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ORIGINAL ARTICLE

Toxic Effects of Carbendazim on Brain of Male albino Rats with Possible Protective Role of Linalool

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ABSTRACT

Background: One systemic fungicide that is regarded as a persistent environmental pollutant is carbendazim (CBZ). Pharmacological activity of linalool has been shown to exhibit a range of characteristics, including antibacterial, antioxidant, and anticancer effects. The current study designed for estimation of the protective mechanism of linalool in CBZ-induced brain damage in rats. **Methods**: This experimental study consisted of negative control, positive control, linalool (50 mg/kg), CBZ (500 mg/kg), and combined CBZ and linalool groups for 8 consecutive weeks. 35 rats were randomly allocated into five groups, seven rats per each as follow G(I): Negative control group regular diet and tap water. G (II): Positive control group: 1 ml corn oil (as a vehicle) once daily by oral gavage for 8 weeks. G (III): Linalool-treated group: linalool 50 mg/kg B.W. according to once daily by oral gavage for 8 weeks. G (IV): Carbendazim-treated group: 500 mg/kg B.W. of carbendazim (1/10 LD50) dissolved in 1ml corn oil once daily by oral gavage for 8 weeks (Oral LD50 of carbendazim in rats equal 5000 mg/kg). G (V): Carbendazim and linalool-treated group linalool 50 mg/kg then after 1 hour received carbendazim 500 mg/kg once daily by oral gavage for 8 weeks. Brain tissues homogenates were used for estimating ACHE, SOD, MDA, IL6, TNF α levels in brain homogenate. **Results**: Linalool co-treatment significantly improved CBZ-induced disturbance in brain level of acetylcholinesterase when compared to CBZ group. Also, linalool alleviated CBZ induced a decrease in SOD levels and a rise in levels of MDA in brain tissues. Rats treated with carbendazim displayed a marked increase in IL-6 and TNF-α compared to the negative control group and the combined carbendazim and linalool-treated group ($p<0.001$). **Conclusion**s: linalool-mediated brain protection in CBZ-treated rats possessed antioxidant and anti-inflammatory effects.

Keywords: Carbendazim; Fungicide;Linalool; Brain;Acetylcholinesterase

INTRODUCTION

Environmental pollution is a significant contributor to health risks globally. Regarding the World Health Organization (WHO), around 25% of the diseases that affect humans are caused by long-term exposure to environmental pollution, such as pesticides, including insecticides, fungicides, and herbicides [1].

Carbendazim (CBZ) is a fungicide that is widely utilized in agriculture worldwide to control fungal infections. It is a broad-spectrum benzimidazole

fungicide and is applied to various crops such as cereals, soybeans, mushrooms, cotton, fruits, bananas, vegetables, grapes, ornamentals, sugar beets, and peanuts [2]. CBZ can be employed as a biocide for preserving a variety of goods, including film, polymerized materials, rubber, fiber, leather, and building facades, in addition to its application in agriculture [3].

Carbendazim is a major global concern due to its frequent transfer via rain to areas where it harms human and animal health, and environment and leaves a residual concentration in soil, fruits, and vegetables. Additionally, CBZ is an enduring ecological contamination agent respecting to its benzimidazole ring, which is difficult to break down, leading to slow degradation. According to reports from many nations, CBZ is frequently present in soil and water for three to twelve months [4].

Respecting to WHO, CBZ is a dangerous substance. Furthermore, because of CBZ's toxicity to a variety of cells, tissues, and organisms in both vitro and in vivo circumstances, the European Commission has added it to its list of critical disruptors of the endocrine system [5,6].

Toll-like receptors (TLRs) are a group of transmembrane proteins that function as signal transduction molecules. Among them, TLR4 is believed to be the primary sensor for recognizing pathogen-associated molecular patterns. The stimulation of TLR4 can activate the nuclear factor kappa beta protein (NF-κB) through either the myeloid differentiation factor 88 (MyD88)-dependent or independent pathway. NF-κB upregulation provokes the expression of genes that encode proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which can lead to cell apoptosis [7].

Natural products are employed for the management of a wide range of human disorders and have an extensive record of therapeutic usefulness. Non-cyclic monoterpenoid linalool (LINL) is frequently produced from neroli oil, lavender, rose, and basil. Numerous investigations conducted in vivo have validated varied impacts of linalool on the nervous system. Linalool have antimicrobial, anti-inflammatory, anticancer, and anti-oxidant properties [8].

This investigation designed to investigate the potential protective effect of linalool administration on the toxic impact of carbendazim on the brain in adult male albino rats.

