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EXPLORING THE POTENTIAL ROLE OF *AMPHORA COFFEAEFORMIS* AND PROPOLIS IN MITIGATION OF NEUROTOXICITY INDUCED BY FLUMETHRIN IN RATS

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Abstract

Flumethrin, a synthetic pyrethroid insecticide, is used to manage a variety of insects, including mites, fleas, and ticks. This study was conducted to investigate the possible neuroprotective potential of *Amphora coffaeformis* and Propolis against Flumethrin-induced neurotoxicity in rat. Sixty male albino rats were allocated into six equal groups as follow: Group 1: (Control group), Group 2: was received Flumethrin (10 mg/kg b.w.), Group 3: was received *Amphora coffeaformis* (772 mg/kg b.w.), Group 4: was received Propolis (400 mg/kg b.w.), Group 5: was received *Amphora coffeaformis* (772 mg/ kg b.w.) followed by Flumethrin (10 mg/kg b.w.) one hour later, Group 6: was given Propolis (400 mg/kg b.w.) followed by Flumethrin (10 mg/kg b.w.) one hour later. The medications were given orally by an intragastric gavage once daily for thirty days. After one month, rats were sacrificed; serum and brain samples were collected for biochemical, molecular and histopathological analysis. The results revealed that Flumethrin exposure precipitated a cascade of oxidative stress, evidenced by elevated malondialdehyde (*MDA*) levels, diminished catalase (*CAT*) and glutathione (*GSH*) activities, and dysregulated *Nrf-2*, *AChE*, and *TNFα* expression. Conversely, *Amphora coffaeformis* and Propolis supplementation exerted a neuroprotective effect, mitigating MDA accumulation, augmenting CAT and GSH activities, and modulating TNFα, Nrf-2, and AChE expression. In conclusion, *Amphora coffaeformis* and Propolis may serve as natural neuroprotective agents, counteracting Flumethrin-induced neurotoxicity through their antioxidant and anti-inflammatory properties.

Key words: *Amphora coffeaformis,* Propolis, Flumethrin, antioxidant, gene expression.

INTRODUCTION

Pyrethroid pesticides are frequently used in agriculture to lessen insect damage to crops. Pyrethroids are analogous to pyrethrins, which are active ingredients isolated from *Chrysanthemum cinerariaefolium* flowers. Pyrethroids are classified into two types based on their physical and toxicological characteristics: Type I and Type II. Exposure to pyrethroid chemicals was found to disrupt antioxidant

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enzymes and nervous system processes (Robea *et al.,* 2017).

It is clear that pyrethroids are harmful to the environment, aquatic life, mammals, and people. Furthermore, because they are not fully eliminated, pyrethroid type II containing cyano groups are more hazardous than type I and are bioretained in the skin and stomach (Patel and Patil, 2016).

Flumethrin is a Type II α-cyano synthetic pyrethroid that primarily targets ticks and other ectoparasites of pets and animals. It is a neurotoxic insecticide, acts by inhibiting sodium channels and blocking voltage-gated chloride channels (Arslan *et al.,* 2021). Flumethrin is used to manage ectoparasites on horses, dogs, sheep, and cattle. It is administered topically as a 1% w/v pour-on and 6% w/v plunging dip in veterinary medicine (Singh *et al.,* 2012). A number of studies on the side effects of this insecticide have been reported, including hepatotoxicity, neurotoxicity and reproductive side effects (Salama *et al.,* 2019; El-Saad and Abdel-Wahab., 2022).

Microalgae and diatoms have received significant attention recently as a natural supply of nutrients (Mekkawy *et al.,* 2020). *Amphora coffeaeformis* and other diatoms are thought to contain a variety of bioactive substances, primarily carotenoids, sulfated polysaccharides, polyunsaturated fatty acids, vitamins C and E, and β-glucans (El-Sayed *et al.,* 2018). In addition to the antioxidant activity of algae, *Amphora coffeaeformis* showed high levels of polyunsaturated fatty acids (PUFAs), including ecosapentaenoic (EPA), docosahexaenoic (DHA), and linoleic acid (El-Bahr *et al.,* 2020). Pennate diatom microalga *Amphora* received attention recently due to its potential as a biofuel and as a very productive and eco-friendly source of vital lipids (Hogan *et al.,* 2021). *Amphora coffeaeformis* is regarded as a strong radical scavenger because it contains fucoxanthin and β-carotene, which are used as food additives and have a variety of nutraceutical uses, including pro-vitamin (Jaswir *et al.,* 2011).

Propolis, a resinous bee product, has been reported to have antimicrobial activities, based on its content of phenolic compounds, flavonoids, and esters of aromatic acids (Bachevski *et al.,* 2020). Propolis has a wide spectrum of activity; it is used as an antibacterial, antiinflammatory, antiviral, antiprotozoal, anti-fungal, antiseptic, analgesic, antitumor, antioxidant, antimutagenic, and antihepato-toxic agent (Anjum *et al.,* 2019). Also, Propolis has been shown to hasten damaged cell repair and tissue regeneration (Fuliang *et al.,* 2005). Additionally, it has been suggested that certain chemical components of Propolis, including galangin and caffeic acid phenethyl ester, may have neuroprotective properties (Nanaware *et al.,* 2017).

Therefore, the objective of the current work was to determine the possible ameliorating effects of *Amphora coffeaeformis* and Propolis on Flumethrin-induced neurotoxicity, as well as biochemical, histopathological, and molecular changes involved in neurotoxicity in male albino rats.

MATERIALS AND METHODOLOGY

Animals:

In our study: Sixty male Wistar albino rats, with an average weight of 160–200 g. were purchased from El-Nahda University, Beni-Suef, *Egypt*. Rats were divided into six groups (10 rats each). The rats were placed in individual cages with five rats per cage. Rats were kept in a ventilated room under controlled laboratory conditions of the normal light–dark cycle (12 h light/dark). Food and water were provided *ad libitum*. The animal handling and experimental design were approved by the Beni-Suef University's Institutional Animal Care and Committee of Use Ethics (IACUC) (Approval number: 022-388). They were kept two weeks before starting the experiment for acclimatization.

