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Original Article

Amelioration of neurotoxicity induced by malathion via modulation of neurotransmitters, apoptosis and mitochondrial potential *in* rats by Sweet Basil *(Ocimum basilicum Lamiaceae)* methanolic extract

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Abstract

Herbicides are used worldwide for protection the crops from weeds, but they still pose health problems. We investigated the mechanism of sweet basil (*Ocimum basilicum L)* methanolic extract (OBLE) against neurotoxicity induced by malathion in rats through the modulation of neurotransmitters, redox changes, cytochrome c release, and apoptosis. This study included sixty male Sprague Dawley rats allocated into six equal groups: Group I, Control; Group II, rats given malathion dissolved in DMSO at the LD50/10 dose (10 µg/kg b.w) orally for 4 weeks; Group III, rats given DMSO orally (0.1 ml/kg b.w) for 4 weeks; Group IV, normal rats treated with OBLE (100 mg/kg) for 4 weeks; Group V (Preventive group), rats administered malathion and OBLE orally (100 mg/kg) for 4 weeks; Group VI (treated group), rats administered malathion for 4 weeks followed by OBLE for another 4 weeks. The phenolic and flavonoid contents of OBLE were found to be 85 mg GAE /g and 82.65 mg catechin E/g, respectively. Rats given malathion showed a significant reduction in brain acetylcholine esterase (AchE) activity, levels of serotonin, noradrenaline, dopamine and elevation of GABA compared to normal rats. However, rats in the preventive or treated groups with OBLE exert a significant elevation in serotonin, dopamine, and NE levels and activation of AChE activity while GABA level decreased compared with untreated. Malathion induced brain tissue Cyt c release, reduced apoptosis markers (caspase 3 and 9) and increased mitochondrial membrane potential (marker of mitochondrial function). OBLE was found to reduce mitochondrial potential, enhance antioxidant activity, reduce the release of Cyt c, and enhanced caspase 3 and 9. The molecular docking analysis showed the potential interactions between the tested compounds target proteins and receptors is reflected by the binding free energies (□G) (measured in kcal/mol), with a lower value indicating a more stable interaction. It was concluded that, the phenolic and flavonoid content of OBLE contributed to the neuroprotection against malathion induced neurotoxicity via neurotransmitters, redox status and apoptosis.

Keywords: Malathion, Neurotoxicity, Apoptosis, Redox status, *Ocimum basilica*

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Introduction

Herbicides poisoning is a significant public health issue in developing countries (Hongoeb et al., 2025). One of the most commonly used herbicides is malathion, which causes adverse effects, including hepatic, renal, neuro, and cardiotoxicity, and an increased incidence of death due to a lack of effective treatments (Richardson et al., 2019). Malathion 's toxicity is linked to its ability to affect the redox system and increased the production of reactive species such as peroxy-radicals and peroxy-nitrite. Malathion is detoxified by the NADPH-cytochrome P450 enzyme system (Tarhoni et al., 2008) and the production of highly reactive oxygen and nitrogen species results in organ toxicity. Medicinal plants have gained significant attention for their potential to improve human health and nutrition due to the presence of variety bioactive compounds and antioxidants. Traditionally used to treat numerous diseases, these plants have risen in prominence with scientific development (Zengin et al., 2021). Currently, up to 30% of medications are produced and extracted from medicinal plants. Free radicals are implicated in many diseases, while antioxidants significantly delay or prevent oxidative damage in target molecules (Dos Santos et al., 2016). Moreover, antioxidants prevent many diseases by reducing free radicals or scavenging reactive oxygen species. The impact of dietary foods on human health may be due to their content of antioxidant potential of functional active compounds (Yamagishi and Matsui, 2011). These biological active compounds contribute in prevention or attenuation the development of chronic diseases (Rashid and Khan, 2017). The critical step that specify the therapeutic purposes is the extraction of active compounds or peptides from raw materials. The extraction process using different methods and solvents is the critical step that determine the quantity and quantity of the active compound with highest biological activity (Chang et al., 2002). The *Ocimum* genus belonging to the *Lamiaceae* family, is characterized by high content of active volatile oil. The most popular species known is *Ocimum basilicum L. (sweet basil)* which has different applications as flavourings, and preservatives. In addition, it has different pharmaceutical and cosmetic applications. It was reported that *Ocimum basilicum L.* has many application as complementary medicine in gastrointestinal disease and as a disinfectant (Sana et al., 2023).

It was reported that, there are about 30 species of the genus *Ocimum* contains (Vieira and Simon, 2000). Some species can be used to repel or kill insects. *Ocimum americanum* (syn. *Ocimum canum*) and *Ocimum basilicum*, were used against mosquitoes in East and West Africa (Pålsson and Jaenson, 1999). The essential oils of *Ocimum basilicum* was used to kill insects and to protect stored cereals from damage by cowpea weevils (*Callosobruchus maculatus*) (Kéita et al., 2001). Furthermore, *Ocimum americanum* volatile oil was shown to repel *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*, under cage conditions, for up to 8 h (Tawatsin et al., 2001). Normally, cytochrome c (Cyt c) is found in the mitochondrial spaces as a component of the electron transport system for oxidative phosphorylation. During apoptosis, the outer mitochondrial membrane facilitates communication between spaces and promotes the mobilization of Cyt c, which is then released into the cytosol. In this study, we investigated the mechanism of OBLE against neurotoxicity induced by malathion in rats via modulation of mitochondrial potential, neurotransmitters, redox changes, cytochrome c release and apoptosis.

Material and Methods

Plant sampling and solvent extract preparation

The aerial parts of *Ocimum basilicum L.* were collected from Taif city, Saudi Arabia, during the winter of 2022. It was identified by staff member in Biology Dep, KAU herbarium with # (KAU-220138). The samples were cleaned, dried at room temperature, and ground into powder using a Molineux mixer. Ten grams of the powder was extracted (1:2, w/v) in high-grade methanol at 60°C for 24 hours. The mixture was then filtered using Whatman #1 filter paper. The solvent was evaporated under vacuum, and the extract was lyophilized and stored for further experiments. Malathion (purity 96%) was obtained from Insecticides company, Jeddah, Saudi Arabia.

Assay of total flavonoids and total phenolic

The total flavonoid content was evaluated according to Akrout et al. (2011). Briefly, 600 µL of $AlCl_3$ (2% in methanol) was mixed with 600 µL of OBLE. After 15 min, the absorbance was measured at 415 nm. The total flavonoid content was expressed as µg quercetin equivalents per mg of OBLE. The phenolic content

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was evaluated using the Folin-Ciocalteu method as described by Oktay et al. (2003). Using gallic acid as standard. 40 µL of OBLE mixed with 200 µL of the Folin-Ciocalteu reagent and 1160 µL of DW. After 3min, 600 µL of 7.5% Na2 CO3 was added to the mixture, kept in the dark for 2 h at room temperature. The absorbance was measured at 760 nm. The results were expressed as µg of gallic acid equivalents per mg (µg GAE/mg) of OBLE.

Identification the components of *Ocimum basilicum L* by GC/MS

One milligram of *Ocimum basilicum L*. extract was dissolved in 1 mL of acetonitrile (99%). The solvent was then evaporated under liquid nitrogen, and the residue was dissolved in 0.5 ml of acetonitrile (99%). Subsequently, 10 µl of this solution was injected into a GC/MS system (Agilent, Santa Clara, USA).

Experimental animals design

The handling of animals was conducted in accordance with the ethical committee of King Abdul-Aziz University. The animals were kept in a 12-hour dark/light cycle. This study included sixty rats, aged 4 weeks and weighing between 100-120 grams was obtained from King Fahd Medical Research Center, Jeddah, Saudi Arabia. divided into six groups, each containing 10 rats. Group I; rats were served as the control group. Group II; rats were received malathion in DMSO at the LD_{50}/10 dose (10 µg/kg body weight) for 4 weeks, according to (Casas et al., 1999). Group III; rats were administered DMSO orally (0.1 ml/kg b.w) for 4 weeks. Group IV rats were given 100 mg/kg b.w of OBLE for 14 days. Group V, the preventive group; rats were administered malathion and OBLE orally (100 mg/kg) for 4 weeks. Lastly, Group VI; the treated group, rats received malathion for 4 weeks and was then given 100 mg/kg body weight of OBLE for 4 weeks. The dose of OBLE was given according to (Okasha et al., 2016).

Methods

At the end of the experiment duration time, all rats were euthanized by 10 mg of thiopental, blood samples were collected. Whole blood, Plasma, and serum were allocated and stored at -80°C for further analysis.

The brain tissue was isolated from skull and blood was removed by sterilized tissue. One gram of tissue was homogenized in 5 ml of phosphate saline buffer (pH 5.5) using a glass homogenizer on ice box for 5 minutes. The homogenate was centrifuged at 6000 RPM for 5 minutes in a cooling centrifuge. Then, 0.5 ml of cell lysate buffer was added to 1 ml of the filtrate, centrifuged at 5000 RPM for 5 minutes. The Filtrate was purified by filtration and stored at - 80°C for further analysis.