METHODS

Carbendazim: A 50% pure commercial product called OCCIDOR, including inactive ingredients, was purchased from a local market in the form of white powder.Linalool, A colorless liquid with over 97% purity of linalool was purchased from Sigma Aldrich Co in Cairo, Egypt and Corn oil was bought from a nearby market.

Animals & Experimental Design: We conducted an experimental study using 35 adult male albino rats weighing around 150-200 gm. These rats were taken from Zagazig University's Faculty of Medicine's animal house. The investigation was carried out in cooperation with the Zagazig University Faculty of Medicine's departments of Histology, Cell Biology, Clinical Toxicology, and Forensic Medicine.

All animal procedures were reviewed by the Institutional Animal Care and Use Committee (IACUC) for appropriateness and gentleness. Approval number: ZU-IACUC/3/F/59/2023. According to "The Guide for the Care and Use of Laboratory Animals" (ILAR, 2011), all animals were cared for in accordance with the ethical regulations and the Animal Care Guidelines. The animals were in good health before the experiment began, and they were subjected to a week of passive preliminary care to help them get used to their new surroundings, make sure they were physically fine, and rule out any sick animals. The animals were kept in plastic cages, free from chemical pollution, under controlled conditions. The ambient temperature ranged from 22 ± 2 ºC, relative humidity was 50 ± 5%, and there was a 12-hour light cycle. Softwood shavings were used as bedding for the animals, and they were changed during cage cleaning to ensure that the animals remained clean. Overcrowding and isolation were avoided. The rats were fed a well-balanced diet that provided all the necessary nutrients to keep up their health both before and after using drugs. The diet included bread and barley. Water was provided in clean and separate containers.

***Grouping of animals*:** After acclimating to their new housing, 35 rats were randomly allocated into five equal groups, G (I): Negative control group Regular diet and tap water. G (II): Positive control group: 1 ml corn oil (as a vehicle) once daily by oral gavage for 8 weeks. G (III): Linalool treated group: linalool 50 mg/kg B.W. according to Nagappana and Jayaram [9] once daily by oral gavage for 8 weeks. G (IV): Carbendazim treated group: 500 mg/kg B.W. of carbendazim (1/10 LD50)

dissolved in 1ml corn oil once daily by oral gavage for 8 weeks (Oral LD50 of carbendazim in rats equal 5000 mg/kg). G (V): Carbendazim and linalool-treated group linalool 50 mg/kg then after 1 hour received carbendazim 500 mg/kg once daily by oral gavage for 8 weeks. The rats were then euthanized, and their brain tissues were dissected from each rat and divided into two equal parts. One part from both the brain tissues was used to prepare tissue homogenates, which were used for estimating acetylcholinesterase (ACHE) levels in brain homogenate, malondialdehyde (MDA), and superoxide dismutase (SOD), IL-6 and TNF-α. The other part of the brain tissues was put in 10% neutral buffered formalin, which was prepared for histopathological examination.

Biochemical analysis of brain homogenates*:* The cerebral hemispheres were isolated from all the groups to prepare a homogenate. An electrical homogenizer was used to prepare the homogenate by mixing 0.5 g of tissue with 5 ml of phosphate-buffered saline (PBS) at 4◦C. The homogenates were then centrifuged at 3000 rpm for 15 minutes. The resulting supernatants were collected and stored at −20◦C until further assessment of the following:

a) ***Acetylcholinesterase level in brain homogenate.*** The activity of ACHE was measured using Habila et al. [10] method. To prepare the mixture, a 0.4 mL aliquot of the homogenate was mixed with 2.6 mL of phosphate buffer (0.1 M, pH 8.0) and 100 µL of (dithiobisnitrobenzioc) DTNB (270 µ M). The mixture was pre-incubated for 2 minutes at 30°C, and then the reaction was initiated by adding 20 µL of ATC (30 mM). The product of the thiocholine reaction with DTNB was measured at 412 nm every 2 minutes for a total of 10 minutes to determine the absorbance per minute.

b) ***Oxidative stress parameters in brain tissue homogenates*:** MDA: Tissue MDA was assayed using Biodiagnostic kits (Egypt), regarding to the method proposed by Ohkawa et al. [11]. MDA activity was expressed in umol/gm tissue. Superoxide Dismutase (SOD): Tissue SOD activity was evaluated utilizing Biodiagnostic kits (Egypt), respecting to Misra and Fridovich [12] method. SOD activity was expressed in U/mg protein.

c) ***Pro-inflammatory cytokines in brain tissue homogenates*:** Tissue TNF-α and IL-6 were measured using commercial ELISA kits (catalog number: RAB0480 for TNF-α and RAB0312 for IL-6, Sigma Aldrich) following manufacturer instructions. The data are expressed as pg/mg protein.