Drugs:

- **Flumethrin (bayticol ™ 10%):**

It was manufactured by Bayer Global Company, *Egypt,* as an oily, yellowish solution with high viscosity. It is soluble in most organic solvents; it is less soluble in water at 20°C.

- ***Amphora coffeaformis* algae:**

Dried extract of *Amphora coffeaeformis* was obtained from Algal Biotechnology Unit, National Research Center (NRC), Dokki, *Cairo*, *Egypt*.

- **Propolis (Biopropolis®):**

It is a commercial product, soluble in water in the form of capsules, and contains 400 mg of pure Propolis. It is manufactured by Sigma Pharmaceutical Industries Company.

Experimental design:

The experimental animals, sixty male albino rats, were divided into six equal groups as follows:

Group 1: (Control group) was given (0.5 ml of D.W./ rat per day).

Group 2: (Flumethrin group) received Flumethrin at a dose of 10 mg/ kg b.w. (Asl *et al.,* 2018).

Group 3: (*Amphora coffeaeformis* group), rats were given *Amphora coffeaformis* at a dose of 772mg/kg b.w. (Shaban *et al.,* 2023).

Group 4: (Propolis group), where rats were given 400 mg/kg b.w. of propolis (Ożarowski *et al.,* 2023).

Group 5: (Flumethrin+*Amphora coffeeaformis* group) rats were given 772 mg/ kg b.w. of *Amphora coffeaformis* and 10 mg/ kg b.w. of Flumethrin an hour later.

Group 6: (Flumethrin+Propolis group): One hour after receiving 400 mg/kg b.w. of propolis, the rats received 10 mg/kg b.w. of Flumethrin.

The medications were given orally by an intragastric gavage suited to an insulin syringe once daily for thirty days.

Sampling and biochemical estimation:

Rats were anesthetized with ketamine (90 mg/kg b.w.) and xylazine (5 mg/kg b.w.) according to (Hohlbaum *et al.,* 2018). Forty-two hours after the last treatment dose. Retro-orbital bleeding was used to collect blood samples, and clotted blood samples were centrifuged at 3000 rpm for fifteen minutes to separate the sera, which were kept at -20 °C until use. The brain was removed after cervical dislocation, and the brain samples were separated into two parts: the first was immediately fixed in 10% formalin to perform the histopathological examination, and the second part was immediately kept at -80 °C for gene expression analysis.

Oxidative/antioxidant indices:

Serum samples were estimated for GSH, MDA and CAT, according to (Meister and Anderson 1983), (Satoh *et al*., 1978) and (Aebi *et al*., 1983), respectively.

Estimation of gene expression using the quantitative real-time (QRT-PCR) for brain assessment:

RNA was extracted using the Qiagen RNeasy Micro Kit (cat. no. 74004) as per the manufacturer's instructions. Thermo Scientific's Revert Aid First Strand cDNA Synthesis Kit (cat. no. K1621) is used for cDNA synthesis (Abdel-Gawad *et al*., 2024). Gene expression relative to one another (RQ) was quantified using the SYBR-Green master mix and the StepOnePlusTM Real-Time PCR System (Thermo Fisher Scientific, US) (Abo El-Ela *et al*., 2024). Table (1) lists the primers specific to each gene. The NCBI platform was used to design the primers. The cycling stage consisted of 40 cycles, with 10 seconds at 95°C denaturation, 15 seconds at 58°C annealing, and 15 seconds at 72°C extension (Noshy *et al*., 2022). We then performed a melting curve to verify specificity. The DDCt method was used to calculate the relative quantification of gene expression. (Mehanna *et al*., 2022) used β-actin, a housekeeping gene, to ensure uniformity in the gene expression data. Every experiment had no template controls for any gene, and each sample underwent two analyses (Moustafah *et al*., 2022).

Table 1: Primer sets of assessed genes for the brain

	Sense	Antisense	Amplicon	Accession no
Nrf-2	TGTAGATGACCATGAGTCGC	TCCTGCCAAACTTGCTCCAT	159	NM_031789.2
TNF-a	ACACACGAGACGCTGAAGTA	GGAACAGTCTGGGAAGCTCT	235	NM_012675.3
AChE	AGGACGAGGGCTCCTACTTT	CATGGCATCTCTCAGGTGGG	200	NM_172009.1
β-actin	CCGCGAGTACAACCTTCTTG	CAGTTGGTGACAATGCCGTG	297	NM_031144.3

Histopathological examination:
By the end of the experiment, the brain of adult male albino rats was removed from all studied groups, sliced as coronal sections, fixed in formalin 10% for 48 h., then subjected to a typical paraffin technique and sectioned at 4 microns thick using an automatic microtome.

According to Bancroft and Gamble (2008), the brain tissue sections were stained with;
1) H&E stain for general histological examination and scoring.
Stained sections were examined and photographed by a LEICA (DFC290 HD) camera connected to the light microscope.
The degree of scoring of different pathological lesions of all groups was estimated in H&E-stained brain coronal sections at X40 (par 500 µm) (Gibson-Corley, 2013). The scoring of lesions was done under a light microscope in degrees as follows: - 0 = no lesions; 1 ≤ 25% minimal lesions; 2 = 26–50% mild lesions; 3 = 51–75% moderate lesions; 4 = 76–100% severe lesions.

2) Bromophenol blue stain for identification of cytoplasmic total protein.
The area percentage of positive Bromophenol blue stain was assessed in brain tissue sections at X200 (par 100 µm) using the Image J program.

Statistical analysis:
Using SPSS software (version 22.0), One-way analysis of variance (ANOVA) and the Tukey test for multiple comparisons were used to establish statistical significance. *P* values deemed significant were those that were less than 0.05. Mean ± standard error of mean (SEM) was used to express the results.