Assay of Acetylcholine esterase (AchE), neurotransmitters, oxidative stress and apoptotic markers in brain tissue

The brain homogenate was subjected for determination of acetylcholinesterase (AChE) activity, neurotransmitters [dopamine (DA), norepinephrine (NE), serotonin (5-HT), and gamma-aminobutyric acid (GABA)], malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase(SOD), glutathione peroxidase (GS-Px), glutathione reductase (GSRase) and reduced glutathione using kits from BIORAD, NY, USA. The apoptotic activity markers as caspase-3 and caspase-9 were evaluated by using an ELISA kit from BIORAD, NY, USA. Cytochrome c from mitochondria was determined using the ab210575 Mouse/Rat Cytochrome C ELISA Kit (Abcam), Cambridge, UK. Protein levels in the brain homogenate were quantified using the Bradford method (1976).

Assessment of mitochondrial membrane potential in brain tissue

Mitochondrial membrane potential was evaluated in brain tissue homogenate according to Baracca et al. (2003). Changes in mitochondrial potential were determined by measuring the quenching rate of the fluorescent dye rhodamine 123. A total of 100 µl of mitochondrial lysate was incubated with 10 µl of 10 µM rhodamine 123 for 20 minutes, then centrifuged at 10,000 × g for 10 minutes at 4°C.

Molecular docking study

The amino acid sequences of rat acetylcholine esterase (AChE), glutathione reductase (GSHR), paraoxonase and superoxide dismutase (SOD) (Accession Numbers: P37136, P70619, P55159 and P07632 respectively) were retrieved from UniProtKB database (uniprot.org/).

Due to the potential limitations of using pre-defined structures, template-based homology modeling was employed to generate 3D models for the target

proteins. SWISS-MODEL (swissmodel.expasy.org/), a web-based server for protein structure prediction, was used for this purpose. This server utilizes a combination of BLASTp and HHBlits algorithms to identify suitable template structures within the Protein Data Bank (PDB) and SWISS-MODEL Template Library (SMTL) databases for the target proteins. The identified templates are then used to build a reliable model for the target protein sequences. The quality of the generated models was evaluated using the Z-scoring functions, General Model Quality Estimate (GMQE), and Qualitative Model Energy Analysis (QMEAN), which are specifically designed for SWISS-MODEL outputs (Waterhouse et al., 2018 and Studer et al., 2021). These scores provide an objective assessment of the model's accuracy and reliability. The 3D models of target proteins bound to the co-crystalized ligands were downloaded in PDB format and prepared as docking receptors.

AutoDock Vina (v.1.2.0) was employed to perform molecular docking simulations, predicting the binding affinity and optimal orientation of the studied compounds (cinnamic acid, menthol, and limonene) within the ligand-binding pockets of the target proteins. For comparative analysis, the co-crystallized ligands (TZ5, GSH, and OCH for AChE, GSHR, and paraoxonase, respectively) along with a known SOD inhibitor, nitroprusside, were also docked as reference ligands.

PyMOL molecular visualization tool (PyMOL v.2.5.4) (Schrödinger, Inc.) was used to extract the target protein chains from their co-crystalized ligands, after adding hydrogen atoms to both. The extracted files were in PDB format (Schrödinger and DeLano, 2020). Auto-Dock (MGL-tools) was used to determine the docking site and the grid box dimensions of ligand binding pockets (Morris et al., 2009). The grid box dimensions were selected by centering the grid box on the co-crystalized ligand, included in crystal structure (Table 1). Moreover, the target proteins and the tested compounds were exported in PDBQT format (AutoDock format) using Open Babel v.2.3.

Table-1. Summary of ligand binding pocket grid box dimensions of target proteins.

Target proteins	n.pts (x; y; z)	Center (x; y; z)
AChE	50; 36; 44	33.568; 25.344; 8.265
GSHR	50; 24; 20	24.855; 14.631; 36.660
Paraoxonase	30; 24; 20	-14.316; -22.694; 26.214
SOD	20; 24; 20	18.122; -14.322; 17.250

The 2D structures of the tested compounds were downloaded from PubChem database (pubchem.ncbi.nlm.nih.gov/). Subsequently, Open Babel v.2.3.1 was employed to generate 3D structures, assign protonation states, calculate partial charges, and perform geometry optimization and energy minimization using the MMFF94 force field for organic molecules and UFF for metal containing compounds to enhance the accuracy of the docking simulation. Moreover, the target proteins and the tested compounds were exported in PDBQT format (AutoDock format) using Open Babel (O'Boyle et al., 2011).

A maximum of 9 poses were considered for each molecule where the target proteins were kept as the rigid receptors while keeping the conformation of the ligands as flexible. Finally, the most favorable poses were selected according to the minimum free energy of the protein–ligand complex and for analyzing the type of interactions between the ligands and the proteins, BIOVIA Discovery Studio (DS) Visualizer v.4.5. was used.

Statistical analysis

One-way ANOVA was employed to using SPSS version 22 to assess variance. Results for all parameters were compared statistically to the control group, with $p < 0.05$ considered significant.

Results

Preliminary study was done to find out the suitable solvent used for extraction of Ocimum *basilicum L* to achieve higher extraction yields. Among the solvents used, methanol showed the highest extract yield at 29%, followed by n-hexane at 18% and chloroform at 16%. Therefore, the further experiments were done using the methanol extract. The phenolic and flavonoid contents are summarized in Table (2),

showing that, total phenolic compounds were 85 mg Gallic acid/g and flavonoids was 82.65 mg GAE/g. The GC/MS components of OBLE are presented in Table (3), highlighting content of flavonoids, phenols, and terpenoids. Biochemical Data obtained indicated that, malathion caused a significant reduction of brain AChE activity compared to the control group ($p < 0.01$). Rats in the treated or preventive OBLE groups showed elevation AChE activity compared to untreated rats, but levels did not return to normal ($p < 0.05$). The preventive effect was more effective than the treatment effect. Additionally, malathion increased brain GABA levels compared to control rats ($p < 0.05$), while treatment or preventive with OBLE reduced GABA levels compared to the untreated group ($p < 0.01$) (Table 4). Rats injected with malathion exhibited significantly reduction of brain levels of serotonin and elevation of noradrenaline and dopamine compared to normal rats. However, rats in the preventive or treated groups with OBLE reversed this action. Normal rats treated with DMSO or OBLE showed no significant changes in the measured parameters compared to normal rats. In current study, malathion increased MDA, NO levels and SOD activity while reducing the level of GSH and the activities (GS-Px, and GS-Rase) compared with control group (Table 5). Treatment or prevention with OBLE improved redox status by decreasing the levels of MDA and NO, increased level of GSH and enhancing activities of (GS-Px, and GS-Rase) with lowering SOD activity. The preventive effect was promising than treated effect. It was found that, malathion stimulated the release of cytochrome c from mitochondria as indicated by elevation level after administration of malathion compared with control while treatment of prevention with OBLE decreased its release compared with untreated (Figure 1). The levels of caspases 3 and 9 (Figures 2,3) and fluorescence intensity in rats injected with malathion (Figure 4) were significantly decreased compared with control group ($p<0.001$). However, caspase 3, and 9 levels and fluorescence intensity were statistically elevated in rats treated or prevented with OBLE compared to the untreated group.

Table-2. The total phenolic and flavonoids content of *Ocimum basilicum L* extract (OBLE) (Mean ±SD).

	Total phenolic (mg gallic acid/ g)	**Flavonoids (mg Quercetin/g)**
Ocimum basilicum L extract	85±4.9	82.65±6.1

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Table-3. The GC/MS identification components of *Ocimum basilicum L* extract.