Histopathological studies: All groups of rats were used to collect brain tissues, which were then formalin fixed at a concentration of 10% for 24 hours. The specimens were dehydrated using ascending grades of ethanol, followed by clearing agents like xylene I and xylene II. They were then impregnated in wax, and 5-micron thicknesses of paraffin sections were obtained using a microtome (Leica RM 2155, London, UK). The sections were stained using hematoxylin and eosin (H&E) for examination [13].

Immunohistochemical staining: Paraffin sections of brain tissues underwent Immunohistochemistry (IHC) staining using an Anti-NF-κB p65 antibody (ab16502) and an Anti-TLR4 antibody (ab22048), obtained from Abcam, Cambridge, UK. The staining was carried out following the manufacturer's protocols and using the DAB chromogenic agent (Expose mouse and rabbit specific horseradish peroxidase/ 3,3'Diaminobenzidine (HRP/DAB) detection kit, Abcam; cat #: ab80436). Counterstaining was done using hematoxylin. For each antigen, three immuno-labeled sections were analyzed per animal, with a total of 7 animals per group.

STATISTICAL ANALYSIS

Data were expressed as mean + SD. The statistical analysis was performed using the Epi-info statistical package program (version 6.04d), and Graph Prism (version 9). One-way analysis of variance (ANOVA or F-test) was used to compare the means of more than two groups, while the Least Significant Difference (LSD) was used for comparison between two groups. Pearson correlation was used to compare between different groups. p value of < 0.05 was considered significant.

RESULTS

Biochemical examination: After conducting ACHE level in brain homogenate, oxidative stress biomarkers (MDA, SOD), TNF-α, and IL-6 in the brain of the negative control, positive control, and linalool-treated groups, no significant changes ($p > 0.05$) were observed among these groups (Table 1). Therefore, we used the negative control group (I) as a point of reference for comparison with the other treated groups.

Acetylcholinesterase (AChE) level in brain homogenate. After 8 weeks of administration, there was a substantial variation in ACHE between the

negative control group, carbendazim-treated group, and carbendazim and linalool-treated group ($p < 0.001$). The LSD test revealed that the mean values of ACHE were substantially reduced in the carbendazim group compared to the other groups of the study ($p<0.001$). The mean values of ACHE were substantially elevated ($p<0.001$) after administering linalool in combination with carbendazim for 8 weeks compared to the carbendazim group. Additionally, the mean values of ACHE were significantly reduced ($p<0.05$) in the carbendazim and linalool-treated group compared to the negative control group (Table 1).

Oxidative stress parameters in brain tissue homogenates : Table (1) indicated that the carbendazim-treated group had notably lower mean values of brain SOD levels compared to the other groups ($p<0.001$). The carbendazim and linalool-treated group revealed remarkable elevation in brain SOD when compared to the carbendazim-treated group ($p<0.001$). However, the carbendazim and linalool-treated group exhibited non-significant low SOD levels in brain tissues compared to the negative control group ($p<0.05$) (Table 1). The results also showed that the mean values of MDA in brain tissues were substantially elevated in the carbendazim group than in both the negative control and carbendazim and linalool-treated group ($p<0.001$). Comparing the carbendazim and linalool-treated group with the negative control group showed substantially increased MDA levels ($p<0.001$) (Table 1). We found significant differences in ACHE, SOD and MDA levels in brain homogenates among the negative control group, carbendazim-treated group, and carbendazim and linalool-treated group ($p<0.001$) (Table 2).

Pro-inflammatory cytokines in brain tissue homogenates: There was substantial variance between the negative control group, the carbendazim-treated group, and the carbendazim and linalool-treated group in terms of TNF-α and IL-6 levels in brain homogenates ($p<0.001$). Rats treated with carbendazim displayed a marked increase in IL-6 and TNF-α compared to the negative control group and the combined carbendazim and linalool-treated group ($p<0.001$). On the other hand, the carbendazim and linalool-treated group revealed substantial increase in TNF-α and IL-6 compared to the negative control group ($p<0.001$ for TNF-α and $p<0.05$ for IL-6) (Fig 1 A, B)

The study on correlation coefficient revealed some important findings. The brain tissues showed a positive association between ACHE and SOD, and a negative relationship between ACHE and MDA, TNF-α, and IL-6 (Table 3).

