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EFFECT OF VITAMIN E AND SELENIUM ON IMMUNITY, NEUROTRANSMITTER LEVEL, HEPATORENAL FUNCTION, AND MEAT QUALITY IN CHLORPYRIFOS-INTOXICATED EXPERIMENTAL RABBITS

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ABSTRACT

Oxidative stress caused by pesticide exposure is one of the main causes of animal toxicity. Additionally, it degrades the quality of meat and liver tissues. This study investigated how vitamin E and selenium influence immunity, the activity of acetylcholinesterase, liver and kidney health of the chlorpyrifos (CPF) intoxicated rabbits , as well as the quality of their meat and liver over a week of chilling. Each of five groups of the total thirty male New Zealand white rabbits received either corn oil, CPF, CPF and vitamin E, CPF and selenium, or a combination of CPF, vitamin E and selenium for 14 days. The study found that combining vitamin E and selenium with chlorpyrifos treatment in rabbits improved hepatorenal function, normalized levels of immunity (lysozyme, nitric oxide, and tumour necrosis factor-alpha), and prevented drops in serum acetylcholine esterase (AChE) levels. This combination also showed protective effects against oxidative stress (elevation of glutathione (GSH) and superoxide dismutase, SOD) and tissue damage, and lowering CPF residues in fresh tissues to below the maximum residue limit, ensuring food safety for human consumption. The supplementation also improved the quality of meat and liver by ameliorating pH, total volatile basic nitrogen (TVB-N), and thiobarbituric acid (TBA) levels to extend shelf life and reduce potential public health risks associated with pesticides. The study concludes that combined administration of vitamin E, selenium has protective effects against oxidative stress and tissue damage induced by chlorpyrifos and elevates the meat and liver quality of rabbits.

Keywords: Pesticides, AChE, antioxidants, meat quality, Residue

INTRODUCTION

Despite being recognized as a significant environmental issue, pesticides remain the most economically viable option for managing various agricultural pests. Livestock can accumulate persistent organic pollutants from contaminated feed and water and pesticides used in regions where animals are raised. Feeding chickens with plant materials treated with pesticides through cultivation and storage may result in contamination. Growing public concern over pesticide residues in animal-derived food has heightened the need for precise technology to identify these chemicals

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(Pagliuca *et al.,* 2005; WHO, 2005; and Jabłońska-Trypuć, 2017).

Chlorpyrifos (CPF) (O, O′-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothionate) is a widely used chlorinated organophosphate insecticide found in agriculture, animal husbandry, horticulture, and domestic settings. It functions as a cholinesterase inhibitor, disrupting the production, release, and breakdown of acetylcholine, resulting in cholinergic toxicity. The symptoms of cholinergic toxicity can vary based on the type of inhibitor, class, or exposure method. The main toxic effects are on the endocrine system, but it can also affect organs such as the liver and kidneys (Pope *et al.,* 2005; Cobilinschi *et al.,* 2020). CPF has moderate toxicity in rats and mice, with a lethal dose (LD50) extending from 50 to 500 mg/kg body weight. It also shows moderate toxicity in sheep, guinea pigs, and pigs (Pope *et al.,* 2005; Ishii *et al.,* 2007). Continuous exposure to pesticides via the food chain in humans is related to negative health effects, including endocrine disruption, cancer, immunosuppression, and reproductive issues in animals and humans. The toxicity of CPF is connected to increased production of reactive oxygen species, producing oxidative stress (Babazadeh and Najafi 2017; Mishra *et al.,* 2019; and Song *et al.,* 2025).

Rabbit meat is renowned for its nutritional profile, essential minerals, high-quality proteins, polyunsaturated fatty acids, and vitamins (Abd-Allah and Abd-Elaziz, 2018). Rabbit meat is highly perishable due to its nutritional composition, a pH level that falls within the optimal range for microbial growth, and the presence of lipids that are particularly prone to oxidation (Moawad *et al.,* 2020). Chlorpyrifos maximum residual limit (MRL) is 0.01 mg/kg in swine meat, liver and kidney according to the European Food Safety Authority (EFSA, 2017). Antioxidants can reduce or prevent the oxidation of other molecules caused by reactive oxygen species (ROS). Vitamin E serves as a cell's crucial membrane-bound antioxidant. It prevents cell membrane lipid peroxidation and preserves mitochondrial enzyme activities in contrast to ROS (Niki, 2015; Habibian *et al.,* 2016; and Viliene *et al.,* 2021). Moreover, selenium is essential for animal growth, reproduction, immunity, metabolism, and organ function. It enhances animal production and improves antioxidant status (Gouda *et al.,* 2021; Abdelmoteleb *et al.,* 2023). Freshness parameters, such as TVBN and TBA values, serve as key indicators of the degradability of proteins and fats. Additionally, the pH level can be an effective marker for identifying the initial stages of decomposition (Li *et al.,* 2019; Luong *et al.,* 2022).

The current study investigates how vitamin E and selenium affect immunity, neurotransmitter levels, and hepatorenal function while also assessing meat and liver quality during a 7-day refrigeration period. Furthermore, it evaluates the tissue distribution of chlorpyrifos in muscle, liver, and kidneys using HPLC assays, providing insights into its residual presence and potential food safety implications.

MATERIALS AND METHODS

Drugs:

Chlorpyrifos-certified reference material, Trace CERT®, (Sigma-Aldrich Production GmbH, Switzerland). Chlorpyrifos 48% EC (Hebei Hontai Biotech Co., Ltd, China). To induce rabbit intoxication, Chlorpyrifos was given at 33.3 mg/kg/day, orally in corn oil (Yassin *et al.,* 2021). Vitamin E (Merck Pharmaceuticals Pvt. Ltd., Karachi, Pakistan) was given at a dose of 50 mg/kg/day, orally, and selenium as sodium selenite (Asia Pharmaceuticals Pvt. Ltd., Faisalabad, Pakistan) was given at 0.5 mg/kg/day, orally (Naseer *et al.,* 2020).

Animals and Experimental Design:
The Institutional Animal Ethics Committee (ARC/AHRI/87/24) approved the experimental protocol. Thirty New Zealand white male rabbits, weighing 1100 ± 75 g, were obtained from Giza farm and divided into 5 groups. Group I received corn oil, which served as the normal control. Group II was a positive control, which received chlorpyrifos; Group III received chlorpyrifos and vitamin E; Group IV received chlorpyrifos and selenium; and Group V received a combination of chlorpyrifos, vitamin E, and selenium. The rabbits were given a balanced diet with unrestricted access to food and tap water during the experiment. The chlorpyrifos was administered once daily in the morning after a food supplement. After an hour, selenium and vitamin E were administered for 14 days. At the end of the experimental period, rabbits were slaughtered for serum separation and for collecting muscle, kidney, and liver to perform different analyses.

Determination of chlorpyrifos residue:
The chlorpyrifos concentration was determined using AGILENT HPLC (1200 series, DE6297628) with a mobile phase consisting of 80% acetonitrile in water. The flow rate was 1 mL/minute, and the separation was performed on a C18 reversed-phase column (Supelco, 25 cm x 4.6 mm, 5 µm, Sigma-Aldrich) at a temperature of 40°C (Bottomley and Baker, 1984). The calibration plot was obtained by serial dilution of chlorpyrifos-certified reference material, in acetonitrile in the range of 10-2000 ppb. The CPF extraction process involved homogenizing 5 grams of tissue samples (liver, kidney, and muscle) with 20 mL of acetonitrile in a 25 mL test tube. The mixture was shaken for 30 minutes and then centrifuged at 7000 rpm for 10 minutes. The resulting supernatant was filtered and transferred to a separatory funnel, where it was combined with 10 mL of dichloromethane (HPLC grade from Thermo Fisher Scientific) and 40 mL of 2.5% sodium sulphate solution. This extraction process was repeated twice, and the collected lower layers were treated with 4 grams of anhydrous sodium sulphate. The samples were then purified using an alumina column eluted with 20 mL of dichloromethane. Afterwards, the samples were dried using a lyophilizer. The dried residue was reconstituted with 1 mL of acetonitrile and filtered through a 0.22 µm nylon syringe filter. A 20 µL of the filtrate was analysed using an HPLC auto-sampler (Singh *et al.,* 2018).