PK	RT	Area Pct	Library/ID	Ref	CAS	Qual
1	6.6049	0.0249	Octane, 2,2-dimethyl-	19676	015869-87-1	64
2	7.8236	0.7186	Silane, cyclohexyldimparquatymethyl-	55014	017865-32-6	70
3	8.2242	0.3449	Oxalic acid, isobutyl nonyl ester	132408	1000309-37-4	64
4	8.5618	0.3526	Decane, 2-methyl-	29360	006975-98-0	72
5	8.6362	0.849	Decane, 2,4,6-trimethyl-	51445	062108-27-4	59
6	8.825	0.329	Cinnamic acid	63625	000629-59-4	86
7	8.8651	0.3074	Hexadecane, 2,6,11,15-tetramethyl-	142256	000504-44-9	80
8	8.928	0.2864	Hexadecane, 1-iodo-	206779	000544-77-4	72
9	8.9795	0.3261	Carbonic acid, prop-1-en-2-yl tetradecyl ester	157798	1000382-90-9	72
10	10.2555	3.1149	Tridecane, 1-iodo-	168440	035599-77-0	90
11	10.3127	0.5834	Octadecane, 2-methyl-	128843	001560-88-9	91
12	10.4615	0.4995	Methoxyyacetic acid, 3-pentadecyl ester	159889	1000282-05-2	90
13	10.5359	1.9834	Menthol	39975	000112-40-3	90
14	10.5988	0.3826	Octadecane, 2-methyl-	128843	001560-88-9	90
15	10.7934	4.8689	2,4-Di-tert-butylphenol	70634	000096-76-4	96
16	11.8176	1.0376	limonene	252712	000630-04-6	90
17	12.0178	1.2591	Methoxyacetic acid, tridecyl ester	132788	1000281-82-0	91
18	12.2467	1.1117	Decane, 3,8-dimethyl-	40006	017312-55-9	94
19	12.3268	0.6714	Pentacosane	207499	000629-99-2	87
20	12.4642	1.7431	Heneicosane	155887	000629-94-7	91
21	12.5157	0.8475	2-methyloctacosane	242320	1000376-72-8	87
22	12.5729	0.4778	Tetracontane	273586	007098-22-8	91
23	12.6301	3.7473	Dotriacontane, 1-iodo-	272203	1000406-32-4	90
24	12.7445	0.8127	Decane, 3,8-dimethyl-	40006	017312-55-9	86
25	12.796	0.7354	Tetratetracontane	273586	007098-22-8	87
26	12.8418	1.7314	Eicosane	142238	000112-95-8	91
27	12.9162	0.9714	Decane, 3,6-dimethyl-	40000	017312-53-7	92
28	13.0135	0.6498	Carbonic acid, eicosyl vinyl ester	219345	1000382-54-3	91
29	13.2824	1.3033	Decane, 3,8-dimethyl-	40006	017312-55-9	91
30	13.3625	0.4071	2-Methylhexacosane	227470	001561-02-0	76
31	13.4712	1.7624	Heptacosane	227468	000593-49-7	91
32	13.58	2.9664	9-Octadecenoic acid	142074	002027-47-6	91
33	13.6314	2.9079	Octadecane	115547	000593-45-3	91

PK: Peak, RT: Retention time, Area Pct: Area of the peak, Library/ID: Library reference compound, Ref: Reference substance, CAS: Registry Number, a unique numerical identifier assigned to a specific chemical substance by the Chemical Abstracts Service, Qual mean: Mean of qualitative analysis.

Table-4. Brain acetylcholine esterase and neurotransmitters levels in all studied groups (Mean ± S.D).

Groups	Acetylcholine esterase (U/gm tissue)	Dopamine (µg/gm tissue)	Norepinephrine (µg /gm tissue)	Serotonin (µg /gm tissue)	GABA (µg /gm tissue)
Group I (Normal)	890 ± 115.7	6.7 ± 0.8	5.4±0.7	8.1 ±0.9	93.1±9.4
Group II (Malathion)	330 ± 45.3	2.8 ± 0.7	2.1 ±0.8	3.3 ±1.5	150 ±13.2
Group III (DMSO)	871 ± 101	6.4 ± 0.9	5.1±0.9	8.3 ±0.8	91.1 ±8.1
Group IV (Normal +OBLE)	874 ± 95	6.2 ± 0.78	5.2±0.5	7.8 ± 0.7	90.1 ±7.8
Group V (Malathion+ OBLE-preventive)	540± 60.4 [a,b]	4.1 ± 0.2 [a,b]	3.7 ±0.5 [a,b]	5.9 ±1.4 [a,b]	113 ±12.3 [a,b]
Group VI (Malathion+ OBLE treated)	449 ± 56.1[a,b]	3.6±0.5 [a,b]	4.1±0.4 [a,b]	5.2 ±0.9 [a,b]	100 ±11.0 [a,b]

(a) Significant versus control. (b) Significant of treated and preventive versus.

Table-5. Brain oxidative stress markers in the studied groups (Mean ± S.D).

Groups	MDA (µmol/mg protein).	Nitric oxide (NO) (µg /mg protein/g tissue).	Superoxide dismutase (SOD) (U/mg protein/g tissue).	Glutathione peroxidase (U/mg protein g tissue /).	Glutathione reductase (U/mg protein/ g tissue).	Glutathione reduced (µg/mg protein/ g tissue).
Group I (Normal).	36 ± 1.7	66.7 ± 28	45 ±2.1	987 ±90	2112 ±154	42 ±1.9
Group II (Malathion)	94 ± 8.3	172 ± 9.7	147 ±11.3	533 ±45	1205±131	22±1.1
Group III (DMSO).	33 ± 2.1	62.3 ± 21	42±3.1	945 ±87	2100 ±111	40 ±1.5
Group IV (Normal +OBLE).	33 ± 2.3	61.4 ± 20.5	40±4.2	940 ±101	2095 ±115	39 ±2.1
Group V (Malathion+ OBLE-preventive) .	77± 6.4 [a,b]	110 ± 9.2 [a,b]	99 ±9.4 [a,b]	780 ±84 [a,b]	1530 ±123 [a,b]	29 ±1.3 [a,b]
Group VI (Malathion+ OBLE treated).	68 ± 8.1[a,b]	105± 8.5 [a,b]	87±8.4 [a,b]	860 ±86 [a,b]	1611±133 [a,b]	32±1.4 [a,b]

(a) Signifiant versus control. (b) Signifiant versus treated.

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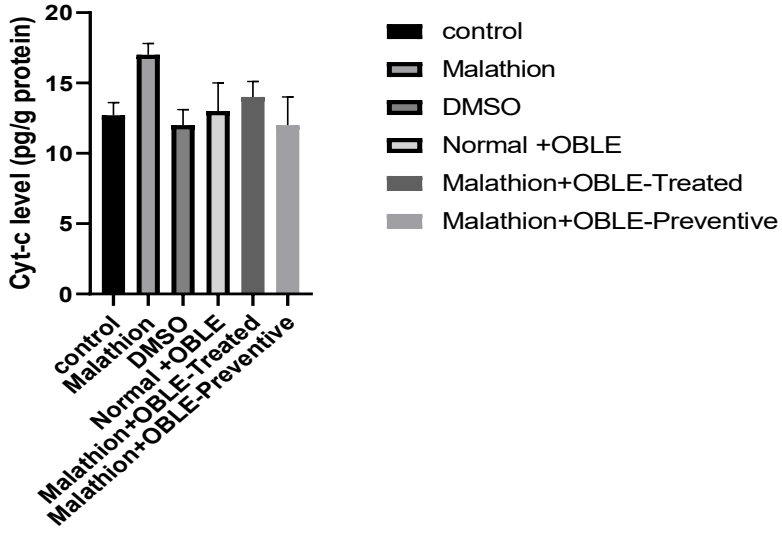

Figure-1. Level of Cyt-c in brain tissue in all studied groups.

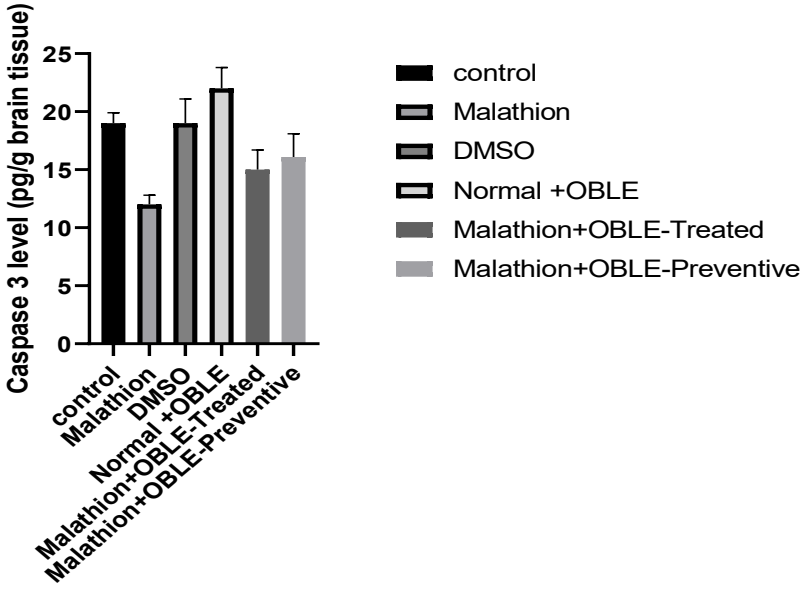

Figure-2. Level of caspase 3 in brain tissue in all studied groups.