Histopathological changes of the brain: The brain tissues of control and linalool groups exhibited normal histology of neuronal cells, glia cells, neuropil, blood vessels, clear perivascular space, and meninges (Fig. 2 A, B)

After 8 weeks of treating animals with carbendazim, different histological alterations were detected which include; focal areas of lytic necrosis represented by central microcavitations surrounded by clusters of glia cells accompanied by inflammatory cells with or without neuronophagia. Lymphocytic meningitis was also seen which is characterized by congestion of meningeal vasculatures with lymphocytic aggregates. Congested cerebral vasculatures with perivascular lymphocytic cuffing & hemorrhages within Virchow Rubin space were noticed. Moreover, pyknotic neurons, neuropil vacuolations, and intense meningeal hemorrhages were the most encountered lesions (Fig. 2 C-E). Carbendazim and linalool treated group revealed that the majority of neurons and cerebral vasculatures are intact. However, few pyknotic neurons surrounded by glia cell aggregations were seen (Fig. 2 F).

Immunohistochemistry: Immunohistochemistry staining of brain sections for TLR4 showed non-observable immunostaining in both the control group and linalool treated group. While immuno-stained sections of brain tissues for the NF-κB marker showed nearly negative stained cells in both the control group and linalool treated group. Carbendazim treated group (Figures 17E, 17F) showed moderate areas of immunostaining for TLR4 mainly in some glia cells and endothelium of cerebral vasculatures, and a higher expression level of NF-κB particularly in glia cell clusters. The expression of TLR4 and NF-κB was remarkably reduced in the carbendazim and linalool-treated group compared to carbendazim-treated group (Fig. 3,4).

Table 1: Comparison of mean values of brain level of ACHE, and levels of SOD, MDA, TNF-α, and IL-6 in brain tissues among negative control, positive control, and linalool-treated groups was conducted using one-way ANOVA**.**

Parameter	Negative Control group	Positive Control group	Linalool treated group	F	p-value
	Mean± SD				
ACHE (Pg/mg)	313.57±21.80	308.71±25.27	309.47±19.57	0.096	0.909 NS
B.SOD (U/mg)	212.85±12.19	209.28±11.70	203.90±16.08	0.784	0.471 NS
B.MDA (umol/gm)	0.55±0.11	0.56±0.09	0.52±0.08	0.309	0.738 NS
B.TNF-α (pg/mg)	59.13±1.65	60.91±4.19	60.14±1.33	0.7586	0.4827 NS
B.IL-6 (pg/mg)	66.49±2.43	65.14±3.03	62.77±3.11	3.001	0.0751 NS

All values are expressed as mean± SD, Number of rats in each group =7 rats. All values are expressed as mean± SD, Number of rats in each group=7 rats. NS: non-significant (p >0.05), ACHE: acetylcholinesterase B.SOD: Brain superoxide dismutase, B.MDA: Brain malondialdehyde,
pmol/l: Pico mol per liter, ng/ml: nanogram per milliliter, umol/gm: micro mol per gram Pg/mg: Picogram per milligram, TNF-α: Tumor necrotic factor alpha, IL-6: Interlukin 6

Table 2: Statistical comparison between the negative control group, carbendazim-treated group, and carbendazim and the linalool-treated group as regarding mean values of ACHE, SOD and MDA in brain tissue using ANOVA test.

N.B :All values are expressed as mean±SD. **ACHE:** Acetylcholinesterase

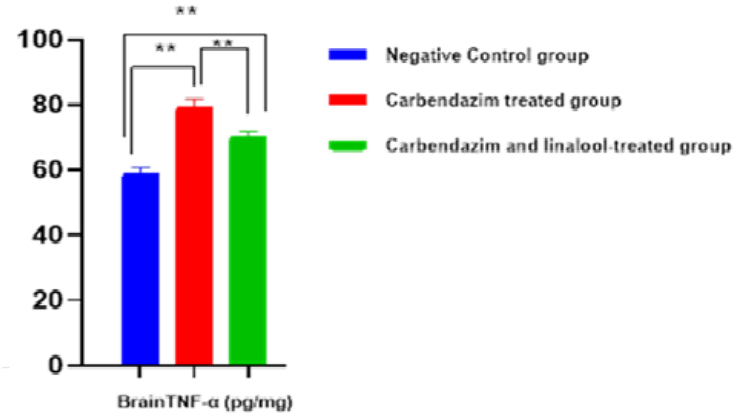
Group / Parameter	Negative control group	Carbendazim treated group	Carbendazim and linalool-treated group	F	P-value
	Mean ± SD				
ACHE (Pg/mg)	313.57±21.80	20.83±5.44	290.00±15.27	752.384	<0.001**

