± 0.222 (6)	1.17	0.002
Albumin 2nd 3 weeks	4.22^{a}± 0.72 (6)	4.20^{a}±0.64 (6)	-0.37	3.11^{b}± 0.86 (6)	-26.2	4.35^{a} ± 0.85 (6)	3.17	<0.001
Albumin 3rd 3 weeks	4.33^{a}± 0.221 (6)	4.48^{a}±0.157 (6)	3.4	3.24^{b}± 0.136 (6)	-25.1	4.26^{a}± 0.252 (6)	1.6	0.001

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.

(5) Kidney Function

The results showed that vitamin C administration to ME toxicity rats showed to some extent improvement in the levels of urea and creatinine compared with methomyl treatment periods **(Table 7).**

Table (7): creatinine and urea levels in control and treated groups

Groups / Parameter	Control	Vit C	% D	Methomyl	%D	Vit C +Methomyl	%D	p-value
Creatinine 1st 3 weeks	0.816^{a}± 0.060 (6)	0.917^{a} ±0.277 (6)	12.34	1.69^{c} ± 0.101 (6)	107.1	1.12 ^{b} ± 0.022 (6)	36.6	<0.001
Creatinine 2nd 3 weeks	0.851^{a}± 0 .055 (6)	1.02^{ab}± 0.046 (6)	19.8	2.29^{c} ± 0.091 (6)	169.09	1.13 ^{b}± 0.014 (6)	32.31	<0.001
Creatinine 3rd 3 weeks	0.825^{a}± 0.054 (6)	0. 807 ^{a}± 0.036 (6)	-2.21	1.08^{b} ± 0.030 (6)	30.9	0.802^{a} ± 0.041 (6)	-2.82	<0.001
Urea 1st 3 weeks	31.50^{a}± 0.846 (6)	35.66^{ab}±1.145 (6)	13.20	48.83^{c} ± 2.68 (6)	55.01	37.50^{b} ± 0.671 (6)	19.04	<0.001
Urea 2nd 3 weeks	30.16^{a}± 0.601 (6)	34.16^{ab}±0.749 (6)	13.26	65.33^{c}± 3.02 (6)	116.6	36.83^{b} ± 0.703 (6)	22.11	<0.001
Urea 3rd 3 weeks	30.33^{a}± 0.882 (6)	32.50^{ab}±0.563 (6)	7.15	36.33^{c}± 1.45 (6)	19.7	35.16^{bc}± 1.19 (6)	15.9	0.004

Values are represented as mean ± S.E, % D: Percentage difference [(Treated value – Control Value) / Control Value] x 100, Different letters indicate significantly different means at p-value < 0.05, Same letters indicate non-significant changes.

DISCUSSION

Methomyl is a systemic insecticide used widely in agriculture. But the higher application rates increase the risk of threaten human health through a direct contact and a long-term bioaccumulation in food or water [9]. Carbamates accentuate oxidative stress in rats, which lead to the overproduction of free radicals that exert serious effects on the liver, kidney, brain, and erythrocytes.

In the present study, we studied the hematological and physiological impact of vitamin C against methomyl toxicity on male albino rats.

HEMATOLOGICAL RESULTS

With reference to table 1 and table 2, there was a significant reduction in key hematological parameters, including red blood cell count, hemoglobin level, and mean corpuscular volume. Vitamin C supplementation, while exhibiting minimal independent effects, appears to attenuate the adverse effects of methomyl exposure, as evidenced by less pronounced decreases in these parameters when administered concurrently. These findings highlight the potential protective role of vitamin C against methomyl-induced hematological toxicity.

Methomyl exposure resulted in a significant elevation in both WBC and PLT counts compared to the control group. While vitamin C alone exhibited minimal influence on these parameters, its co-administration with methomyl led to a significant reduction in the methomyl-induced increase in PLT count. Notably, vitamin C did not mitigate the methomyl-induced elevation in WBC count. These findings suggest that methomyl exerts a stimulatory effect on hematopoiesis, particularly megakaryopoiesis, and vitamin C may have a selective inhibitory effect on platelet production. Further investigation is warranted to elucidate the underlying mechanisms and explore the potential clinical implications of these findings.

Our results are consistent with the observations of **Mossa and Abbassy** [10], who noted decreased red blood cell counts, hemoglobin concentration, and packed cell volume in rats treated with methomyl (1.70 mg/kg bw) over 90 days. The anemia observed in the methomyl-treated group may stem from acute hemolysis or oxidative stress affecting red blood cells, leading to increased RBC breakdown and potentially impacting bone marrow function. Erythrocytes from male rats exposed to methomyl exhibited signs of hemolysis. Carbamates are known to inhibit acetylcholinesterase (ACHE), present in both erythrocytes and plasma [10].

The improvements observed in the combined ME and vitamin C group may be attributed to vitamin C's role as a water-soluble antioxidant, providing protection against organophosphorus pesticides [11]. Furthermore, vitamin C has been shown to reduce chromosomal damage and oxidative DNA harm caused by certain toxic substances. It also diminishes fragmentation and rearrangements induced by pesticides, and both forms of vitamin C—ascorbic and dehydroascorbic acid—have been reported to alleviate cellular oxidative stress [12]. Vitamin C is integral to various physiological processes, acting primarily as a reducing agent (donating electrons) and regulating copper and iron metabolism in the body, including enhancing iron absorption [13]. The increase in white blood cell count may indicate activation of the animal's immune defenses [10].