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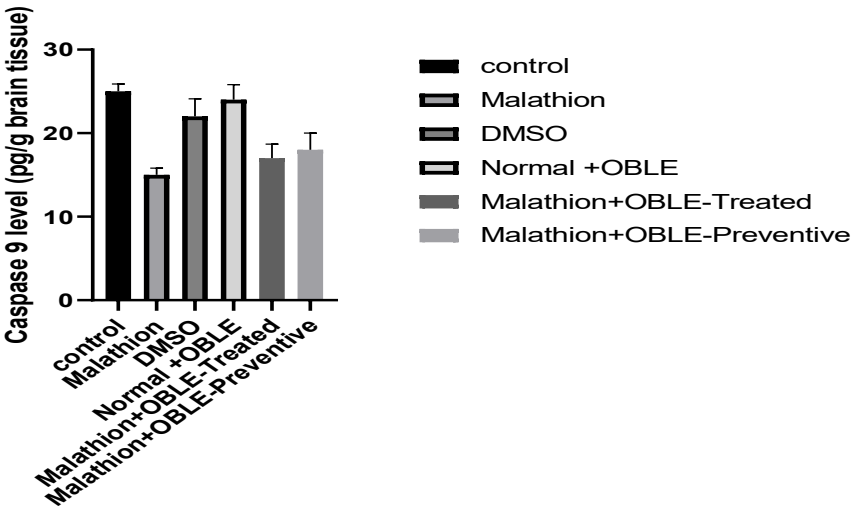

Figure-3. Level of caspase 9 in brain tissue in all studied groups.

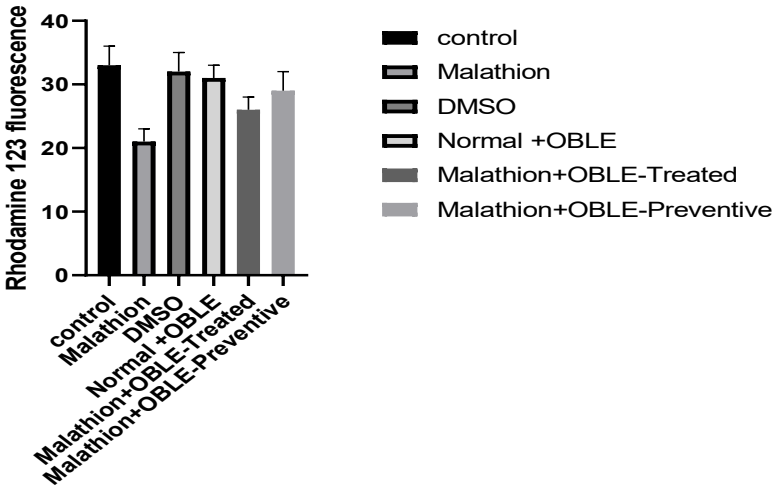

Figure-4. Mitochondrial potential in brain tissue in all studied groups.

Normally, Rhodamine 123 can enter mitochondria and emit bright yellow-green fluorescence; when the mitochondrial membrane potential of cells decreases, Rhodamine 123 is released from the mitochondria, causing the yellow-green fluorescence in the mitochondria to weaken or disappear.

Molecular docking study

The 3D structures of rat acetylcholine esterase (AChE), glutathione reductase (GSHR), paraoxonase and superoxide dismutase (SOD) were modeled using protein homology modeling since they were not available in the PDB database at the time of the study. The models were constructed and evaluated for their accuracy in resembling the natural rat proteins.

For the construction of protein models, high-quality template structures were selected based on their strong sequence similarity and coverage. The AChE model was built using the crystal structure of mouse acetylcholinesterase (PDB ID: 1q83), which exhibited a very high sequence identity of 98.1% with 94% query coverage. Similarly, the GSHR model utilized the human glutathione reductase structure (PDB ID: 3dk8), showing 87.14% identity and 99% coverage. The paraoxonase and SOD models were derived from human paraoxonase-1 (PDB ID: 9r0q) and human SOD1 (PDB ID: 8ccx), respectively, both templates providing complete (100%) query coverage with high sequence identities of 84.51% and 83.12%.

Several parameters assessed the quality of the models. The GMQE score (ranging from 0 to 1) indicated good structural reliability for models (0.91 for the AChE, 0.94 for GSHR, 0.94 for paraoxonase and 0.92 for SOD). MolProbity analysis of the Ramachandran plot showed a high percentage of favored residues for models (96.09% for the AChE, 96.45% for GSHR, 94.94% for paraoxonase and 99.01% for SOD). The low percentage of outliers (0.09% for the AChE, 0% for GSHR, 0.3% for paraoxonase and 0% for SOD) suggests good models' stability (Figures 5-9).

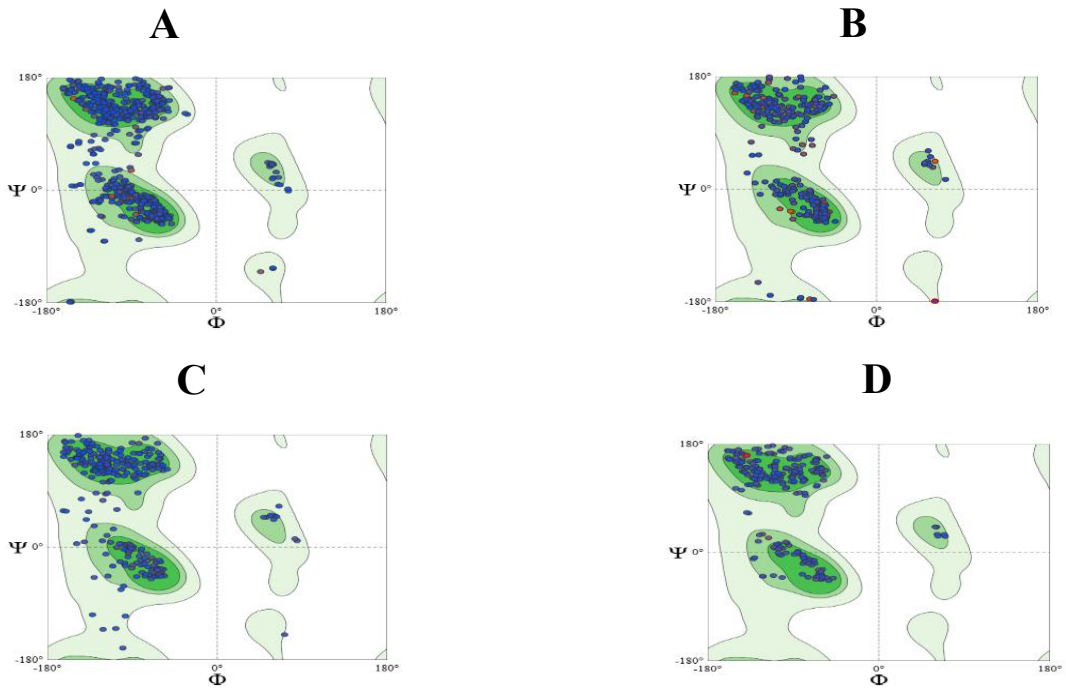

Figure-5. The Ramachandran plot by MolProbity analysis highlights the amino acids' preferred and forbidden zones. (A) AChE; (B) GSHR; (C) paraoxonase and (D) SOD.