RESULTS

A. Effect of *Amphora coffeaeformis* and Propolis on Flumethrin-induced changes on oxidant/antioxidant status in rats.
The obtained results in (Table 2) and (Figures 1, 2, 3) showed that Flumethrin diminished the antioxidant capacity of rats' brain tissue as noticed by a substantial elevation in Malondialdehyde (MDA) level (P<0.05) and a substantial decrease in catalase (CAT) and reduced glutathione (GSH) enzymatic activities (P<0.05) when compared to the control group. Pre-treatment with *Amphora coffeaformis* or Propolis noticeably sets the Flumethrin-provoked oxidative stress via a statistically significant decrease in MDA (P < 0.001) and a statistically significant increase in CAT and GSH (P< 0.05), compared to the Flumethrin group.

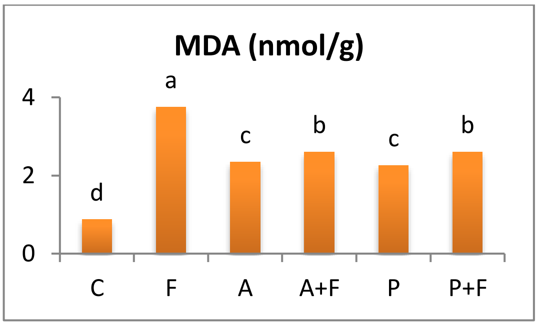

Figure (1): Effect of Flumethrin alone or pretreated with either Amphora coffeaeformis or Propolis on malondialdehyde (MDA) level in rats. C: Control group; F: Flumethrin group; A: Amphora treated group; P: Propolis treated group; A+F: Amphora with Flumethin treated group; P+F: Propolis with Flumethrin treated group

Table 2: Effect of *Amphora coffeaeformis* and Propolis on Flumethrin-induced changes on oxidant/antioxidant status in rats.

Groups	MDA (nmol/g)	CAT (U/L)	GSH (µg/mg)
Control group (Group1)	0.87±0.03^{d}	3.24±0.04^{a}	8.12±0.06^{a}
Flumethrine group (Group2)	3.75±0.04^{a}	0.93±0.02^{d}	2.99±0.09^{d}
***Amphora* group (Group3)**	2.34±0.03^{c}	1.95±0.04^{b}	5.29±0.08^{b}
Propolis group (Group4)	2.26±0.04^{c}	2.05±0.04^{b}	5.51±0.06^{b}
***Amphora*+Flumethrine group (Group5)**	2.60±0.02^{b}	1.51±0.05^{c}	4.27±0.09^{c}
Propolis+Flumethrine group (Group6)	2.60±0.04^{b}	1.61±0.05^{c}	4.54±0.10^{c}

Data are expressed as Mean ± SD (N = 5). MDA: malondialdehyde; CAT: catalase; GSH: reduced glutathione peroxidase. The different superscript symbols (a, b, c, d) indicate that the means are significantly different (*P<0.05*).

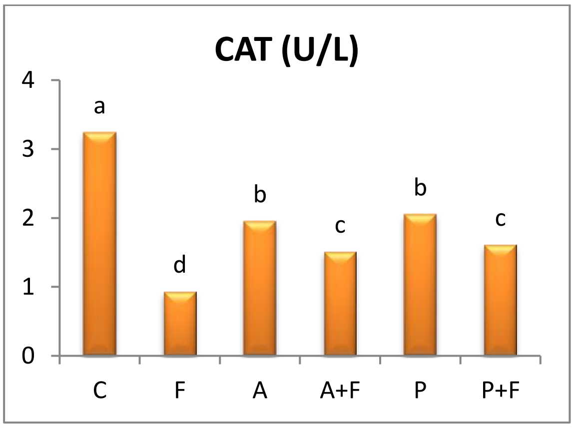

Figure (2): Effect of Flumethrin alone or pretreated with either *Amphora coffeaeformis* or Propolis on catalase (CAT) level in rats. C: Control group; F: Flumethrin group; A: Amphora treated group; P: Propolis treated group; A+F: Amphora with Flumethin treated group; P+F: Propolis with Flumethrin treated group

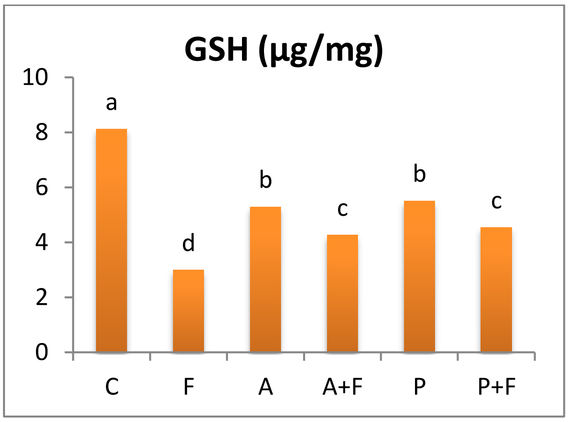

Figure (3): Effect of Flumethrin alone or pretreated with either *Amphora coffeaeformis* or Propolis on reduced glutathione peroxidase (GSH) level in rats. C: Control group; F: Flumethrin group; A: Amphora treated group; P: Propolis treated group; A+F: Amphora with Flumethin treated group; P+F: Propolis with Flumethrin treated group

B. Gene expression findings:

The effect of *Amphora Coffeaformis* and Propolis against brain toxicity induced by Flumethrin on some gene expression was shown in Figures (4, 5, 6) representing the transcript levels of (a) *AChE* (acetylcholinesterase), (b) *TNF- α* (Tumor necrosis factor), (c) *Nrf-2* (nuclear factor erythroid 2- related factor 2) among the experimental groups in brain tissue. The expression level of the Nrf-2 and AChE revealed significant downregulation in the Flumethrin group due to the oxidative stress and neurotoxicity induced. While the TNF-α expression showed upregulation. However, the *Amphora* or Propolis alleviated the neurotoxic effect induced by the Flumethrin by increasing the expression level of Nrf-2 and AChE and decreasing the TNF-α expression.