Group / Parameter	Negative Control group	Carbendazim treated group	Carbendazim and linalool-treated group	F	P-value
	Mean ± SD				
SOD (U/mg)	212.85±12.19	17.59±4.15	200.28±15.72	606.882	<0.001**
MDA (umol/gm)	0.55±0.11	4.31±0.55	1.20±0.35	188.580	<0.001**

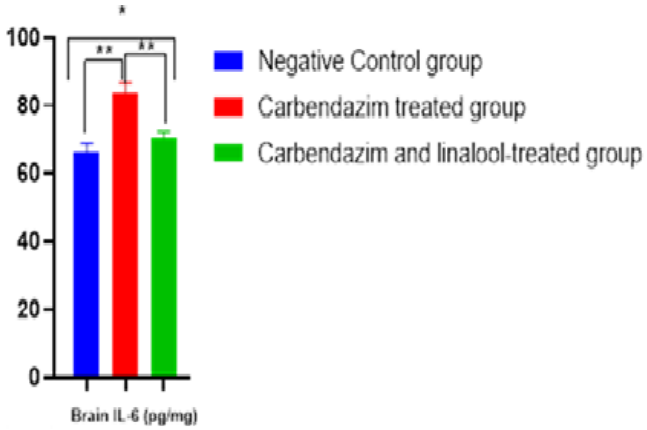
SOD: superoxide dismutase **MDA**: malondialdehyde
Number of rats in each group=7 rats**.** umol/gm: micro mol per gram
**: highly significant (P<0.001).
U/mg: Unit per miligram

Table (3): Correlation Coefficient between acetylcholinesterase activity, SOD, and MDA levels in brain tissues

	B. SOD (U/mg)		B. MDA (umol/gm)		B. TNF-α (pg/mg)		B. IL-6 (pg/mg)	
	r	p	r	P	r	p	r	p
ACHE (Pg/mg)	0.965	<0.001	-0.960	<0.001	-0.8435	<0.001	-0.8986	<0.001



(A)



(B)

Figure 1: (**A**): Effect of carbendazim on level of TNF-α in brain tissues and role of linalool among negative control, carbendazim, and combined carbendazim and linalool treated groups, (**B**): Effect of carbendazim on level of IL-6 in brain tissues and role of linalool among negative control, carbendazim, and combined carbendazim and linalool treated groups, All values are expressed as mean ±SD. *: significant ($p<0.05$); **: highly significant ($p<0.001$).