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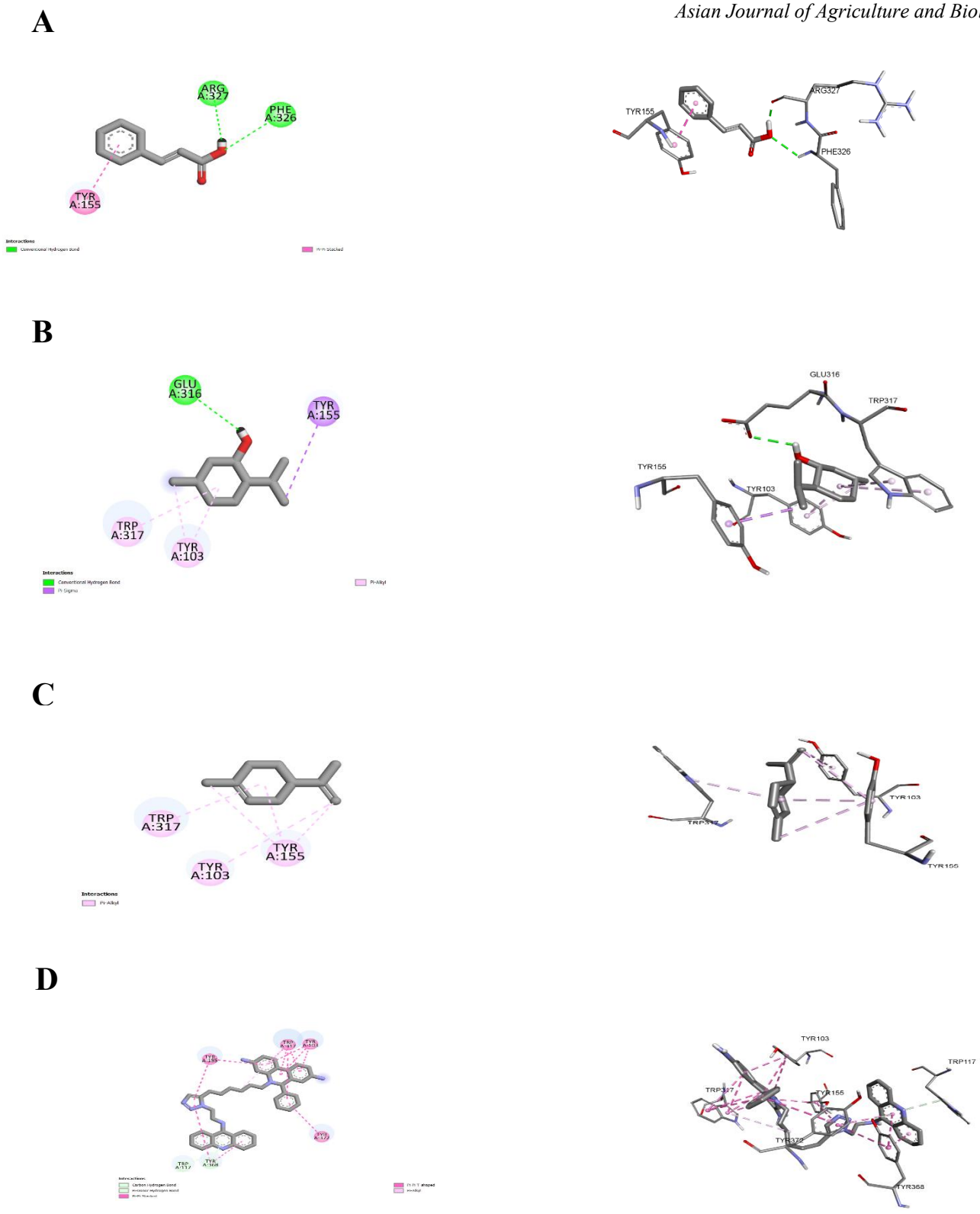

Figure-6. Interactions of AChE with cinnamic acid, menthol, and limonene and TZ5 (A-D respectively). (3D (right) and 2D (left).

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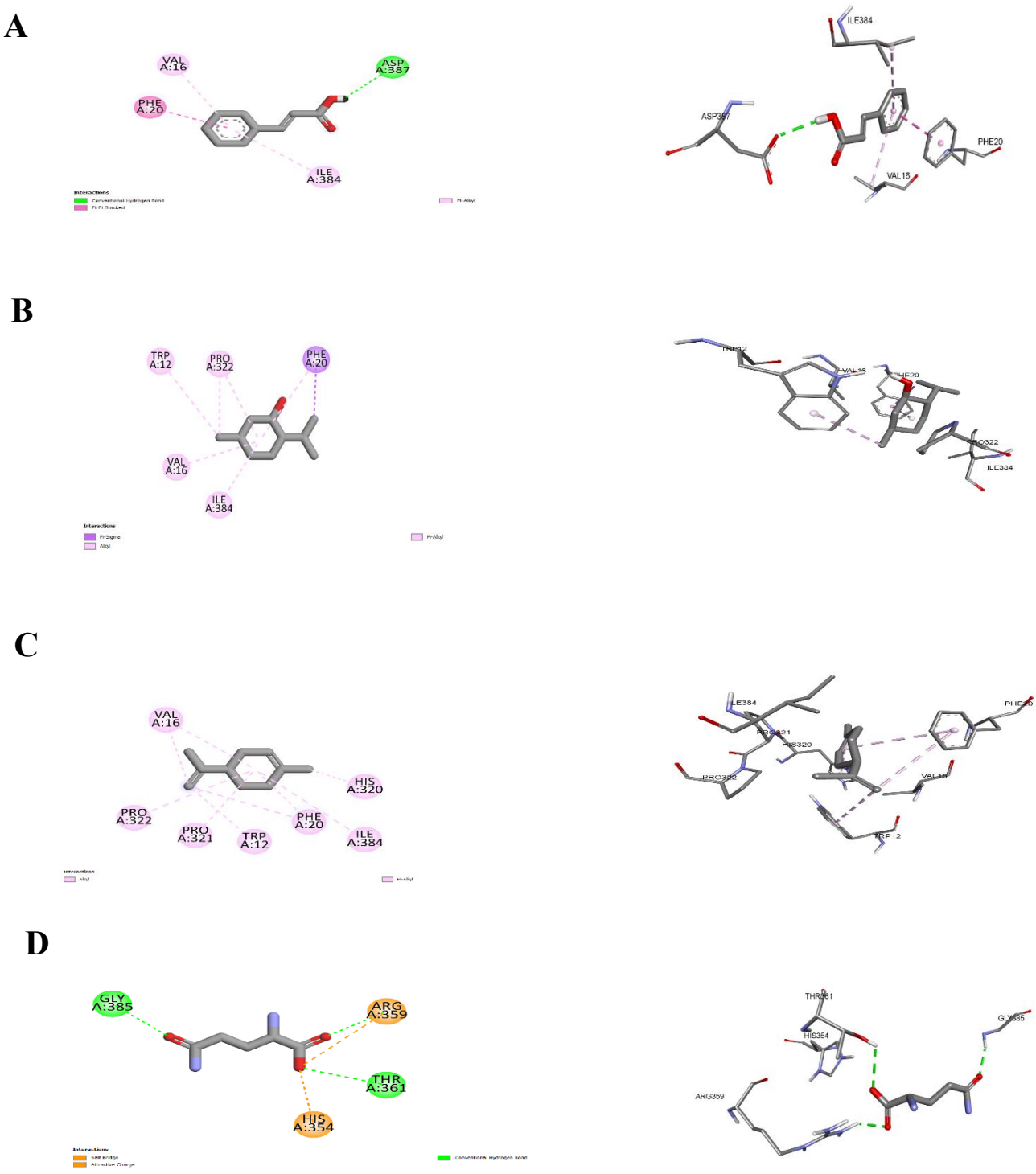

Figure-7. Interactions of GSHR with cinnamic acid, menthol, and limonene and GSH (A-D respectively). (3D (right) and 2D (left).

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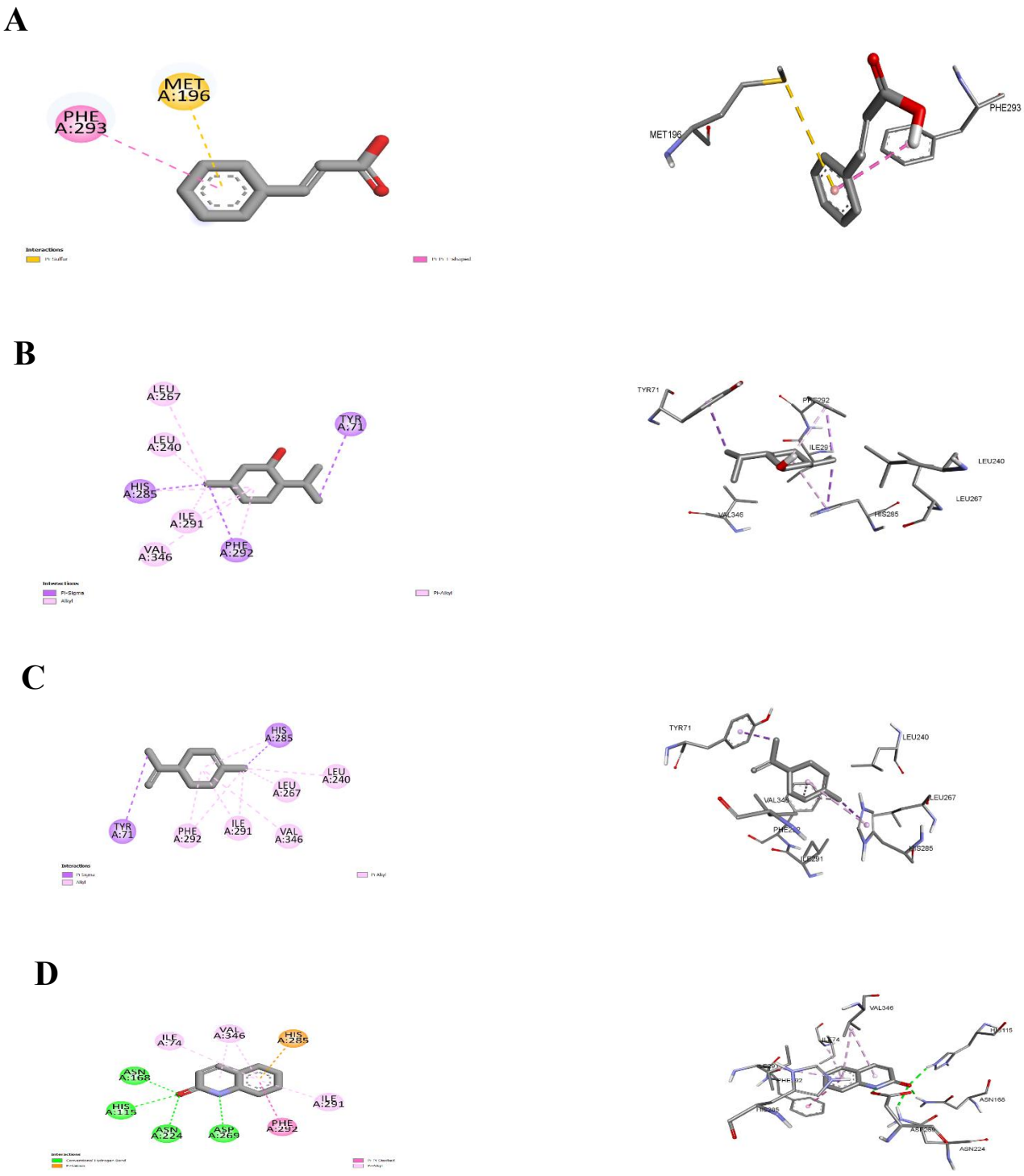