Determination of serum Acetylcholine esterase (AChE) activity:
The AChE activity was measured using a spectrophotometer (UV/VIS, T80) at 412 nm, following the method described by Ellman *et al.* (1961) and using acetylthiocholine iodide as the substrate. The intensity of the yellow color indicates the level of AChE activity. The percentage of AChE inhibition was calculated using the equation: AChE = [(EAC-EAT)/EAC] ×100, where EAC and EAT represent the activity of the acetylcholine esterase enzyme in the control and treatment groups, respectively (Commission of the European Communities, 2000).

Determination of serum hepato-renal function:
According to Anderson and Cockayne (1993)**,** serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and total protein were measured in the laboratory using commercial kits obtained from Sigma Company. Additionally, creatinine, urea, and uric acid were estimated spectrophotometrically, respectively.

Determination of serum oxidant/ antioxidant biomarkers:
The spectrophotometric determination of malondialdehyde (MDA), an indicator of oxidative stress, glutathione (GSH), and superoxide dismutase (SOD), biomarkers

of antioxidant parameters, was estimated according to Mistura and Medura 1987; Ellman 1959; and Nishikimi *et al.,* 1972, respectively.

Determination of immunological parameters:

Serum lysozyme activity assay:

It was determined as described by Schultz (1987). The lysoplates were prepared by dissolving 1% agarose in 0.06 M PBS (pH 6.3) with 500 mg/L *micrococcus lysodeikticus*. The concentrations of lysozyme were obtained from the logarithmic curve of standard lysozyme.

Serum Nitric Oxide (NO) Assay:

The concentrations were determined as described by Lee *et al.* (2011) and were calculated from a standard curve with sodium nitrate.

Molecular detection of tumour necrosis factor-alpha (TNF-α) mRNA gene expression in liver tissue by quantitative real-time PCR (qRT-PCR):

RNA extraction was applied using the QIAamp RNeasy Mini kit (Qiagen, GmbH, Germany). Oligonucleotide primers supplied from Metabion (Germany) are listed in Table (1). The SYBR green qRT-PCR reaction was achieved in a Stratagene MX3005P real-time PCR machine. Analysis of results was determined by the amplification curves and threshold cycles (CT) values (Stratagene MX3005P software). The variation of fold change in mRNA gene expression of different samples was estimated, and the CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method stated by Yuan *et al.* (2006).

Determination of meat and liver chemical quality:

Different samples of rabbit meat and liver were packaged and labelled inside sterile polyethylene bags and then stored in the refrigerator at 4±1°C. The experiment was repeated three times. All sample groups were subjected to chemical examination at (zero, 1st, 3rd, 5th, and 7th) days. The chemical quality was evaluated by estimation of pH, total volatile basic nitrogen (TVBN) and thiobarbituric acid (TBA) as follows:

pH determination (ES:63-11/2006):

Ten grams of minced rabbit meat or liver samples were added to one hundred ml of cooled distilled water that had been boiled previously. The flask was left for ten minutes. Apart from the hydrous layer added to another flask. The pH was measured by a calibrated digital pH meter (Jenway pH meter, 3310).

Total volatile basic nitrogen (TVB-N) determination (ES: 63-9/2006):

Minced rabbit meat or liver samples (10 grams) were put in a conical flask with 2 grams of magnesium oxide, 300 mL of distilled water and a glass bead antifoaming agent. 125 mL of distillate was received in a flask containing twenty-five mL of 2% boric acid solution. The distillate was titrated by using 0.1N sulphuric acid solution until the endpoint. The blank detection occurred using distilled water instead of the samples, using the same steps. TVB-N (mg N/100g) of rabbit meat or liver sample was calculated as follows: TVB-N (mg N/100g) = 14 × (D – A), where D is the sulphuric acid volume that was used for the titration of the sample and A is the sulphuric acid volume that was used for the titration of the blank.

Thiobarbituric acid (TBA) determination (ES:63-10/2006):

Approximately 10 grams of prepared rabbit meat sample were mixed with 97.5 mL of distilled water, 2.5 mL of HCl solution (4 N), and glass beads in a distilling flask. 50 ml of distillate was collected in an empty flask. Five ml of TBA reagent were mixed with 5 ml of distillate in a test tube that was put into a

boiling water bath for 35 min. 5 ml of distilled water was added to 5 ml of TBA reagent in another test tube instead of the sample as a blank. The optical density (R) of the sample was read on the spectrophotometer at 538 nm, a wavelength against the blank. TBA was calculated as follows:
TBA (mgA/kg) =7.8 × R

Statistical Analysis:
Statistical analysis was carried out using analysis of variance (ANOVA) using SPSS (version 2020).

RESULTS

Chlorpyrifos residue in different tissues:
The analytical method used was linear with a regression equation of Area = 98.2× Amount +49.5 and a correlation coefficient of 0.99997 (Figure 1). The limits of detection and quantification are 0.015 and 0.045 ppb, respectively. The retention time for the separation of CPF was 6.213 minutes, as shown in Figure (2). It was found that CPF was detected in muscle, liver, and kidney with different residue concentrations. The highest concentrations were found in the kidneys. Vitamin E or selenium administration (G-III or G-IV) significantly declines chlorpyrifos residue in tested tissues (p-value < 0.05), except in the rabbit muscle of group III. Administration of vitamin E and selenium (G-V) showed the lowest chlorpyrifos residue in muscle and kidney and non-detected residue in the liver, as illustrated in Table (2). According to the European Food Safety Authority, the minimum residue limit (MRL) for chlorpyrifos and chlorpyrifos-methyl in food and feed was lowered, which can be measured by analytical laboratories, to 0.01 mg/kg. Our results showed the highest CPF residue found in the kidney, followed by the liver. The lowest values were detected in muscle (EFSA, 2017).

Table 1: Primer sequences, target genes and cycling conditions for SYBR green qrt-PCR

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation	
GAPDH	TGACGACATCAAGAAGGTGGTG	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	60˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	60˚C 1 min.	94˚C 1 min.	**Schnupf and Sansonetti (2012)**
	GAAGGTGGAGGAGTGGGTGTC									
TNF-α	GTCTTCCTCTCTCACGCACC				60˚C 30 sec.			60˚C 1 min.		**Godornes et al., (2007)**
	TGGGCTAGAGGCTTGTCACT									

Serum acetylcholinesterase (AChE) activity:

A strong decrease in AChE activity was observed in the CPF-intoxicated rabbits (GII) at the end of the study. However, the rabbits treated with vitamin E and selenium (GV) showed a non-significant decline compared to the control group (G1), which only received corn oil. There were significant increases in AChE activities (P<0.05) observed in the G III, G IV, and G V groups when compared to the G II group (Table 3).