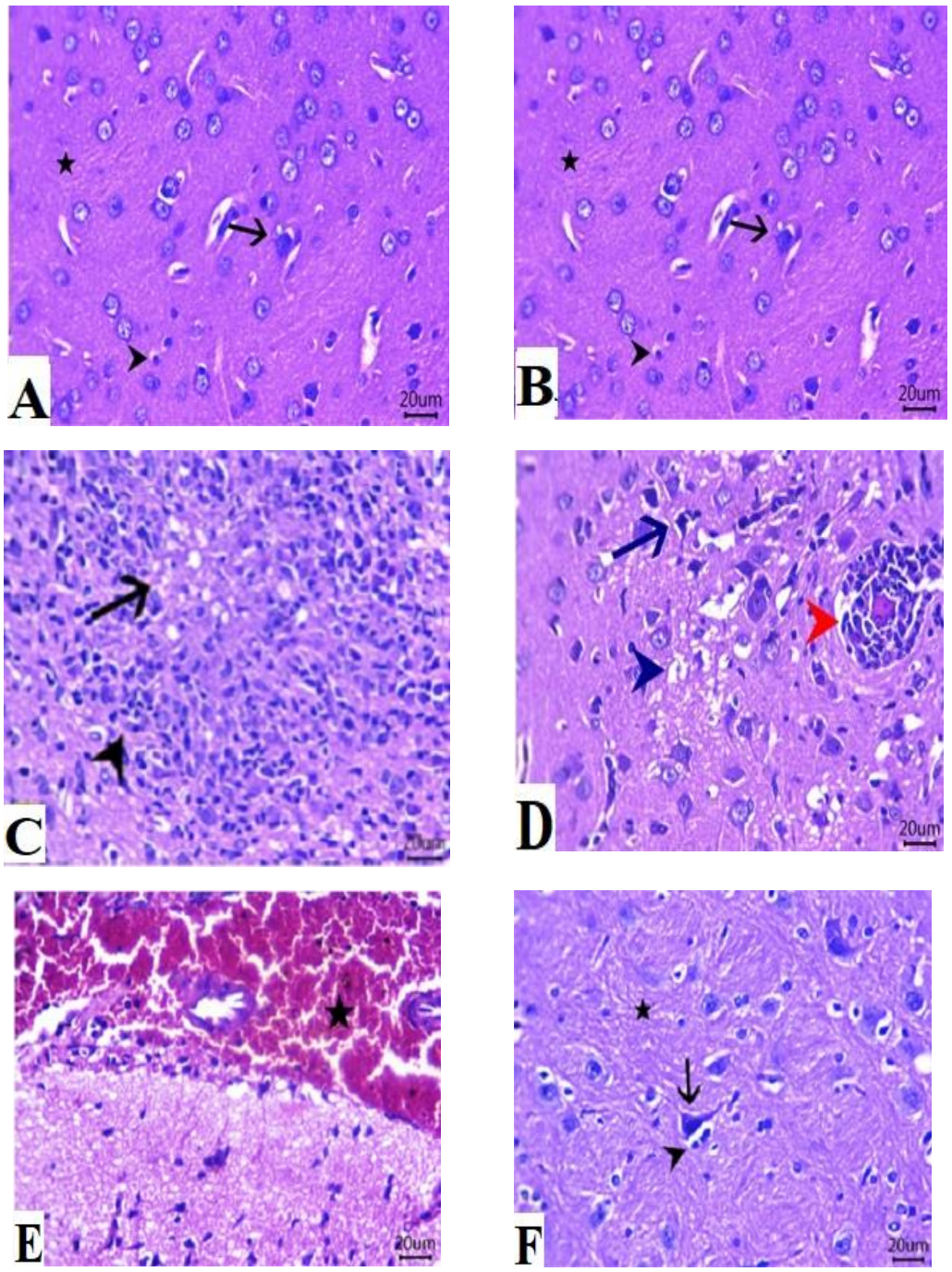


Figure. 2. Photomicrograph of H&E-stained sections of the control group from cerebral cortex showing: **(A)** Normal histomorphology of neuronal cells (arrow), glia cells (arrowhead), neuropil (star) and clear perivascular space. **(B)** Photomicrograph of H&E-stained sections of linalool treated group from cerebral cortex showing normal histology of neurons (arrow), glia cells (arrowhead), and neuropil (star). (**C-E**) Photomicrograph of H&E-stained sections of carbendazim treated group from cerebral cortex showing: (**C)** focal area of lytic necrosis formed from central microcavitations (black arrow) surrounded by clusters of glia cells and inflammatory cells (black arrowhead). **(D)** neuropil vacuolations (blue arrowhead) and perivascular cuff (red arrowhead). **(E**) intense meningeal hemorrhages (star). (**F**) Photomicrograph of H&E-stained sections of carbendazim and linalool treated group from cerebral cortex showing intact the majority of neurons, neuropil (star), few pyknotic neurons (arrow) surrounded by glia cell aggregations (arrowhead).

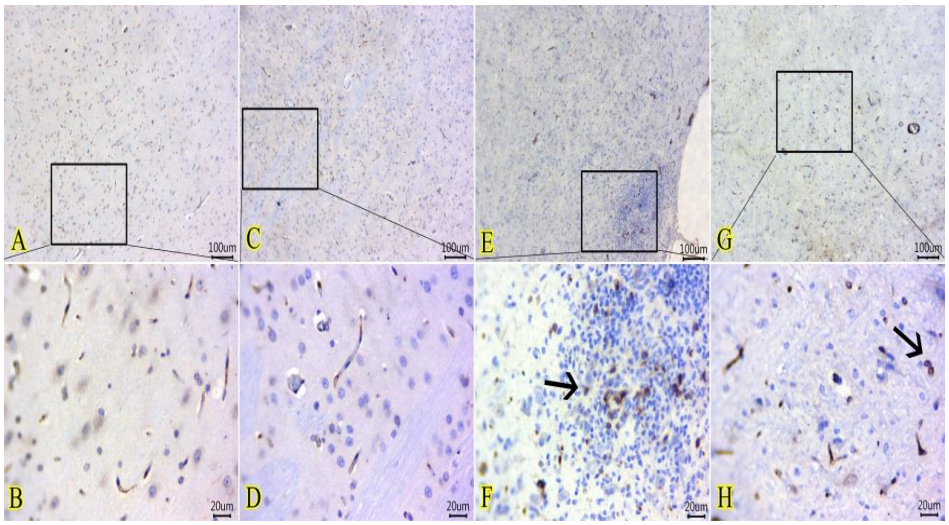


Figure 3. Photomicrographs of immunostained brain sections for TLR4 protein showing: non-observable immunostaining in both the control group **(A, B)** and linalool-treated group **(C, D).** Moderate areas of immunostaining in carbendazim treated group **(E, F).** Mild immunoexpression in combined carbendazim and linalool treated group **(G, H).** (The positive stained cells exhibited brown color) (The arrow demonstrates the positive cells). IHC counterstaining with Mayer's hematoxylin. **A, C, E, G**: TLR4 protein X100; scale bar=100 µm, **B, D, F, H:** TLR4 protein **X400**; **scale bar**=20 µm.