Figure-8. Interactions of paraoxonase with cinnamic acid, menthol, and limonene and OCH (A-D respectively). (3D (right) and 2D (left).

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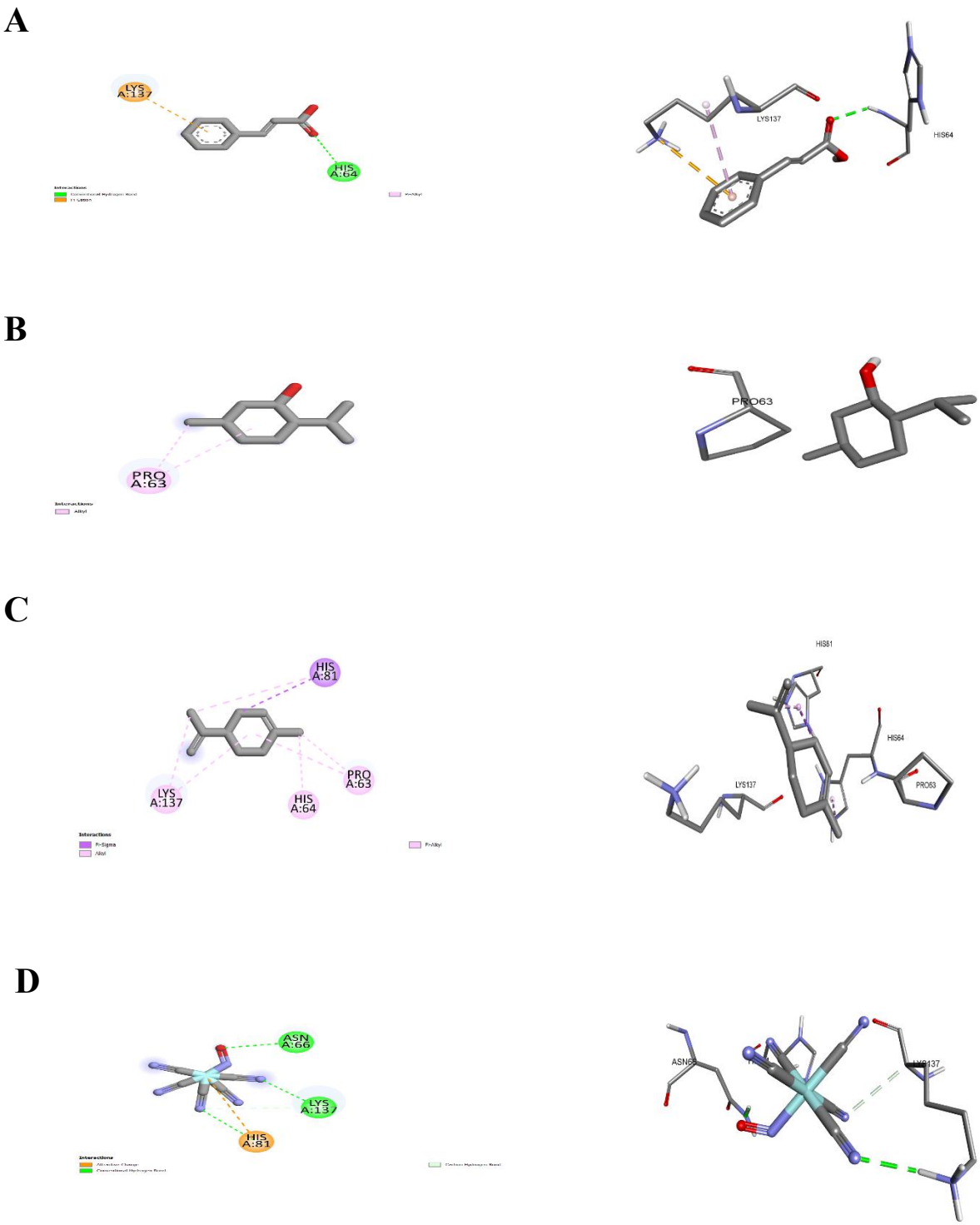

Figure-9. Interactions of SOD with cinnamic acid, menthol, and limonene and nitroprusside (A-D respectively). 3D (right) and 2D (left).

The results of the molecular docking analysis (Table 6) offer valuable insights into the potential interactions between the tested compounds and the target proteins. The binding affinity between tested compounds and the receptors is reflected by the binding free energies (□G) (measured in kcal/mol), with a lower value indicating a more stable interaction.

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Table-6. The docking binding free energies (□G).

Target proteins	Tested compounds	Binding free energies (□G).
AChE	Cinnamic acid	-6.965
	Menthol	-6.741
	Limonene	-6.773
	TZ5 (co-crystalized ligand)	-13.66
GSHR	Cinnamic acid	-6.635
	Menthol	-6.027
	Limonene	-6.177
	GSH (co-crystalized ligand)	-5.369
Paraoxonase	Cinnamic acid	-4.701
	Menthol	-6.604
	Limonene	-6.019
	OCH (co-crystalized ligand)	-7.81
SOD	Cinnamic acid	-4.538
	Menthol	-4.347
	Limonene	-4.098
	Nitroprusside (reference ligand)	-3.508

Discussion

The exposure of living organisms to pesticides, specially malathion was reported to affect several key activities of such as the metabolic pathway, redox status and cholinergic activity. This impact on several abnormalities of corresponding markers in the brain function. Therefore, preservation of these markers is the targets for the management of neurodegenerative diseases (Gupta et al., 2023). The rational of the current study was the possible therapeutic effect of OBLE against neurotoxicity of malathion in rats.

Azizah et al. (2023) reported that, the phytochemical components of Sweet Basil (*Ocimum basilicum L.*) as polyphenols, terpenes, and phytosterols were correlated with its antimicrobial and biomedical activities. In addition, the antiviral activity due to carvacrol, α-guaiene, ursolic acid, apigenin, stigmasterol, and campesterol. Moreover, the exploration of new neuroprotective medicines may be facilitated by novel compounds, such as 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone and 3-hydroxy-3′,4′,5′-trimethoxyflavone.

Preliminary study was done to find out the suitable solvent; different solvents were used for extraction of *Ocimum basilicum L* to achieve higher extraction yields. Among the solvents analyzed, methanol showed the highest yield at 29%, followed by n-hexane at 18% and chloroform at 16%. Therefore, we decided to proceed with the experiment using the methanol extract. The phenolic and flavonoid contents are summarized in Table 1, showing total phenolic compounds was 85 mg Gallic acid/g and flavonoids was 82.65 mg GAE /g. These results support previous reports indicating that plants contain significant levels of phenolics, which are effective against oxidative stress. The GC/MS components of OBLE are presented in Table 2, highlighting high levels of flavonoids, phenols, and terpenoids. Several bioactive compounds may enhance antioxidant activity, including phenolic, alkaloids, terpenoids, glycosides, flavonoids, triterpenes, tannins, curcuminoids, lignans, stilbenes, and quinones. Additionally, the overall composition of the extract can vary based on the plant parts used, harvesting time, plant species, storage type, and drying methods (Efenberger-Szmechtyk et al., 2021). Data obtained indicated that, malathion caused a significant reduction of brain AChE activity compared to the control group ($p < 0.01$). Rats in the treated or preventive OBLE groups showed a significant elevation in the activity of AChE compared to untreated rats, although it did not return to normal ($p < 0.05$). The preventive trail was more effective than the treatment trail. Additionally, malathion increased brain GABA level compared to control rats ($p < 0.05$), while prevention or treatment with OBLE reduced GABA level compared to the untreated group ($p < 0.01$) (Table 3). In this study, the significant changes induced by malathion, particularly disturbances in neurotransmitter levels, were