Table 2: Effect of vitamin E and selenium on chlorpyrifos residue (ppb) in experimental intoxicated rabbits

	Muscle	Liver	Kidney
G I	Nd	Nd	Nd
G II	1.37 ± 0.06^{a}	4.03 ± 0.25^{a}	48.47 ± 3.46^{a}
G III	1.12 ± 0.07^{a}	3.53 ± 0.06^{b}	27.67 ± 2.52^{b}
G IV	0.76 ± 0.05^{b}	1.58 ± 0.11^{c}	10.97 ± 1.04^{c}
G V	0.18 ± 0.02^{c}	Nd	3.97 ± 0.23^{d}

Values were expressed as mean ± standard deviation (n=6).
The different uppercase alphabetical letters in the same column mean significance (p-value < 0.05).

Table 3: Effect of vitamin E and selenium on serum AChE levels in experimental intoxicated rabbits

	AChE (U/L)
G I	4421.8 ± 410.9^{a}
G II	191 ± 15.4^{b}
G III	1359 ± 15.4^{c}
G IV	2425.5 ± 171.4^{d}
G V	4072.2 ± 269.6^{a}

Values were expressed as mean ± standard deviation (n=6).
The different uppercase alphabetical letters in the same column mean significance (p-value < 0.05).

Serum hepato-renal function:
Levels of ALT and AST enzymes in the experimentally intoxicated rabbits (GII) were significantly higher, compared to the control negative group (GI). Group II had the highest urea, uric acid, and creatinine levels, while the total protein concentration decreased significantly. Vitamin E and selenium supplementation helped maintain normal levels of hepatorenal parameters (Table 4).

Table 4: Effect of vitamin E and selenium on hepatorenal function in experimental CPF-intoxicated rabbits

	ALT U/L	AST U/L	Urea mg/dL	Creatinine mg/dL	Uric acid mg/dL	Total protein g/dL
G I	34.56 ± 1.10^{a}	42.5 ± 1.92^{a}	25.53 ± 1.4^{a}	0.97 ± 0.08^{a}	2.91 ± 0.24^{a}	4.14 ± 0.12^{a}
G II	51.76 ± 2.51^{b}	107.24 ± 3.41^{b}	35.31 ± 1.6^{b}	1.51 ± 0.06^{b}	5.12 ± 0.27^{b}	2.53 ± 0.17^{c}
G III	36.33 ± 2.423^{a}	42.75 ± 2.25^{a}	24.73 ± 1.9^{a}	1.04 ± 0.05^{a}	3.13 ± 0.21^{a}	3.11 ± 0.17^{b}
G IV	40.82 ± 2.4^{c}	40.83 ± 2.46^{a}	24.82 ± 1.7^{a}	1.10 ± 0.08^{a}	3.22 ± 0.21^{a}	3.22 ± 0.23^{b}
G V	32.84 ± 2.34^{a}	40.51 ± 1.96^{a}	23.73 ± 1.4^{a}	0.99 ± 0.05^{a}	2.83 ± 0.22^{c}	3.41 ± 0.08^{b}

Values were expressed as mean ± standard deviation (n=6).
The different uppercase alphabetical letters in the same column mean significance (p-value < 0.05).

Serum oxidant/antioxidant biomarkers:
Data in Table 5 indicated that rabbits intoxicated with chlorpyrifos (G-II) had the highest levels of MDA. Conversely, SOD and GSH concentrations were significantly reduced, indicating a compromised antioxidant defence system. Administration of vitamin E (G-III) or selenium (G-IV) had a positive influence by decreasing values of MDA and increasing values of GSH and SOD in comparison with G-II. Supplementation with vitamin E and selenium had succeeded in maintaining normal levels of these biomarkers.

Table 5: Effect of vitamin E and selenium on oxidant/antioxidant biomarkers in experimental CPF-intoxicated rabbits

	MDA U/mL	GSH mg/dL	SOD U/mL
G I	3.38 ± 0.11[a]	42.51 ± 1.87[a]	19.5 ± 1.38[a]
G II	13.13 ± 0.95[b]	10.75 ± 0.36[b]	4.77 ± 0.35[b]
G III	4.72 ± 0.33[c]	21.33 ± 0.82[c]	10.67 ± 0.82[c]
G IV	4.15 ± 0.61[d]	31.17 ± 1.47[d]	12.33 ± 1.21[d]
G V	3.25 ± 0.14[a]	40.33 ± 2.33[a]	23.33 ± 1.21[e]

Values were expressed as mean ± standard deviation (n=6).
The different uppercase alphabetical letters in the same column mean significance (p-value < 0.05).

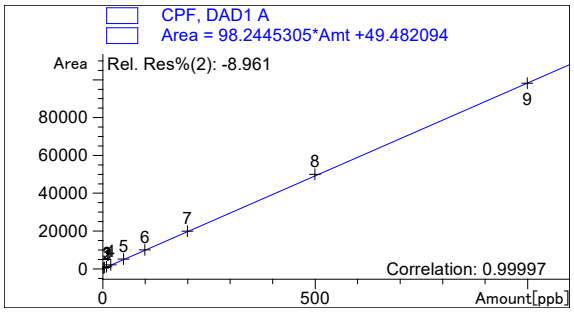

Figure 1: Calibration plot of CPF by HPLC

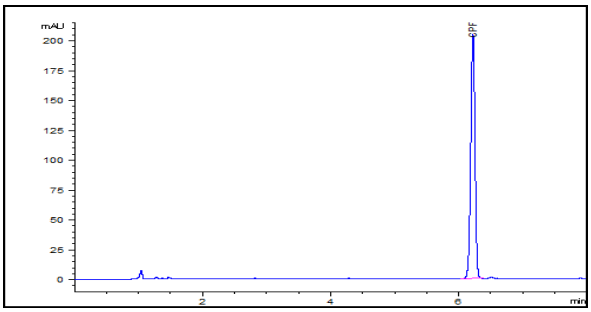

Figure 2: The chromatogram shows 10 ppb of CPF at a retention time of 6.213 minutes.

Lysozyme concentration:
The data obtained revealed a significant decrease in levels of lysozyme (Figure 3) in chlorpyrifos-intoxicated rabbits (G-II), compared with the negative control one (G-I). Although those of G-III and G-IV exposed a clear rise in lysozyme concentration in comparison with group G-II, nevertheless, it did not reach the normal values as in G-I. The influence of vitamin E and selenium (G-V) on lysozyme was promising and was evidenced by the values of lysozyme as those of G-I.

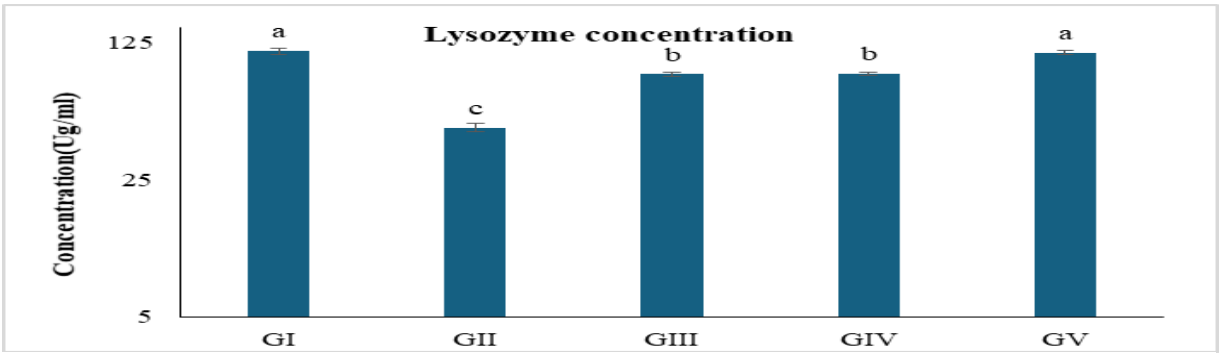

Figure 3: Effect of vitamin E and selenium on lysozyme concentration in rabbits intoxicated with CPF
Data were expressed as mean ± standard error.
Different uppercase alphabetical letters indicate significance between groups at p < 0.05.