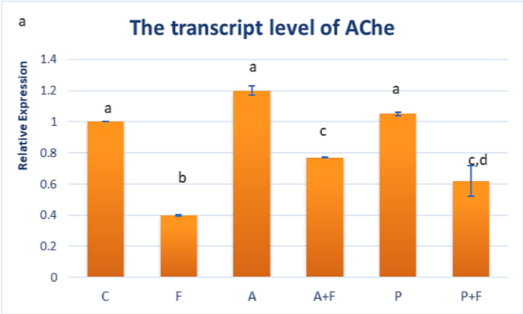

Figure 4: Effect of Flumethrin alone or pretreated with either *Amphora coffeaeformis* or Propolis on the brain mRNA expression levels of AChE gene. Values are presented as Mean±SE (n=10). Different superscript letters indicate a significant difference at (P<0.05).

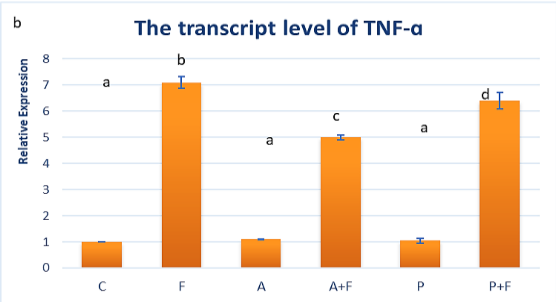

Figure 5: Effect of Flumethrin alone or pretreated with either *Amphora coffeaeformis* or Propolis on the brain mRNA expression levels of the TNF-α gene. Values are presented as Mean ± SE (n=10). Different superscript letters indicate a significant difference at (P<0.05).

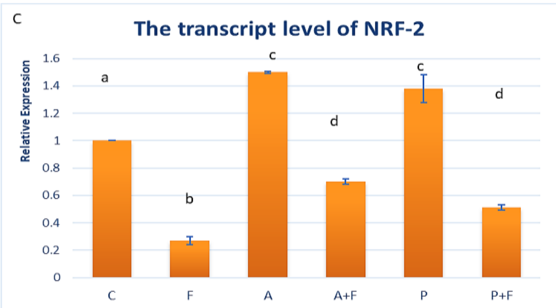

Figure 6: Effect of Flumethrin alone or pretreated with either *Amphora coffeaeformis* or Propolis on the brain mRNA expression levels of the Nrf-2 gene. Values are presented as Mean ± SE (n=10). Different superscript letters indicate a significant difference at (P<0.05).

C. Histopathological results:

a- H&E stain of brain tissue coronal sections from adult male albino rats for general microscopic examination and scoring.

In the control group, brain sections stained with H&E revealed normal architecture. It showed normal meninges, intact cerebral and hippocampal layers of normal basophilic neurons with vesicular nuclei and neuroglia cells, as well as capillaries (Fig. 7 (a1, a2)). On the contrary, the Flumethrin group showed highly pathological pictures, including separation and degeneration of meninges with dilated and congested capillaries, marked degenerative changes and reduction in cerebral and hippocampal neurons, pyknotic neuroglia cells, as well as numerous capillary dilations and congestion. These latter-mentioned lesions appeared in severe degree, compared to the control and distributed all over the brain tissue, in addition to numerous cerebral multifocal gliosis, pericellular vacuolations, as well as perivascular edema (Fig. 7 (b1, b2)). While the *Amphora* group (Fig. 7 (c1, c2)) exhibited a normal picture of brain tissue, as the control. On the other hand, pretreatment with *Amphora*, to a great extent, alleviated lesions caused by Flumethrin alone. It showed minute areas of gliosis with a minimal number of degenerated neurons with pericellular vacuolations and pyknotic glia cells (Fig. 7(d1, d2)). In the Propolis group (Fig. 7 (e1, e2)), the brain tissue looks like control. While the pathological lesions in Propolis with Flumethrin group were reduced to a lesser extent by the protective administration of Propolis before Flumethrin, compared to *Amphora* with Flumethrin group. The brain tissue appeared with some degenerated neurons, as well as glia cells with pericellular vacuolations, in addition to a few gliosis and moderate capillary dilation with perivascular edema (Fig. 7 (f1, f2)). The degree scores of pathological lesions in all treated groups compared with the Control and the evaluation of *Amphora* and Propolis treatment in alleviation of induced Flumethrin brain tissue lesions are shown in Figure 8. It was assessed in H&E-stained brain tissue sections at X40 according to lesion scores.