normalized by OBLE pretreatment. Thus, OBLE was found to activate AChE activity, demonstrating its potential as neuroprotective (Gueli and Taibi, 2013). Rats injected with malathion exhibited a significantly reduction of brain levels of serotonin, noradrenaline, and dopamine compared to normal rats. However, rats in the preventive or treated groups with OBLE showed a significant elevation in serotonin, dopamine, and NE levels. Our study in line with previous study that reported, the degree of pesticide inhibition of AChE depends on dose that affect cholinergic activity (Namba, 1971). Similarly, Organophosphates in mice reduced hippocampal AChE by 45%, (Dos Santos et al., 2016). Herbicides were found to stimulate free radical production, damage cell components, lipid peroxidation and formation of MDA. Cell defense by antioxidant activity was enhanced by some natural products as thymoquinone and L carnitine (Soliman et al., 2024). Increased reactive oxygen species was mitigated by the antioxidant enzymes and reduced glutathione. Organophosphate administration in rats lead to decreased SOD, GS-px and GSRase activities (Fortunato et al., 2006). In addition, high dosing of malathion at 200 mg/kg body weight for seven days triggered increased total brain CAT and SOD activity (Ranjbar et al., 2010), presumably reflecting increased protein expression in response to redox stress. In line with our data, organophosphates induce cellular redox stress and affect mitochondrial permeability of cell membrane and release of ROS that affect the function of cellular proteins, lipids and DNA. Lipid peroxidation measured by MDA was elevated in response to the OPs, this is in agreement with reported study as increased lipid peroxidation in brain tissue after malathion exposure (Dos Santos et al., 2016). Normal rats treated with DMSO or OBLE showed no significant changes in the measured parameters compared to normal rats (Tables 3 and 4). The antioxidant capacity of the tissue is associated with antioxidant enzymes such as SOD, GSRase, and GS-Px. In this study, malathion increased MDA and NO levels while reducing antioxidant enzyme activities (SOD, GS-Px, and GS-Rase) (Table 4). Rats treated with OBLE showed improved antioxidant capacity, reflected in lowered MDA and NO levels, with preventive effects being superior to treatment alone (Akbel et al., 2018).

Reactive oxygen species and oxidative stress are the trigger for pathogenesis of different abnormalities as neurodegenerative diseases. Elevated free radicals is abrogated by the antioxidant enzymes capacity as catalase, SOD, reduced glutathione. Previous study reported that, administration of malathion to rats reduced the activities of catalase and SOD in the different brain regions (Fortunato et al., 2006). On the other hand, high dosing of malathion daily given 200 mg/kg for one week stimulated total brain catalase, superoxide dismutase activities (Ranjbar et al., 2010), reflecting stimulation in response to oxidative stress. The induction of oxidative stress by pesticide is mediated via mitochondrial dysfunction and production of ROS that can damage cellular components.

Brain tissues are most sensitive to the ROS due to its low mitotic activity (Floyd and Hensley, 2002). In addition, its low content of antioxidants (Guignet et al., 2020). The enzyme Carboxyl esterases involved in breakdown of malathion was found to be lower in the brain (Hazarika et al., 2003). This make the brain when exposed to malathion release ROS. Our data was found to be in agreement with previous study that report, in brain tissue, malathion caused a significant elevation of MDA level (Akbel et al., 2018). However, the rats treated or prevented with OBLE showed a protection of rat brain from malathion lipid peroxidation. In parallel to this, a significant reduction in of GSH in brain tissue indicated. The malathion caused a reduction in the content of GSH in rat brain, which was protected by OBLE. The OBLE found to keep the normal content of GSH from depletion in malathion exposure. Increased ROS and reduction of GSH can leads to the apoptosis, and necrosis (Brocardo et al., 2005). The GSH was reduced by due to its consumed in removing ROS produced by malathion. The treatment or protection with OBLE inhibit peroxidation in rat brain. The enzymes involved in the attenuation of oxidative damage including CAT, SO, GST, and GPx (Costa et al., 2021). Abnormalities in the activities of these enzymes may lead to severe pathological disorders (Gupta et al., 2019). Data obtained showed that, the malathion has modulated the SOD activity in the brain. Brocardo et al. (2005) stated that, the antioxidant enzymes activities may be elevated, decreased, or not changed in the malathion exposed brain. It may be suggested that involvement of ROS is responsible for the neuronal damage induced by malathion exposure. Our results are in agreement with similar data reported in rats exposed to malathion (Akbel et al., 2018). The antioxidant enzymes Grase and GPx are also known to be involved with the rapid removal of the ROS formed due to pesticide exposure. In current study, the

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reduction in GS Rase and GPx activities in the malathion exposed rat brain suggested the potential defense of glutathione redox system. Similar data have been reported in rats exposed malathion (Selmi et al., 2018). However, the rats treated with OBLE showed attenuation of malathion toxicity as the activities of GSR ase and GPx compared with untreated.

Oxidative stress caused by malathion led to autophagy, mitochondrial dysfunction and apoptosis. Previous study reported that, in hippocampus, malathion produced ROS which inhibit mitochondrial electron transport chain complex for ATP production (Karami-Mohajeri et al., 2014). In addition, malathion decreased the levels of antiapoptotic mediators, such as phosphorylated AKT and BCL-2. For that, it induced apoptosis via BAX /BCL2, and caspase 3 in the brain (Salama et al., 2019).

A number of polyphenols, including flavonoids, anthocyanins, and phenolic acids, have been shown to be effective against malathion-induced tissue damage. Akbel et al. (2018) demonstrated that resveratrol reduced the amount of malaoxon (breakdown product of malathion) in brain tissues, neutralized the harmful effects of malathion, and preserved the antioxidant defense.

The protective effect of OBLE is attributed to its high content of terpenoids and phenolics, which act as oxygen scavengers to reduce free radicals. Another potential explanation is that the solvent used in the extraction process, the sample preparation technique, extraction time, and temperature could influence the concentration of bioactive compounds in plant extracts, thereby affecting their antioxidant activity (Rathour et al., 2017). Data presented showed a significant decrease in fluorescence intensity in rats injected with malathion, while fluorescence levels were elevated in rats treated with OBLE compared to the untreated group. Cytochrome c mediates the apoptosis via caspase-9 and caspase-3, with activated caspases involved in cell death. In this study, OBLE stimulated the release of cytochrome c (Figure 1) and activated caspases 3 and 9 (Figure 2), indicating increased mitochondrial permeability and potential attenuation (Figure 3). The release of cytochrome c from mitochondria is a marker of the apoptotic mechanism associated with malathion, while OBLE inhibits this release and preserves mitochondrial potential, reducing apoptosis. Previous study reported that, malathion given to rats resulted in reduced mitochondrial complex I activity. But mitochondrial complex III is a major source of peroxide radical production induced by paraquat as oxidative stress in rats (Castello et al., 2007). The parameters evaluated the quality of the models by docking study. The GMQE score (ranging from 0 to 1) indicated good structural reliability for models (0.91 for the AChE, 0.94 for GSHR, 0.94 for paraoxonase and 0.92 for SOD). MolProbity analysis of the Ramachandran plot showed a high percentage of favored residues for models (96.09% for the AChE, 96.45% for GSHR, 94.94% for paraoxonase and 99.01% for SOD). The low percentage of outliers (0.09% for the AChE, 0% for GSHR, 0.3% for paraoxonase and 0% for SOD) suggests good models' stability. The obtained data focus on role of mitochondria in ROS release in response to oxidant as malathion and reflected the environmental pollution that implicated in neural function and protective effect of natural products.

Conclusion

It was concluded that, the phenolic and flavonoid content of OBLE is promising in prevention against malathion neurotoxicity via modulation of neurotransmitters, redox status and apoptosis pathway in brain tissue.

Limitations of The Study

The limitations of this study include lack of gold standard treatment for comparison effect of extract and monoclonal antibodies for investigation the signal transduction mediated the malathion toxicity and mechanism of protection of OBLE.

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Conflict of Interest: None.

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Ethical Approval Statement

The study protocol was approved by the Ethics Committee of King Abdulaziz University, Jeddah, Saudi Arabia. The protocol was done according to the ethical guidelines of the 1975 Declaration of Helsinki.

Data Availability Statement

All datasets generated or analyzed during this study are included in the manuscript.

Contribution of Authors

Kumosani TA, Abulnaja KO & Moselhy SS: Conceived idea and designed research protocol and wrote the manuscript.
Alsunusi S & Huwait E: Conducted experiments and collected data and edited manuscript.
Kumosani TA, Abulnaja KO, Moselhy SS & Yaghmoor SS: Analyzed and interpretated data and edited manuscript.

All authors read and approved the final draft of the manuscript.