Nitric oxide:
Results of NO (Figure 4) conveyed a notable elevation in NO concentration in G-II compared with those of G-I. However, the levels of NO in G-III and G-IV were also high, but they were numerically lower than G-II. Results of G-V reflect the positive impact of the vitamin E and selenium combination in returning NO to its normal levels as compared with G-I.

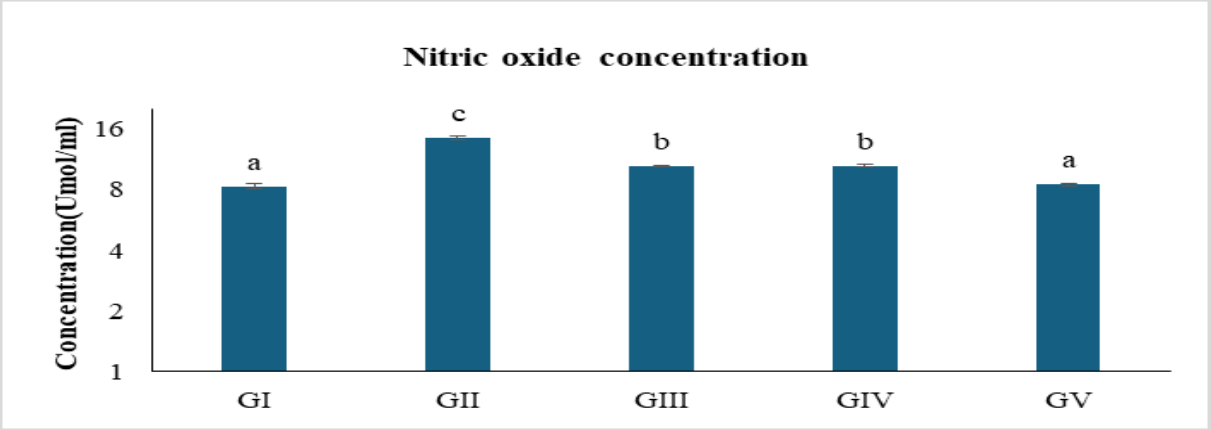

Figure 4: Effect of vitamin E and selenium on NO concentration in rabbits intoxicated with CPF
Data were expressed as mean ± standard error.
Different uppercase alphabetical letters indicate significance between groups at P<0.05.

Tumour necrosis factor-alpha (TNF-α):
The representative data (5) illustrated a significant increase in TNF-α in CPF-intoxicated rabbits (G-II) compared with that of G-I. The gene expression was sharply downregulated in G-III and G-IV; however, it was still higher than the values of G-I. The combination of vitamin E and selenium (G-V) had succeeded in returning levels of TNF-α to be similar to those of G-I.

Meat and liver quality results:
Regarding the Figures (6 and 7) data, it is indicated that the initial pH value in intoxicated rabbit meat and liver in G-II was higher than all other groups; then these values were decreased on the 1st examination day; after that, these values were raised in all groups on the other examination days. G-II showed significant differences with other samples on all days of examination. The reported results in figures (8 and 9) revealed that G-I had the lowest initial TVB-N content, followed by GV, while GII had the highest initial TVB-N content. The content of TVB-N in all examined refrigerated rabbit liver and rabbit meat groups increased along with the refrigeration period. There were significant differences (P<0.05) on all days of examination among G II with all sample groups. The recorded data in figures (10 and 11) indicated that the TBA (mg MAD/kg) in rabbit liver and meat was at the lowest value at GV and the highest value at GII in the initial and last days of examination. There were significant differences (P<0.05) on all days of examination among GII with all sample groups, except on the third day of GIII rabbit meat examination.

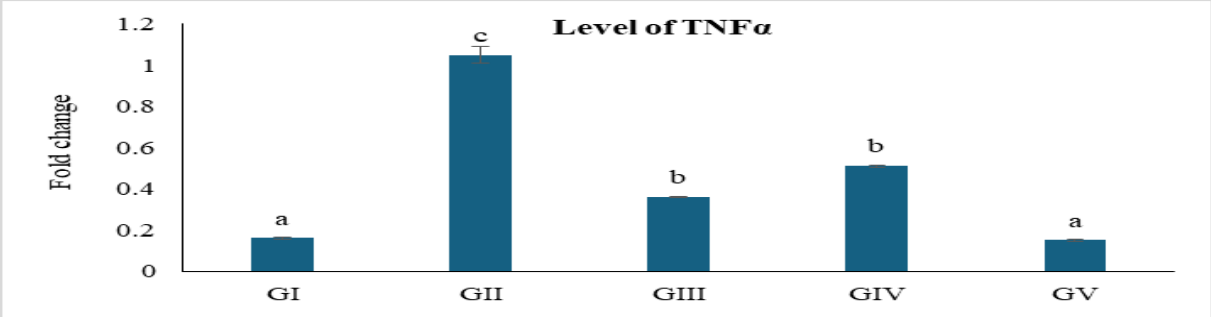

Figure 5: Effect of vitamin E and selenium on TNF-α levels in rabbits intoxicated with CPF.
Data were expressed as mean ± standard error.
Different uppercase alphabetical letters indicate significance between groups at P<0.05.

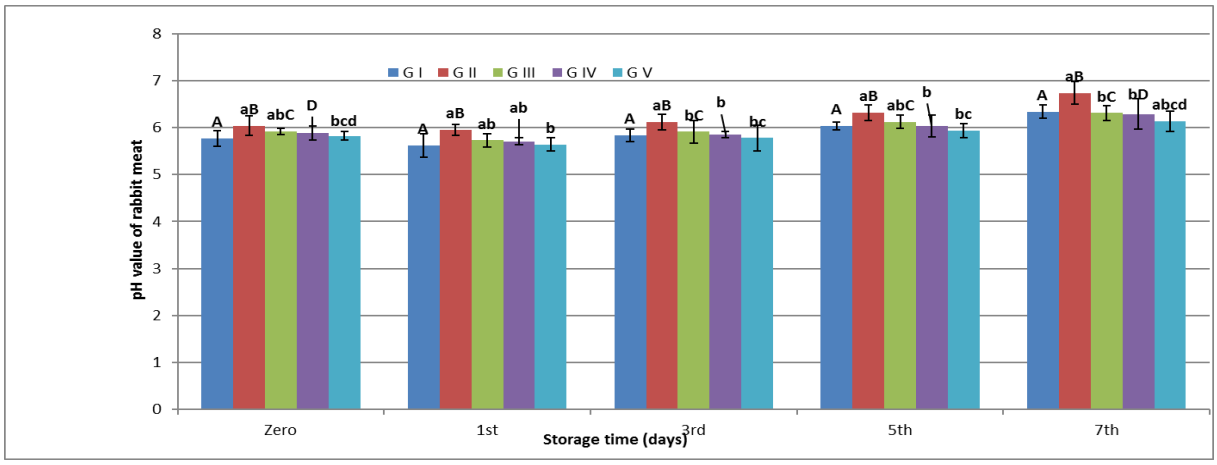

Figure 6: Changes in pH value in rabbit meat of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