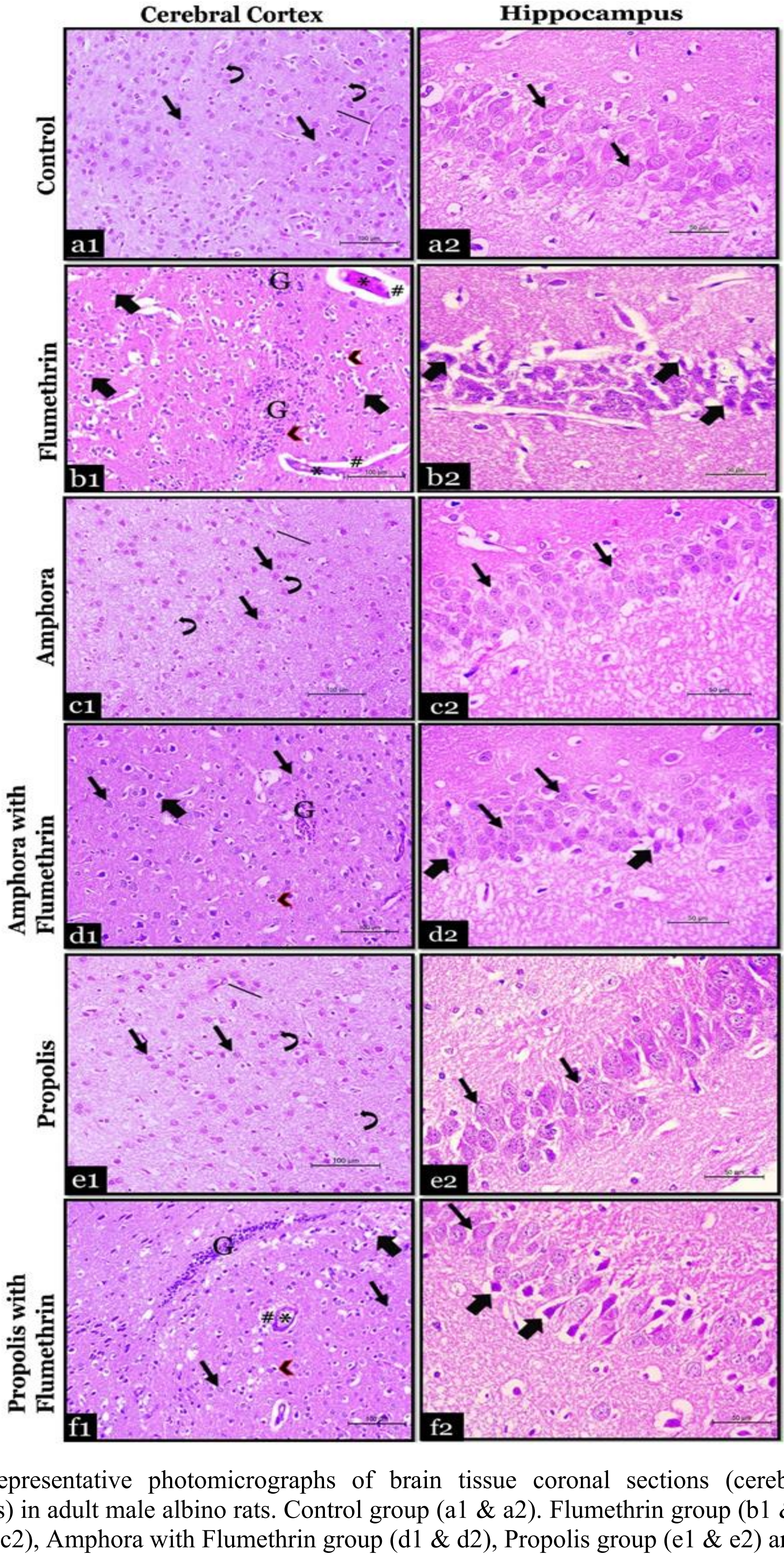

Figure 7: Representative photomicrographs of brain tissue coronal sections (cerebral cortex and hippocampus) in adult male albino rats. Control group (a1 & a2). Flumethrin group (b1 & b2), Amphora group (c1 & c2), Amphora with Flumethrin group (d1 & d2), Propolis group (e1 & e2) and Propolis with Flumethrin group (f1 & f2). Notice, normal neurons (thin arrows), normal neuroglia cells (curved arrows), normal capillaries (straight line), degenerated neurons with pericellular vacuolations (thick arrows), pyknotic neuroglia cells (arrowheads), dilated and congested capillaries (*) with perivascular edema (#) and gliosis (G). H&E stain, cerebral cortex X 200 par 100µm and hippocampus X400 par 50µm).

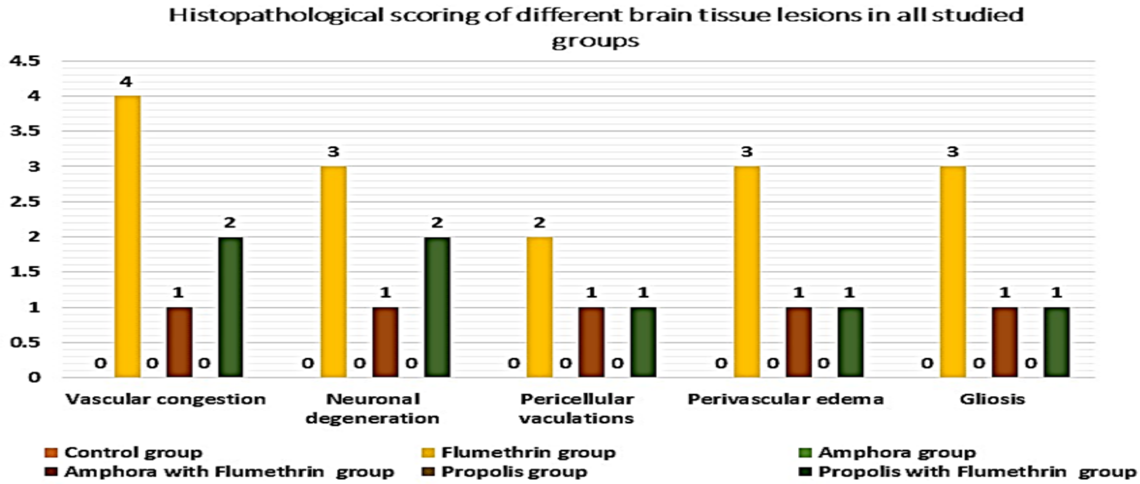

Figure 8: Chart showing the different brain tissue injury scores assessed in H&E-stained sections X40 of all treated groups compared with the control.

b-Bromophenol blue stain of brain tissue coronal sections in adult male albino rats for demonstration of cytoplasmic total proteins.

The difference between all studied groups in the reaction intensity of Bromophenol blue-stained sections is shown in Figures (9 and 10). The reaction intensity appeared high, with intense blue color within all neuronal cytoplasm in the control (Fig. 9a), Amphora (Fig. 9c), as well as Propolis (Fig. 9e) groups, indicating high total protein content. On the contrary, the exposure to Flumethrin revealed degenerated neurons with low total protein content in the form of faint blue cytoplasmic reaction and other neurons with no cytoplasmic coloration in the Flumethrin group. (Fig. 9b). While the protective treatment with Amphora as well as Propolis before Flumethrin administration showed high blue reaction in normal neurons and moderate blue coloration within a few degenerated neurons, indicating moderate alleviation of Flumethrin toxicity effects in Amphora with Flumethrin (Fig. 9d) and Propolis with Flumethrin (Fig. 9f) groups.