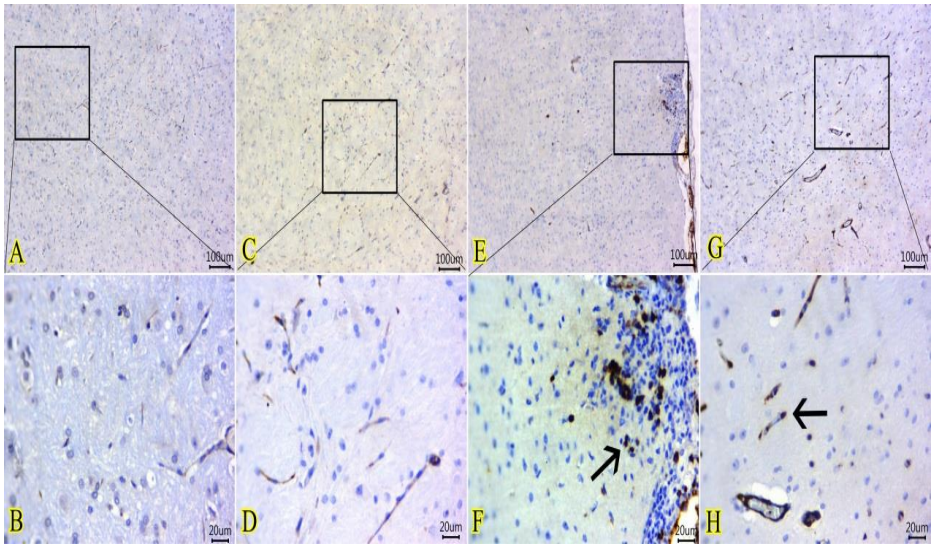


Figure 4. Photomicrographs of immunostained sections from brain tissues for NF-κB marker showing: Nearly negative stained cells in both the control group (**A, B**) and linalool-treated group (**C, D**). Higher expression level in glia cell clusters in carbendazim treated group (**E, F**). low immunoexpression in combined carbendazim and linalool treated group (**G, H**). (The positive stained cells exhibited brown color) (The arrow demonstrates the positive cells). IHC counterstaining with Mayer's hematoxylin. **A, C, E, G:** NF-κB marker X100; scale bar=100 µm, **B, D, F, H**: NF-κB marker **X400; scale bar**=20 µm.

DISCUSSION

Pesticides in particular are environmental pollutants that are currently causing a variety of disorders in both humans and wildlife [1]. Within the benzimidazole class, CBZ is a systemic broad-spectrum fungicide. Because of its widespread use as an industrial fungicide, CBZ was overused in fields, which had negative consequences on both people and wildlife [6]. Many aromatic plants yield the well-known medicinal compound linalool. Its capacity to neutralize reactive oxygen species (ROS) and excite other antioxidants has led to its application in both conventional medicine and the perfume sector [6,14]. The study designed to assess the potential protective effect of linalool administration on the toxic impact of carbendazim on the brain in adult male albino rats. The decrease in ACHE levels of the present work was in agreement with the findings of Palanikumar et al. [15], Omonona and Jarikre [16], Ezeoyili et al [2], and Patil et al [17] who observed that ACHE levels decreased significantly in rats treated with carbendazim when compared with control groups. They referred this ACHE activity inhibition to direct CBZ activity on the ACHE enzyme's active sites, reducing the enzyme's ability to bind to acetylcholine.

The brain is an essential organ for investigating the negative impacts of oxidative stress. It has a high concentration of unsaturated fatty acids and requires a lot of oxygen /unit weight. ACHE is a hydrolase enzyme found mostly in nervous and muscular tissues of organisms [18].