References

Akbel E, Arslan-Acaroz D, Demirel HH, Kucukkurt I and Ince S, 2018. The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: The protective role of resveratrol. Toxicol. Res. 7: 503–512.

Akrout A, Alarcon GL, El Jani H and Madrid PC, 2011. Antioxidant and antitumor activities of Artemisia campestris and Thymelaea hirsuta from southern Tunisia. J. Food Chem. Toxicol. 49: 342-347.

Azizah NS, Irawan B, Kusmoro J, Safriansyah W, Farabi K, Oktavia D, Doni F and Miranti M, 2023. Sweet Basil (*Ocimum basilicum L.*) A Review of Its Botany, Phytochemistry, Pharmacological Activities, and Biotechnological Development. Plants. 12(24): 4148.

Bradford MM, 1976. A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 72: 248-254.

Brocardo S, Pandolfo P, Takahashi RN, Rodrigues ALS and Dafre AL, 2005. Antioxidant defenses and lipid peroxidation in the cerebral cortex and hippocampus following acute exposure to malathion and/or zinc chloride. Toxicol. 207: 283–291.

Casas M, Prat G, Robledo P, Barbanoj M, Kulisevsky J and Jane´ F, 1999. Malathion prevents tolerance to the effects of caffeine on rotational behavior in 6-hydroxydopamine-denervated rats. Eur. J. Pharmacol. 366: 1–11.

Chang CC, Yang MH, Wen HM and Chern JC, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10: 3.

Castello PR, Drechsel DA and Patel M, 2007. Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. J. Biol. Chem. 282: 14186–14193.

Costa TJ, Barro PR, Arce C, Santos JD, Neto JS, Egea G, Dantas AP, Tostes RC and Jiménez-Altayó F, 2021. The homeostatic role of hydrogen peroxide, superoxide anion and nitric oxide in the vasculature. Free Radic. Biol. Med. 162: 615–635.

Dos Santos AA, Naime AA, de Oliveira J, Colle D, Dos Santos DB, Hort MA, Moreira EL, Suñol C, de Bem AF and Farina M, 2016. Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: Evidence of hippocampal mito-chondrial dysfunction, astrogliosis and apoptotic events. Arch. Toxicol. 90: 647–660.

Efenberger-Szmechtyk M, Nowak A and Czyzowska A, 2021. Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. Crit. Rev. Food Sci. Nutr. 61(1): 149-178.

Floyd RA and Hensley KL, 2002. Oxidative stress in brain aging Implications for therapeutics of neurodegenerative diseases. Neurobiol. Aging 23: 795–807.

Fortunato JJ, Feier G, Vitali AM, Petronilho FC, Dal-Pizzol F and Quevedo J, 2006. Malathion-induced oxidative stress in rat brain regions. Neurochem. Res. 31: 671–678.

Gueli MC and Taibi G, 2013. Alzheimer's disease: amino acid levels and brain metabolic status. Neurol. Sci. 34(9):1575-9.

Guignet M, Dhakal K, Flannery BM, Hobson BA, Zolkowska D, Dhir A, Bruun DA, Li S, Abdul Wahab, Harvey DJ, Silverman JL, Rogawski MA and Lein PJ, 2020. Persistent behavior

deficits, neuroinflammation, and oxidative stress in a rat model of acute organophosphate intoxication. Neurobiol. Dis. 133: 104431.

Gupta VK, Siddiqi NJ, Ojha AK and Sharma B, 2019. Hepatoprotective effect of Aloe vera against cartap- and malathion-induced toxicity in Wistar rats. J. Cell. Physiol. 234: 18329–18343.

Gupta VK, Park U, Kim E, Kim S, Siddiqi NJ, Huh YS and Sharma B, 2023. Antioxidative effect of Aloe vera against malathion induced neurotoxic response in Wistar rats. Arab. J. Chem. 16(10): 1-14.

Hazarika A, Sarkar SN, Hajare S, Kataria M and Malik JK, 2003. Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicol. 185: 1-8.

Hongoeb J, Tantimongcolwat T, Ayimbila F, Ruankham W and Phopin K, 2025. Herbicide-related health risks: key mechanisms and a guide to mitigation strategies. J. Occup. Med. Toxicol. 20: 6.

Karami-Mohajeri S, Hadian MR, Fouladdel S, Azizi E, Ghahramani MH, Hosseini R and Abdollahi M, 2014. Mechanisms of muscular electrophysiological and mitochondrial dysfunction following exposure to malathion, an organophosphorus pesticide. Hum. Exp. Toxicol. 33(3): 251–263.

Kéita SM, Vincent C , Schmit JP , Arnason JT and Bélanger A, 2001. Efficacy of essential oil of Ocimum basilicum L. and O. gratissimum L. applied as an insecticidal fumigant and powder to control Callosobruchus maculatus (Fab.) [Coleoptera: Bruchidae]. J. Stored Prod. Res. 37(4): 339-349.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS and Olson AJ, 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 30(16): 8785-8791.

Namba T, 1971. Cholinesterase inhibition by organophosphorus compounds and its clinical effects. Bull. WHO. 44: 289–307.

O'Boyle N, Banck M, James CA, Morley C, Vandermeersch T and Hutchison GR, 2011. Open Babel: An open chemical toolbox. J. Cheminform. 3(1): 33.

Oktay M, Gulcin I and Kufrevioglu OI, 2003. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. Food Sci. Technol. 36: 263-271.

Okasha MA, Gabr NM, Algendy A and Magaji RA, 2016. Effects of the methanolic extract of ocimum basilicum (green peas) leaves on ethanol-induced gastric mucosal injury in adult male albino rats. Al-Azhar Med. J. 45(2): 465-473.

Pålsson K and Jaenson TG, 1999. Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Trop. 72(1): 39-52.

Rashid U and Khan MR, 2017. Fagonia olivieri prevented hepatorenal injuries induced with gentamicin in rat. Biomed. Pharmacother. 88: 469-479.

Ranjbar A, Ghahremani MH, Sharifzadeh M, Golestani A, Ghazi-Khansari M, Baeeri M and Abdollahi M, 2010. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. Hum. Exp. Toxicol. 29: 851–864.

Rathour M, Malav OP, Kumar P, Chatli MK and Mehta N, 2017. Storage stability of chevron rolls incorporated with ethanolic extracts of aloe vera and cinnamon bark at refrigeration temperature (4±1° C). J. Animal Res. 7(1): 183-190.

Richardson JR, Fitsanakis V, Westerink RHS and Kanthasamy AG, 2019. Neurotoxicity of pesticides. Acta Neuropathol. 138: 343–362.

Salama OA, Attia MM and Abdelrazek MA, 2019. Modulatory effects of swimming exercise against malathion induced neurotoxicity in male and female rats. Pestic. Biochem. Physiol. 157: 13-18.

Sana A, Naveed S and Faizi S, 2023. Phytochemical characterization, antioxidant activity and antihypertensive evaluation of Ocimum basilicum L. in L-NAME induced hypertensive rats and its correlation analysis. Heliyon. 9: e14644

Selmi S, Rtibi K, Grami D, Sebai H and Marzouki L, 2018. Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicol. Rep. 5: 189–195.

Soliman A, Alkafafy M, Hassan FW, Zakaria M, Abduljabbar MH, Mohamed K and Aboubakr

M, 2024. Investigating the protective role of L-carnitine and thymoquinone against methotrexate-induced testicular damage in rats. Pak. Vet. J. 44(2): 314-321.

Studer G, Tauriello G, Bienert S, Biasini M, Johner N and Schwede T, 2021. *ProMod3 - A versatile homology modelling toolbox.* PLOS Comp. Biol. 17(1): e1008667.

Tawatsin A, Wratten SD, Scott RR, Thavara U and Techadamrongsin Y, 2001. Repellency of volatile oils from plants against three mosquito vectors. J. Vector Ecol. Jun;26(1): 76-82.

Tarhoni MH, Lister T, Ray DE and Carter WG, 2008. Albumin binding as a potential biomarker of exposure to moderately low levels of organophosphorus pesticides. Biomarkers 13: 343–363.

Vieira RF and Simon JE, 2000. Chemical Characterization of basil (Ocimum Spp.) found in the markets and used in traditional medicine in Brazil. Econo. Botany (54); 207–216.

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R and Schwede T, 2018. *SWISS-MODEL: homology modelling of protein structures and complexes.* Nucl. Acids Res. 46: W296-W303.

Yamagishi SI and Matsui T, 2011. Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy friend or foe? Pharmacol. Res. 64(3): 187-194.

Zengin G, Mahomoodally MF, Sinan HK, Sadeer N, Maggi F, Caprioli G, Angeloni S, Mollica A, Stefanucci A, Gunes AK, Cakılcıoglu U, Rıdvan Polat R and Akan H, 2021. Evaluation of chemical constituents and biological properties of two endemic Verbascum species. Process Biochem. 108: 110-120.