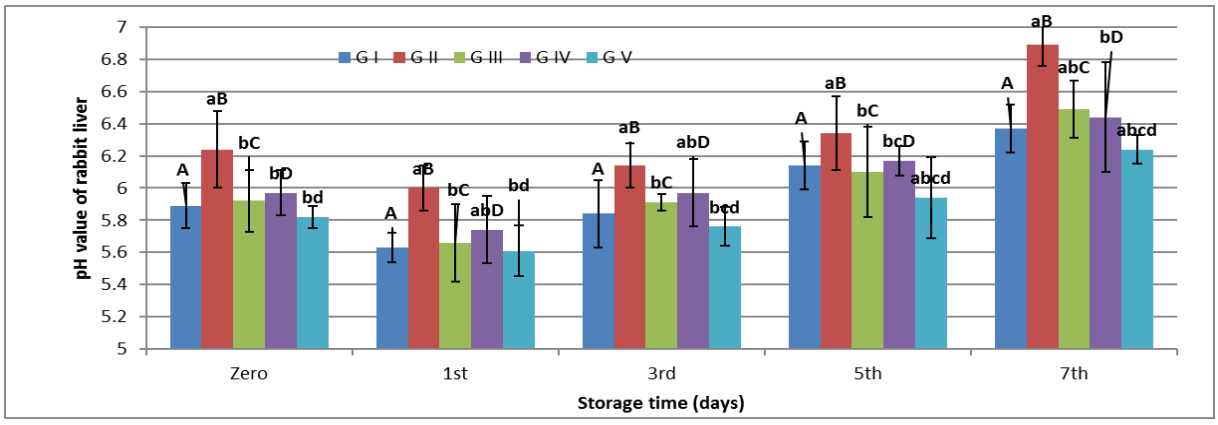

Figure 7: Changes in pH value in rabbit liver of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

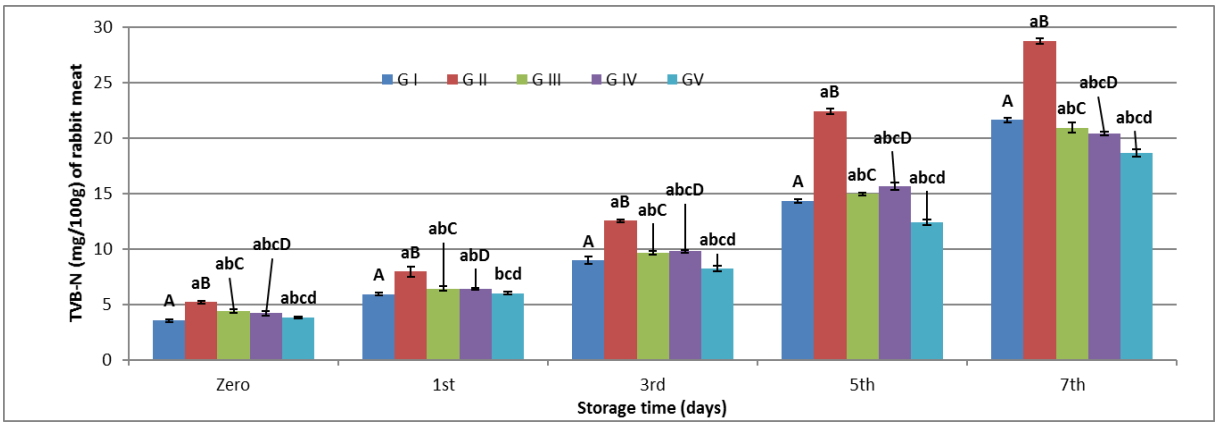

Figure 8: Changes of TVB-N value in rabbit meat of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

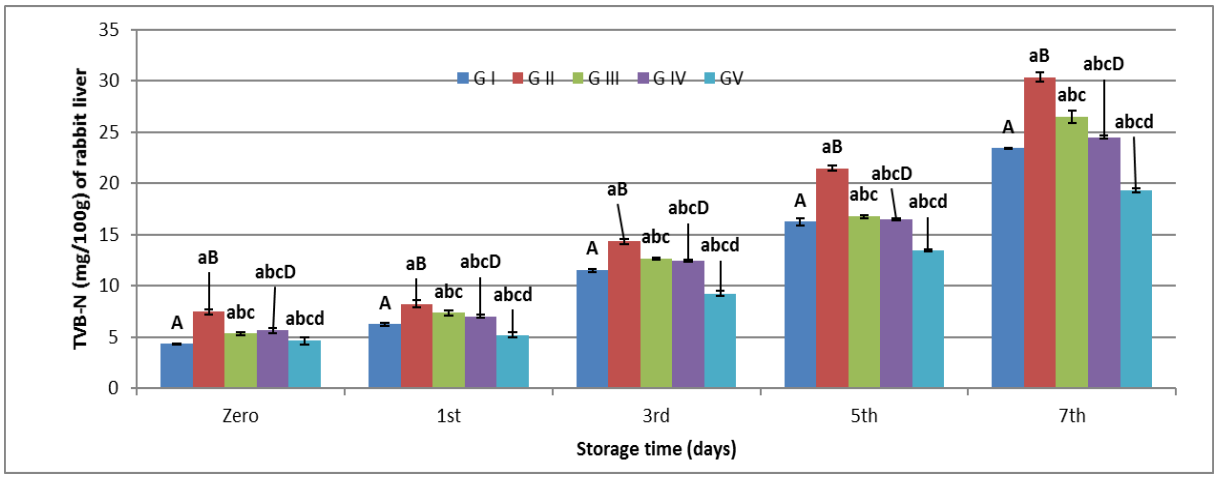

Figure 9: Changes of TVB-N value in rabbit liver of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

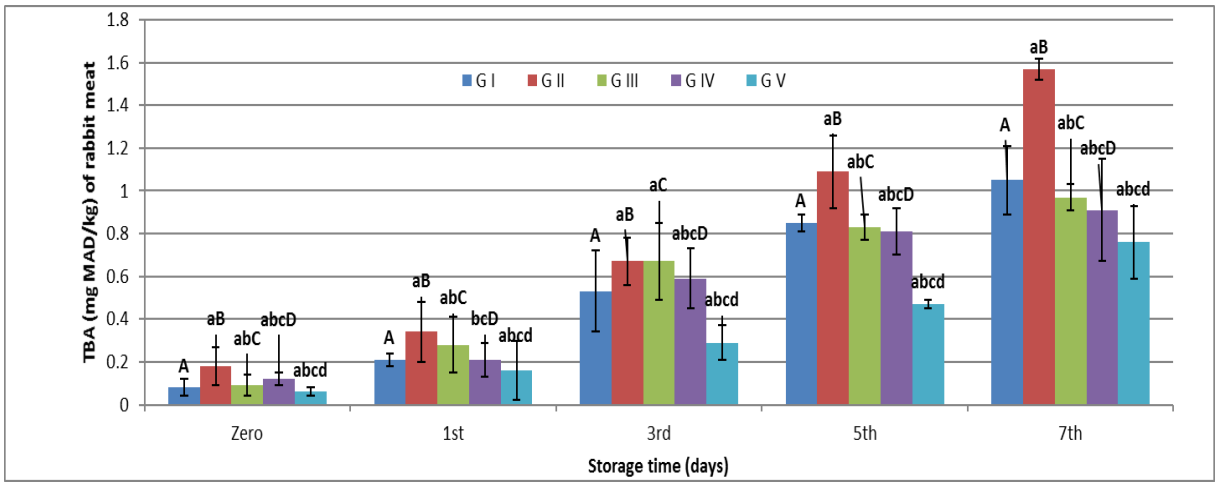

Figure 10: Changes of TBA value in rabbit meat of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