The amelioration occurred in WBCs count by Vit. C may relate to the of vitamin C in the protection against infections. It is important for the differentiation and function of immune cells and epithelial barrier cells. Patients with infections have low levels of vitamin C and the animal models showed a protective effect of vitamin C on different infections or the intoxications using toxins of bacteria [14].

PHYSIOLOGICAL RESULTS

Biochemical parameters

(1) Glucose level

In the present study, serum glucose levels did not significantly differ in the ME group and other treated groups across the three treatment periods when compared to the control group. This contrasts with earlier research, such as the findings by **Groswald *et al.*** [15], which indicated that methomyl affects carbohydrate metabolism by reducing fasting blood glucose levels. These discrepancies may arise from variations in ME concentration or treatment duration.

(2) Liver function

AST and ALT are essential enzymes involved in amino acid catabolism into α-keto acids, crucial for metabolic processes, including the Krebs cycle and electron transport chain [16].

In the present study, the ME-intoxicated group exhibited a significant increase in ALT, AST, and ALP enzyme activities relative to the control group. Notably, the combination of ME and vitamin C resulted in a recovery of ALT and AST activities throughout all treatment periods, while ALP enzyme activity only returned to normal during the self-recovery period in the ME and vitamin C treated group compared to controls. Carbamate exposure can disrupt biochemical parameters in experimental animals [17].

Our findings align with those of **Chabane *et al.*** [18], who reported that oral administration of methomyl led to a marked increase in AST and ALT activities in male rats, likely due to methomyl's capacity to induce oxidative stress, resulting in hepatotoxicity, cellular damage, and enzyme leakage into the bloodstream [18].

The elevated enzyme activities suggest increased permeability or necrosis of hepatocytes. However, the administration of vitamin C to ME-treated rats mitigated the rise in these enzyme activities, likely due to its hepatoprotective properties. Previous studies, such as those by **Bashandy and Alwasel** [19], have supported

the notion that vitamin C can alleviate hepatic damage induced by various chemical agents.

Ascorbic acid was also able to preserve 100% of cell integrity and modulated alanine aminotransferase and aspartate aminotransferase. Similar results were reported when deltamethrin (1.28 mg/kg) was administered to rats as the alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase were significantly increased [20]. Pretreatment with vitamin C (200 mg/kg) normalized the activities of these enzymes [21].

(3) Lipid profile

In our findings, ME treatment elevated total lipids, cholesterol, and triglyceride levels, aligning with **Hashish and Elgaml's** [22] study. The increase in serum cholesterol levels may result from pesticides affecting liver cell membrane permeability [23].

Additionally, the rise in cholesterol levels due to ME is consistent with **Ashgar *et al*.** [24], who found elevated total cholesterol in male rabbits treated with methyl parathion. This increase likely stems from enhanced cholesterol synthesis in the liver.

Moreover, the observed increase of the serum cholesterol level, which is associated with the increase in triglycerides, may recommend a possible membrane lipid peroxidation [25]. The increased serum total cholesterol and triglyceride level in ME treated rats may be due to the increasing of catecholamine concentration, which may raise lipolysis and the formation of fatty acids that directly affect lipoprotein metabolism.

In our experimental conditions, combination between Vit. C and ME were found to inhibit the effects of ME poisoning on the metabolism of lipids. These findings are compatible with the results of the previous studies by **Saoudi *et al.*** [25], showing the treatment of rats with ME plus vitamin C decreased the serum cholesterol and triglycerides levels compared with the rats treated with ME only.

(4) Total protein and albumin level

The present results revealed a significant hypoproteinemia and hypoalbuminemia in ME-intoxicated rats **(Table 6)**. This may be a result of a decrease in the synthesis of albumin in the liver because of liver damage caused by methomyl administration, catabolism of protein, malfunction of the liver [26]. Also, the results showed the improvement that occurred by the administration of vitamin C with methomyl to the rats.

(5) Kidney Function

Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism, whose measurement considered a useful marker of the kidney function. As stated by **Ran *et al.*** [27], the increase in the urea level could be as a result of increasing in nitrogen retention and/or owing to kidney dysfunction. A clear elevation was noticed in the serum urea level in acute defect of glomerular filtration.

The elevation of serum creatinine and urea is considered as significant sign of renal dysfunction [28], raised blood urea is associated either with an increased protein catabolism in the mammalian body or with an extra transformation of ammonia to urea, along with increased synthesis of enzymes involved in urea production [29].

Renal biomarkers were also evaluated in the serum of experimental rats to confirm the renal damage. The treatment with vitamin C shows a non-significant change in theses parameters. The intoxicated rats by methomyl group exhibited a significant increase in the urea and creatinine levels compared to control, this was related to oxidative stress which may describe the renal affection [30]. The results also showed that vitamin C administration to ME toxicity rats showed to some extent improvement in the levels of urea and creatinine compared with methomyl treatment periods **(Table 7).** Similar changes in creatinine and urea values were reported in rabbits by ME-treated rats [23].

CONCLUSION

Our study's findings point to the potential risks of methomyl treatment on the hematological and biochemical parameters of treated rats, as well as liver and renal malfunction. The effects of prolonged exposure are more pronounced. Therefore, in order to minimize potential risks, the use of methomyl should be restricted to a program that has been well planned and handled. Our research showed that the hematological, hepatic, and renal functions were improved when vitamin C and methomyl were administered together. According to these findings, vitamin C therapy may offer some protection against oxidative damage brought on by methomyl. We suggest that vitamin C might offer a long-term solution without negative side effects to oxidative damage brought on by pollutants.

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