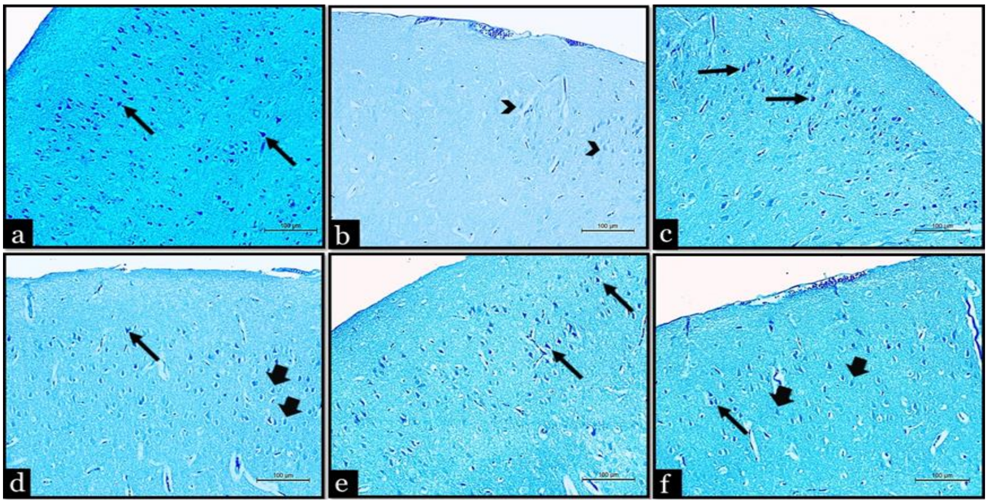

Figure 9: Photomicrographs of brain tissue coronal sections in adult male albino rats stained with Bromophenol Blue stain X 200 par 100µm, showing the difference in reaction intensity of cytoplasmic total protein content between all studied groups. Control (A), Amphora (C) and Propolis (E) groups showing numerous neurons with high positive cytoplasmic reaction of intense blue color (thin arrows). Flumethrin group (B) showed a minimal reaction of faint blue color within some degenerated neurons (arrowheads) and others with no cytoplasmic color. Amphora with Flumethrin (D) and Propolis with Flumethrin (F) groups showing high blue reaction in normal neurons (thin arrows) and moderate blue coloration within a few degenerated neurons (thick arrows).

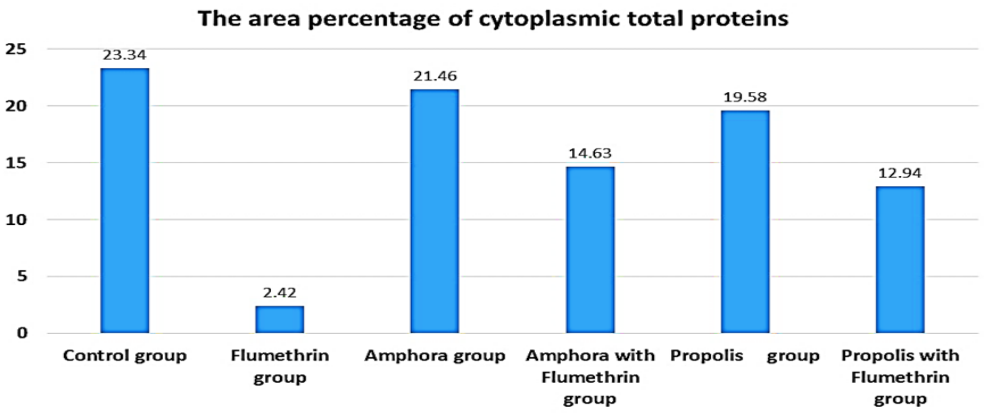

Figure 10: Histomorphometry chart comparing the area percentage of cytoplasmic total proteins in all treated groups measured in Bromophenol Blue-stained brain tissue sections. Data expressed as Mean ± SE (n = 3); the P value<0.0001, which is considered extremely significant.

DISCUSSION

Flumethrin has a broad spectrum of acaricidal and insecticidal activity. It is used to manage ectoparasite infestations on many animal species. It acts primarily on the sodium channel in nerve membranes, making it a neurotoxin to insects (Hayes and Laws 1991). In the present study, the effects of Flumethrin toxicity on the brain, as well as the protective roles of *Amphora coffeaeformis* and Propolis were evaluated.

Malondialdehyde (MDA), the end product of lipid peroxidation, is used as an indicator to determine the oxidative damage caused by pesticides (Jin *et al.,* 2011). Our results revealed that Flumethrin induced a substantial increase in MDA levels, compared to the control group. The elevation in MDA levels may result from the inhibition of the antioxidant enzymes by the free radicals induced by Flumethrin (Kanbur *et al*., 2010; Mishra *et al.,* 2012). The antioxidant enzyme system is the body's first line of defense against free radicals. Similar results were reported before, where the Flumethrin induced increases in MDA levels and decreases in CAT and GSH activities in the rat brain and liver (Salama *et al.,* 2019). Our findings supported the antioxidant effects of *Amphora coffeaeformis* and Propolis as indicated by the substantial reduction in MDA levels and the marked elevation in CAT and GSH levels in all treated groups, compared with Flumethrin-treated rats.