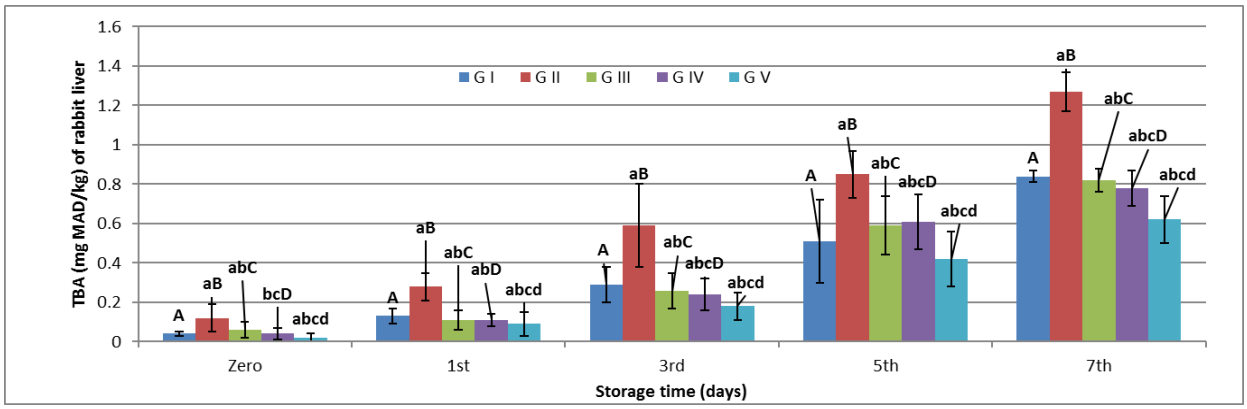

Figure 11: Changes of TBA value in rabbit liver of CPF-intoxicated rabbits treated during chilling storage. Data were expressed as mean ± standard deviation.
Small and capital letters of the same letter indicate significant differences between groups within the same period.

DISCUSSION

Chlorpyrifos residues were detectable in various organs following chronic exposure for 14 days, with the highest concentrations typically found in the kidneys. These results were compared with Kiranmayi *et al.* (2018), who reported a higher chlorpyrifos residues in chicken meat. CPF was detected in high kidney concentrations comparable to liver and muscle after repeated oral administration of CPF to rabbits. This pattern is attributed to the kidneys' role in filtering and excreting toxins, leading to greater accumulation of chlorpyrifos compared to other tissues such as muscle and liver **(**Gupta, 2019).

While muscle tissue generally showed low levels of chlorpyrifos as detected by El-Nahhal *et al.* (2020)**,** the liver, despite being a primary site for metabolism, also exhibits lower residue levels due to its efficient detoxification processes. The high kidney concentration underscores the organ's critical function in excretion and highlights the potential for nephrotoxicity with chronic exposure. Muhammad *et al.* (2010) observed that during winter, chlorpyrifos residue levels in cattle liver were greater than in both muscle and kidney tissues, whereas in summer, higher levels were noted in cattle meat compared to kidney and liver.

CPF tends to accumulate more in the kidneys than in the liver, despite the kidneys having a lower fat content, due to several reasons related to the body's detoxification and excretion processes. Kidneys are primary organs for filtering blood and excreting waste products, including toxins like chlorpyrifos. When chlorpyrifos enters the body, it is metabolised in the liver and converted into more water-soluble metabolites. These metabolites are then excreted through the kidneys. The high blood flow through the kidneys and their role in filtering and concentrating urine mean that CPF is more likely to accumulate there (Kamalesh Das *et al.,* 2014). Additionally, the kidneys' function in excreting these substances leads to higher concentrations of chlorpyrifos residues in kidney tissue compared to the liver, which primarily focuses on metabolism and detoxification. This accumulation in the kidneys underscores the importance of monitoring chlorpyrifos exposure and understanding its potential nephrotoxic effects, especially with chronic exposure **(**Wołejko *et al.,* 2022).

Our study suggested that selenium has a more pronounced effect in decreasing the concentration of chlorpyrifos residues in tissues. Selenium's role in enhancing the activity of the glutathione peroxidase antioxidant enzyme helps in detoxifying chlorpyrifos more effectively. Glutathione (GSH) (which is a coenzyme for glutathione peroxidase) functions as a reducing agent, facilitating the removal of organophosphate metabolites from the body via urine. However, both vitamin E and selenium combinations are often recommended for optimal protective effects, as synergistic effects reduce oxidative stress and enhance detoxification processes **(**Ozturk Kurt *et al.,* 2022; Ozturk Kurt and Ozdemir, 2023).

According to the Regulation of (EC) No. 396/2005 established in February 2005, the maximum residue limit (MRL) for chlorpyrifos and chlorpyrifos-methyl in food and feed is set at 10 ppb. All examined rabbit meat and liver samples from various groups fell within this limit. However, kidney samples from intoxicated groups exceeded the MRL for chlorpyrifos, except for the kidney samples from group GV. The combination of vitamin E and selenium appeared to play a significant role in reducing chlorpyrifos residue levels across all rabbit sample groups. Severe inhibition of AChE activity was observed in chlorpyrifos-intoxicated rabbits. The organophosphorus of chlorpyrifos has a

high affinity for binding acetylcholine-esterase: chlorpyrifos may go through metabolic processes and produce the oxon metabolite, which significantly inhibits AChE. Similarly, the organophosphate pesticide dichlorvos significantly inhibits AChE in male domestic rabbits (El-Nahhal, 2017**).** There was a clear link between endosulfan exposure and notable decreases in AChE activity after rabbits were given a sublethal dosage of endosulfan (1 mg/kg b.wt.) in maize oil orally daily for six weeks (Mor and Ozmen (2010**)**. Also, El-Nahhal *et al.* (2020) and Coppola *et al.,* (2025) also reported repeated-dose toxicity of chlorpyrifos in rabbits significantly reduced serum AChE levels.

Our study has shown that vitamin E and selenium have protective effects on serum acetylcholinesterase (AChE) activity in rabbits intoxicated with chlorpyrifos, including rabbits. The antioxidant properties of vitamin E and selenium help mitigate this effect by reducing oxidative stress and restoring AChE activity. Vitamin E acts as an antioxidant, protecting cell membranes from oxidative damage, while selenium, a glutathione peroxidase component, helps reduce peroxides. Together, these antioxidants help maintain the structural integrity and function of AChE, thereby alleviating the neurotoxic effects of chlorpyrifos and improving health status in the affected rabbits **(**Aly and El-Gendy *et al.,* 2015). The oxidative stress caused by chlorpyrifos exposure in rat tissues (brain, liver, and kidney) and the selenium's healing influence was observed. The results indicated that selenium increased the AChE activity, enhanced antioxidant defence systems, and declined malondialdehyde levels, thereby protecting against chlorpyrifos toxicity (Ozturk Kurt and Ozdemir, 2023). Vitamin E ameliorates chlorpyrifos toxicity in Atlantic salmon and suggesting a moderate protective effect against chlorpyrifos-induced oxidative stress (Olsvik *et al.,* 2015).

When liver cells are impaired, ALT and AST are released into the bloodstream, leading to raised levels. In the experimentally intoxicated rabbits with chlorpyrifos, significant increases in levels of liver enzymes against the control groups (GI). For instance, Yassin *et al.* (2021) reported that ALT level in chlorpyrifos-treated rabbits increased from an average of 49.5 U/L in control rabbits to 69.4 U/L after several weeks of exposure. Similarly, AST levels increased from 34.2 U/L in rabbits of the control negative group to 48.9 U/L in treated rabbits. These elevated enzyme levels indicate liver damage or stress caused by chlorpyrifos exposure. The increased ALT and AST levels suggest that chlorpyrifos may cause hepatocellular injury in rabbits **(**Shaheen and Yousafzai (2017); El-Nahhal *et al.* (2020).

Supplementation of vitamin E and selenium can modulate levels of liver enzymes in rabbits exposed to chlorpyrifos. Vitamin E is a potent anti-oxidant that protects cells from oxidative stress, while selenium is an essential trace element that plays a crucial role in antioxidant defence mechanisms. Together, they can mitigate the oxidative damage caused by chlorpyrifos exposure. In studies, rabbits treated with chlorpyrifos and supplemented with vitamin E and selenium showed significantly declining levels of ALT and AST compared to those treated with chlorpyrifos alone **(**Amin and El-Matarawy 2007; El-Kholy *et al.,* 2019**)**. This suggests that the combination of these nutrients helps to reduce liver damage and recover overall liver function in chlorpyrifos rabbit toxicity.

Exposure to chlorpyrifos usually leads to elevated levels of urea, uric acid, and creatinine while significantly decreasing total protein concentration, demonstrating

metabolic and renal stress. However, vitamin E, a powerful antioxidant, and selenium, necessary for antioxidant enzyme function, work together to decrease oxidative stress, regulate gene expression, and improve overall metabolic health. This supplem-entation helps to keep these biochemical parameters within normal ranges, ultimately fostering improved liver and renal function in intoxicated rabbits (Ebeid *et al.,* 2013; Ismail *et al.,* 2018; Viliene *et al.,* 2021).

The elevated MDA level in chlorpyrifos-intoxicated rabbits indicated extensive lipid peroxidation and oxidative damage. The reduction in SOD and GSH levels suggests a weakened antioxidant defence mechanism, making the cells more susceptible to oxidative stress. The oral exposure to chlorpyrifos caused significant oxidative stress, indicated by elevated levels of MDA, a marker of lipid peroxidation (Al-Rikaby *et al.,* 2023). Additionally, there was a notable reduction in the levels of SOD and GSH, suggesting a weakened antioxidant defence mechanism. Supplementation with vitamin E and selenium can help regulate the levels of oxidative stress biomarkers in rabbits intoxicated with chlorpyrifos (Yadav *et al.,* 2018; Ozturk Kurt *et al.,* 2022).

Decreasing lysozyme concentrations in CPF-intoxicated rabbits (Group II) reflect the influences of CPF on systems other than the nervous system. It has a cytotoxic effect on haematological and immune systems, resulting in immunotoxicity that is characterised by leukopenia, lymphopenia, and a decreasing number of absolute neutrophils in CPF-treated birds, as proved by Singh *et al.* (2016). Albuquerquea *et al.* (2017) also observed the disturbing effects of CPF, such as genotoxicity, reduced immunity, disturb-ance in the balance between oxidant and antioxidant and neurobehavioural changes. The repairing role of vitamin E on the immune system was reflected by elevating lysozyme levels in groups III and V. Such improvement is attributed to the nature of vitamin E as a lipid-soluble antioxidant that directly affects immunity through preventing lipid peroxidation, decreasing hydrogen peroxide, scavenging of ROS, and keeping the integrity and signal transduction of immune cells (Lewis *et al.,* 2019). The anti-inflammatory function of selenium (Se) that was accomplished in groups IV and V conveys its ability to modulate macrophage activity (Mahmood-poor *et al.,* 2022).

Nitric oxide is a ubiquitous free radical molecule which is liberated from L-arginine with the aid of the nitric oxide synthase (NOS) enzyme (Singh *et al.,* 2016). The oxidative stress in G-II was confirmed by an obvious elevation in NO levels. Repeated exposure to CPF was proven by the massive production of NO (Imam *et al.,* 2018) that resulted in many violent organ diseases (Jabłońska-Trypuć, 2017). The ameliorative outcome of vitamin E toward NO in group III and group V is because of its ability to inhibit the expression of NF-κB, which is responsible for inflammation (Luan *et al.,* 2019). In addition, the protective effect of vitamin E against oxidative injury in rat hepatocytes that are induced by organo-phosphates. Selenium completed its anti-inflammatory properties, as shown in groups IV and V, via decreasing levels of NF-κB, which is achieved by preventing the separation of IκB proteins from it (Nettleford and Prabhu 2018; Al-Omar *et al.,* 2020).

TNF-α is a multifunctional cytokine that has a crucial role in immune responses and inflammation. It is formed by various cells, including macrophages, natural killer cells, monocytes, endothelial cells, B lymphocytes, T lymphocytes, mast cells, and osteoclasts. The advantageous anti-inflammatory effects are achieved by reducing levels of TNF-α (Chatzigeorgiou and Chavakis, 2016). In Group II, a

notable increase in TNF-α levels was observed following oral exposure to chlorpyrifos as recorded by Al-Rikaby et al., (2023). The metabolism of CPF leads to the generation of a significant amount of free radicals, which subsequently trigger the transcription of inflammatory markers, including TNF-α **(**Thandar *et al.,* 2021). Additionally, Ruíz-Arias *et al.* (2023) confirmed the stimulatory effect of toxic substances on macrophages and neutrophils, resulting in the production of inflammatory mediators. The indirect effects of vitamin E in regulating immune cells were evident in Groups III and V, where there was a down-regulation of TNF-α level (Lewis *et al.,* 2019). Vitamin E manibulate Th1 cells by increasing levels of IFN-γ and reducing TNF-α levels in healthy mice. Further-more, the reduction of NF-κB levels induced by selenium in Groups IV and V significantly contributes to limiting the inflammatory response and the pro-inflammatory mediators' gene expression **(**Radhakrishnan *et al.* (2013); Mahmood-poor *et al.,* 2022).

pH is a predictor of meat freshness indicator of protein breakdown. Group II rabbit meat and liver had considerably higher initial pH values compared to other groups, indicating CPF-induced muscle and liver injury, greater bacterial growth, and higher TVB-N release. Okeyo *et al.* (2009) stated that higher pH may come from total volatile bases rising due to the degradation of nitrogenous substances by enzymes, consistent with enhanced TVB-N levels. Rabbit meat with 1,1-dimethyl-hydrazine (1,1-DMH) toxicosis had a higher pH compared to the control, representing proteolysis and the presence of free amino acids and ammonium salts (Maikanov *et al.,* 2020). The pH values decreased on the first day of storage. Owing to the fact that the primary energy store in living animals is glycogen in the muscles. Acidification of meat results from the conversion of glycogen to lactic acid after slaughter. A lower pH is essential because it prevents undesired bacterial development in rabbit meat products and in broiler chicken meat (Kozioł *et al.,* 2015). Increasing storage time over the third day significantly raises pH levels regardless of treatment conditions, as noted by Moawad *et al.* (2020); El Bayomi *et al.* (2023); and Wu *et al.* (2023). Vitamin E and selenium supplementation effectively reduced the pH of rabbit liver and meat samples in CPF-intoxicated rabbits; results from G-III, G-IV, and G-V. Maikanov *et al.* (2020) and Imbabi *et al.* (2021) supported this impact. The lowest pH levels were in rabbit meat and liver of GV. This resulted from the combined effects of vitamin E and selenium, which reduced CPF residue and effects, delayed microbial growth, decreased TVBN, and improved meat quality.

TVB-N is a crucial indicator of fresh meat quality and is an endogenous enzyme product that is used to evaluate shelf life and rabbit meat quality. Studies showed that TVB-N values increase during refrigerated storage, signifying microbial growth and proteolytic enzymes leading to increased protein breakdown and TVB-N content, as supported by Moawad *et al.* (2020) and El Bayomi *et al.* (2023).