The effect of *Amphora coffeaeformis* for scavenging lipid peroxidation was due to exhibiting iron chelating activity (Halliwell, 1991). The oral administration of *Amphora coffeaeformis* before Flumethrin exerted marked improvement in MDA levels, which gives a hence about its validity as a tissue protectant and antioxidant agent. our investigation agrees with (Badr *et al.,* 2017), who found that the addition of 100µl/ml *Amphora coffeeeformis* to the cryopreserved spermatozoa led to decreased lipid peroxidation (MDA) and this may be due to having antioxidant capacity against reactive oxygen species (Jao and Ko, 2002). In our study, supplementation of *Amphora* before Flumethrin induced a marked decrease in MDA level and such findings coincide with those obtained by (El-Bahr *et al*., 2020), who found that MDA level decreased in breast muscle supplemented with *Amphora microalgae* due to having a higher concentration of β-carotene and fucoxanthin, which have antioxidant effects (Goiris *et al.,* 2012). Salim *et al*. (2019) revealed that *Amphora* supplementation of rabbit drinking water resulted in a decrease in plasma MDA, which is a measure of lipid peroxidation and oxidative stress.

Pretreatment of rats with Propolis before Flumethrin ameliorated its effect on MDA level, which agreed with Marzouk *et al.* (2007), who revealed that Propolis caused a substantial decrease in the MDA levels in plasma and tissues (liver, kidney, and brain). Propolis was reported to have reactive oxygen species (ROS) scavenger activity due to its polyphenol and flavonoid content (Aldemir *et al.,* 2014) which reduce the synthesis of free radicals (Saeed *et al.,* 2017). Propolis was employed to mitigate the decline in antioxidant properties (GSH, CAT and GPx) caused by CCl4, because of its anti-inflammatory and antioxidant enzymatic and non-enzymatic measures and reduced oxidative stress indicators (Akhlaghi *et al.,* 2009; Hermenean *et al.,* 2017).

The breakdown of the neurotransmitter acetylcholine into choline and acetate requires AChE, which can be utilized to detect the effects of dangerous substances (Schmidel *et al*., 2014). Flumethrin lowered AChE activity in treated rats' brains in the current investigation. Similarly, Khan *et al*. (2018) demonstrated that rats exposed to deltamethrin had considerably lower AChE activity than the control group. AChE is mostly inhibited by reactive oxygen species (ROS), which damages neurotransmission in cholinergic synapses (Muthulakshmi *et al.,* 2018). Kumar *et al.* (2009) reported similar findings, where γ-cyhalothrin is a strong inhibitor of AChE in both brain and muscle. This happens as pyrethroids depolarize membranes and delay and prolong the opening of sodium channels by increasing permeability to sodium ions (Narahashi, 1996).

TNF-α is a pro-inflammatory cytokine that was upregulated in response to various factors, including neurotoxic agents like Flumethrin. Increased TNF-α can cause neuroinflammation, which in turn can cause neuronal loss and cognitive impairments (Fayeq *et al*., 2023). The relationship between ROS and TNF-α is complex; ROS causes an increase in TNF-α release, and TNF-α causes an increase in ROS generation (Blaser *et al.,* 2016). TNF expression is usually elevated in Flumethrin-treated mice, indicating an inflammatory response that may worsen oxidative stress in the brain. In our study, the Flumethrin-treated group showed up-regulation of mRNA expression of TNF-α (Fayeq *et al*., 2023). Nrf_2 is a transcription factor that regulates the expression of antioxidant proteins that protect against oxidative damage (Mo *et al.,* 2022). It is essential for controlling oxidative stress, detoxification and cellular defense against oxidants (Mohammadi *et al.,* 2019). Our findings revealed that the Flumethrin-treated group showed a significant downregulation of the mRNA expression level of Nrf2. This finding agrees with (Imam *et al.,* 2024), who revealed that Cypermethrin inhibited the expression of the regulatory protein Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2). Otherwise, this result doesn't agree with Li *et al.* (2011), who revealed that deltamethrin induced upregulation of Nrf2, most likely as a result of translocation from the cytoplasm.

The obtained results clarified that *Amphora coffeaeformis* has a protective effect in brain tissue by enhancing the levels of AChE, TNF-ɑ and Nrf2 and this could be attributed to its bioactive compounds, which include sulfated polysaccharides, flavonoids, β-glucans, carotenoids, such as astaxanthin and canthaxanthin, and polyunsaturated fatty acids. Vitamins C and E, which have antioxidant activity against oxidative stress, are also abundant in it, along with other polyphenolic substances, gave it advantages as an antioxidant, antiviral, antifungal, and anti-inflammatory properties as mentioned before (El-Sayed *et al.,* 2018). Similar results were obtained by (El-sonbaty *et al.,* 2021), who suggested that *Amphora coffeaeformis* algal extract had increased Nrf-2 expression due to its ability to scavenge free radicals, restore oxidative balance and suppress inflammatory processes.

In the current research, Propolis administration prior to Flumethrin led to an ameliorating effect in AChE activity in the brain, similar to a previous study (Newairy *et al*., 2013), where the activity of AChE restored the normal levels after treatment with Propolis and chlorpyrifos. The treatment with Propolis for thirty days could decrease the level of TNF-ɑ, which agreed with Fatahinia *et al*. (2012), who showed that Propolis administration for seven days lowered TNF with a dose of 100 mg/kg. Propolis administration prior to Flumethrin induced neuroprotection by modulating the level of the evaluated genes and this may result from a decrease in the expression of a number of inflammatory cytokines such as TNF-ɑ, protection against oxidative damage to proteins, DNA/RNA, lipids, and carbohydrates, and positive effects on immunological responses. It is therefore regarded as a strong neuro-protective agent because of its biological activity derived from flavonoids (Farooqui *et al.,* 2012). Our current results are consistent with those reported by Saito *et al*. (2015), who announced that water extract of propolis (WEP) and its primary components cause nuclear translocation of Nrf2 and early up-regulation of HO-1 to protect against oxidative stress. Comparable results were introduced by Hotta *et al.* (2020), who declared that ethanol extract of Brazilian red Propolis (EERP)-induced ARE-mediated Nrf2 protein transactivation and nuclear accumulation, along with an increase in Nrf2-regulated antioxidant enzyme gene expression *in vitro*.