The study found that G-II results showed significantly higher TVB-N values than other groups, indicating tissue toxicity by chlorpyrifos through reactive oxygen species generation, leading to cell and organ degeneration and necrotic changes (Yadav *et al.,* 2018). The redox potential in meat microbial growth is highly affected by factors like the gaseous atmosphere, pH, and reductant presence. Reactive oxygen species increase the redox potential in muscle tissue, creating a suitable environment for bacterial growth, which increases TVB-N value via protein decomposition elevation (Bezerra *et al.,* 2020). Results from G-III and G-IV showed significantly lower TVB-N values than G-II, attributed to the redox regulation

effects of selenium and vitamin E. Vitamin E reduces bacteria in meat samples and has strong antioxidant properties, which may explain the lower TVB-N values (Albonetti *et al.,* 2017; Nemati *et al.,* 2020). The permissible limit of TVB-N is 20 mg per 100g (ES2019). G-II and (G-I, G-III, G-IV) rabbit meat and liver samples exceeded this limit by the 5th and 7th days, while G-V samples stayed below the limit until day 7. Chlorpyrifos intoxication increases protein breakdown, raising TVB-N levels and reducing shelf life. However, vitamin E and selenium can mitigate these effects and extend the shelf life of contaminated rabbit meat and liver, with their combined use yielding the best results.

Chlorpyrifos causes oxidative stress in rabbits that can harm cell membranes and mitochondria via lipid peroxidation, potentially escalating oxidative reactions in meat products. The lipid peroxidation can be quantified by measuring the content of thiobarbituric acid (TBA) that serves as an indicator of lipid oxidation during storage in meat products (Estévez, 2015). The levels of TBA in both control and intoxicated rabbit meat and liver samples increased during refrigeration. TBA in G-II values were higher compared to others because of the effect of chlorpyrifos on polyunsaturated fatty acids in rabbit samples (Chwastowska-Siwiecka *et al.,* 2016; Wani *et al.,* 2017 and Moawad *et al.,* 2020).

Vitamin E and selenium antioxidant properties prevent lipid oxidation and protect particularly polyunsaturated fatty acids. Selenium supplementation enhances antioxidant activity, increases selenium levels in various muscles, reduces lipid oxidation, and improves meat quality **(**Bellés *et al.,* 2018; Pečjak *et al.,* 2022).

The TBA results of G-V were significantly lower on all examination days, attributed to the synergistic effects of vitamin E and selenium, as reported by Habibian *et al.* (2016).

The allowable TBA limit is set at 0.9 mg MAD/kg, according to ES (2006). During the refrigeration storage period of seven days, all rabbit liver samples remained within this limit except for those from GII, while rabbit meat samples from all groups except G-V had exceeded the permissible TBA limit on the fifth and seventh days of examination. Vitamin E and/or selenium can normalise the TBA values in all intoxicated samples of rabbit meat and liver, which contributes to extending the shelf life of these intoxicated samples during refrigeration storage (Marković *et al.,* 2018).

Even low levels of contaminant residues in meat products can pose serious negative health dangers, including hormonal disruption, genotoxicity, and carcino-genicity. Humans are primarily exposed to these pesticides through meat and meat products. Organophosphates, particularly chlorpyrifos, have irreversible neurotoxic effects, increasing risks for Alzheimer's, Parkinson's, and autism. Chlorpyrifos inhibits acetylcholinesterase, causing oxidative damage. Repeated exposure to chlorpyrifos can lead to anaemia and increased oxidative stress in rat erythrocytes. Treatments with vitamin E and selenium may help address exposure to these residues (Sheikh and Shekarchizadeh 2024).

CONCLUSION

Vitamin E or selenium supplementation significantly reduced CPF residues and elevated acetylcholinesterase levels in rabbits intoxicated with chlorpyrifos, with improved meat demonstrating protective effects against oxidative stress and tissue damage. The combination of both vitamin E and selenium restored these liver and renal biomarkers and normalised lysozyme, nitric oxide, and tumour necrosis factor-alpha levels, enhancing

fresh rabbit tissues to ensure safety for human consumption by effectively reducing CPF residues in all tissues to below MRL. Additionally, slowing down protein denaturation and lipid oxidation during refrigeration aids in preserving rabbit meat and liver while extending their storage life in refrigeration. Finally, it is recommended that combined selenium and vitamin E be added to rabbit rations to maximise consumer benefits by increasing the safety and quality of rabbit meat and other tissues.

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تأثير فيتامين E والسيلينيوم على المناعة ومستوى النواقل العصبية ووظائف الكبد والكلى وجودة اللحوم في ارانب التجارب المسممة بالكلوربيريفوس

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إن الإجهاد التأكسدي الناتج عن التعرض للمبيدات الحشرية هو سبب مهم للتسمم لدى الحيوانات. وقد قامت هذه الدراسة بتقييم تأثيرات فيتامين هـ والسيلينيوم على المناعة ونشاط الأستيل كولين استريز (AChE) ووظائف الكبد والكلى وتوزيع الكلوربيريفوس (CPF) في انسجة الأرانب المسممة. تم تقسيم ثلاثين أرنبًا أبيضًا من الذكور النيوزيلندي إلى خمس مجموعات: تلقت واحدة زيت الذرة، وتلقت الثانية CPF، وتلقت الثالثة CPF وفيتامين هـ، وتلقت الرابعة CPF والسيلينيوم، وتلقت الخامسة مزيجًا من CPF وفيتامين هـ والسيلينيوم، على مدار ١٤ يومًا. تم العثور على CPF في المقام الأول في الكلى، يليه الكبد والعضلات. قلل فيتامين هـ والسيلينيوم بشكل كبير من بقايا CPF في الأنسجة ومنع انخفاض مستويات AChE. تسبب التعرض لـ CPF في ارتفاع مستويات ALT وAST واليوريا وحمض البوليك والبروتين الكلي، مع انخفاض مستويات الكرياتينين. ساعد تناول مكملات فيتامين هـ والسيلينيوم في الحفاظ على وظائف الكبد والكلى الطبيعية. بالإضافة إلى ذلك، أدى CPF إلى زيادة مستويات مالونديالدهيد (MDA) وانخفاض مستويات أكسيداز الفائق (SOD) والجلوتاثيون (GSH)، مما يشير إلى انخفاض دفاعات مضادات الأكسدة. وقد أدى الجمع بين فيتامين E والسيلينيوم إلى استعادة هذه المؤشرات الحيوية وتطبيع مستويات الليزوزيم وأكسيد النيتريك وعامل نخر الورم ألفا (TNF-α) في الأرانب. يمكن أن يؤدي زيادة مستويات النيتروجين الأساسي المتطاير الكلي (TVB-N) والمواد التفاعلية الثيوباربيتورية (TBA) في أنسجة العضلات والكبد لدى الأرانب المعرضة للسموم وفيتامين E والسيلينيوم إلى تقليل مستويات TVB-N وTBA بشكل كبير في أنسجة العضلات والكبد لدى الأرانب المسمومة بالكلوربيريفوس. أخيرًا، خلصت الدراسة بأن المكملات الغذائية المشتركة من فيتامين E والسيلينيوم لها تأثيرات وقائية ضد الإجهاد التأكسدي وتلف الأنسجة الناجم عن الكلوربيريفوس لدى الأرانب.

الكلمات الدالة: المبيدات الحشرية ، المتبقيات -جوده اللحوم ،الارانب