Our histological observations are consistent with all the biochemical parameters studied. Flumethrin exposure caused meningeal separation and degeneration with dilated and congested capillaries, significant degenerative changes and reduction in cerebral and hippocampus neurons, pyknotic neuroglia cells, as well as multiple cerebral multi focal gliosis and perivascular edema. These findings are consistent with those of (Salama *et al*., 2019), who reported that the group treated with Flumethrin showed neuronal degeneration, neuro-phagia, and multi focal gliosis in the cerebrum. Moreover, Ashafaq *et al*. (2023) described neuronal cell death shown by cypermethrin therapy as vacuolation, pyknotic nuclei, and infiltrating cells. Also, Deltamethrin showed considerable edema and swelling of brain cells, apoptosis, degeneration of neural cells and a decrease in the overall number of neural cells, (Abdel-Daim *et al*., 2016). Likely, our data from Bromophenol Blue stain showed degenerated neurons with low total protein content caused by Flumethrin in the form of a faint blue cytoplasmic reaction, and other neurons without cytoplasmic coloration. These findings are consistent with a recent study, where the exposure to deltamethrin significantly reduced the levels of protein and lipid in the brain tissues of brown trout, *Salmo trutta fario* (Karatas *et al*., 2024).

Pretreatment with *Amphora* before Flumethrin alleviated lesions caused by Flumethrin, included minute areas of gliosis with minimal number of degenerated neurons with pericellular vacuolations and pyknotic glia cells. These findings agreed with previous findings that adding *Amphora coffeaeformis* plus Monosodium glutamate significantly improved the radial pattern of the neuronal processes in the stratum radiatum (SR), and restored of the ordered layered neurons in the stratum pyramidale (SP) with mild degenerative alterations (Yousof *et al*., 2021).

On the other hand, neurotoxicity was mitigated after Propolis administration in the form of some degenerated neurons as well as glia cells with pericellular vacuolations in addition to few gliosis and moderate capillary dilation with perivascular edema. These results are in line with the findings demonstrated in brain samples treated with Monosodium glutamate with Propolis, where mild pericellular edema and mild neuronal were shown (Hussein *et al*., 2017).

CONCLUSION

Depending on our biochemical and histopathological results, Flumethrin induced brain damage as implied by the elevation of serum biochemical parameters, reduction of the antioxidant activity and increase in the inflammatory mediators. However, pretreatment with *Amphora coffeaeformis* or Propolis reduced the Flumethrin-induced neurotoxicity. The neuroprotection was associated with the modulation of the TNF-α and Nrf-2, which play a substantial role in diminishing oxidative stress and inflammation. Therefore, *Amphora coffeaeformis* or Propolis has promising therapeutic roles against brain toxicity induced by Flumethrin. So, we recommend using *Amphora coffeaeformis* or Propolis as food supplements to guard against Flumethrin-induced neurotoxicity.

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استكشاف الدور المحتمل للامفورا كوفيفورمس والبروبوليس فى التخفيف من السمية العصبية الناجمة عن الفلومثرين فى الجرذان

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الفلومثرين، هو مبيد حشري صناعي من مجموعة البيرثرويد، يُستخدم لمكافحة مجموعة واسعة من الحشرات، بما في ذلك العث والبراغيث والقراد. لقد بحثت هذه الدراسة في الخصائص الوقائية المحتملة لأمفورا كوفيفورميس والبروبوليس ضد السمية العصبية التي يسببها الفلومثرين في الجرذان. وُزّع ستون جرذًا أبيض ذكرًا على ست مجموعات متساوية، على النحو التالي: المجموعة الأولى هي المجموعة الضابطة، وتلقت المجموعة الثانية فلوميثرين (١٠ ملغ لكل كيلوغرام)، وتلقت المجموعة الثالثة أمفورا كوفيفورميس (٧٧٢ ملغ لكل كيلوغرام)، وتلقت المجموعة الرابعة البروبوليس (٤٠٠ ملغ لكل كيلوغرام)، وتلقت المجموعة الخامسة أمفورا كوفيفورميس (٧٧٢ ملغ لكل كيلوغرام) ثم فلوميثرين (١٠ ملغ لكل كيلوغرام) بعد ساعة واحدة، وأُعطيت المجموعة السادسة البروبوليس (٤٠٠ ملغ لكل كيلوغرام) ثم فلوميثرين (١٠ ملغ لكل كيلوغرام). أُعطيت الأدوية عن طريق الفم عن طريق الانبوب المعدى المخصص مرة واحدة يوميًا لمدة ثلاثين يومًا. بعد شهر واحد، ذبحت الجرذان، وأُخذت عينات من أدمغتها ومصلها لإجراء فحوصات بيوكيميائية وجزيئية ونسيجية مرضية. أظهرت النتائج أن التعرض للفلومثرين أدى إلى سلسلة من الإجهاد التأكسدي، وهو ما يتضح من ارتفاع مستويات مالونديالدهيد (MDA)، وانخفاض أنشطة الكاتالاز (CAT) والغلوتاثيون (GSH) ، واختلال التعبير عن Nrf-2 وAChE و TNF-α في المقابل، كان لمكملات أمفورا كوفيفورميس والبروبوليس تأثير وقائي عصبي، حيث خففت من تراكم MDA، وعززت أنشطةCAT وGSH، وعدّلت التعبير عن TNFα وNrf-2 و AChE. ختامًا: تشير هذه النتائج إلى أن أمفورا كوفيفورميس والبروبوليس قد يكونان بمثابة عوامل وقائية عصبية طبيعية، حيث يُعاكسان السمية العصبية الناجمة عن الفلومثرين من خلال خصائصهما المضادة للأكسدة والالتهابات.

الكلمات المفتاحية: أمفورا كوفيافورميس، البروبوليس، فلوميثرين، مضادات الأكسدة، التعبير الجيني.