Acetylcholine-mediated neurotransmission is fundamental for nervous system function. Its sudden blockade is fatal, while its gradual loss, seen in conditions such as Alzheimer’s disease and multiple system atrophy, is linked to a progressive decline in cognitive, autonomic, and neuromuscular functions [19]. Acetylcholinesterase controls the neuronal activity of cholinergic synapses. It is a crucial neurotransmission-related enzyme that is extensively used as a biomarker for detecting pollutants' neurotoxic impacts. Metals, herbicides, insecticides, medicines, and other types of contaminants have all been shown in research to impede ACHE activity [2].

ROS are known to cause a wide range of oxidative damage, including undesirable health impacts and illnesses. Excessive ROS formation is well recognized to have a deleterious effect on the principal cellular macromolecules, proteins, and lipids, resulting in genotoxicity, protein and lipid oxidation, and, ultimately, apoptosis. [5] The antioxidant defense mechanism consists of molecular antioxidants, metallic chemical agents, and antioxidant enzymes. Antioxidant enzymes including glutathione peroxidase (GPx), CAT, SOD, and glutathione reductase (GR) are located in practically every part of cells and provide a potential role in the neutralizing or detoxifying of hazardous ROS molecules [20]. Our findings revealed elevation in oxidative stress markers in the carbendazim-treated group as reflected by a remarkable reduction in the mean values of SOD levels and a remarkable higher MDA, IL-6, and TNFα levels in brain tissues when compared with control groups. Many studies observed a remarkable reduction in SOD levels and a substantial elevation in MDA levels in various tissues such as the testes, kidney, liver, brain, and thyroid gland after exposure to carbendazim [1,21]. A possible reason for the decrease in SOD activity is the elimination of free radicals inside the cells. CBZ's inhibitory effect on SOD can also be explained by an overproduction of ROS. The reduced SOD activity could potentially be caused by a rise in the number of ROS and hydroxyl radicals that can deactivate SOD's chemical structure, ultimately leading to enzyme activity loss [2]. Superoxide dismutase increases the conversion of endogenous cytotoxic superoxide molecules to H2O2, and increasing SOD concentrations might lead to enhancing enzyme activity to neutralize the superoxide radicals generated by CBZ and avoid the development of cellular damage during CBZ exposure [22]. CBZ induces oxidative stress by reducing antioxidant enzymes like CAT, SOD, GPx, GR, and GST, as well as decreasing nonenzymatic antioxidant glutathione (GSH). This leads to increased generation of free radicals and ROS affecting both enzymatic and nonenzymatic antioxidant responses. CBZ additionally raises the level of malondialdehyde, H2O2, and nitric oxide (NO) activity by decreasing the nonenzymatic antioxidants, such as vitamins E, C, and A, and inhibiting antioxidant enzymes [5,23].

These results are consistent with elevated inflammatory markers NF-κB, IL-6, and TNFα reported in other CBZ exposure studies [21,24].

The biochemical changes observed after exposure to CBZ in this study were associated with histopathological changes with predominant inflammatory cells in brain tissues. These results were in accordance with Omonona and Jarikre [16], and Patil et al. [17] who found histopathological changes in the brain tissue after carbendazim administration. They attributed the tissue deterioration to oxidative stress caused by a disparity between antioxidants and pro-oxidants in the biological system. These results were in line with oxidative stress caused by CBZ in this study.

Inflammation common protective response against a range of stimuli and pathogenic disorders, which can be triggered by toxic chemicals, radiation, viral and microbial infections, chronic and autoimmune diseases, and unhealthy eating habits [25]. There is a documented association between oxidative stress and inflammation, with scientific evidence suggesting that oxidative stress contributes to the development of chronic inflammatory diseases [26]. When the body is exposed to stress, it produces an excessive amount of ROS, which in turn triggers an inflammatory response.

Nuclear factor kappa beta is transcription factor which controls the expression of several genes, including cytokine genes, which are involved in inflammatory and immunological responses. When cellular homeostasis is upset, inhibitory kappa beta (IκB) is broken down by proteases, which activates NF-κB. NF-κB will migrate into the nucleus as a consequence of its stimulation, inducing its proinflammatory cytokines [27]. Depending on the degree of stress, the nuclear factor kappa B can have an anti- or prooxidant function in modulating oxidative stress. Numerous physiological functions, including neural growth, infection reaction apoptosis and cell growth and inflammation, are mediated by NF-κB. Chronic inflammatory diseases such as malignancies and neurological disorders are brought on by NF-κB dysfunction. To treat such conditions, the pathway must be regulated [28]. The nuclear factor kappa B pathway is triggered by several stimuli, the most important of which is the activation of Toll-like Receptors (TLRs), including TLR4. Toll-like receptors are components of the innate immune system, which mounts a successful defense in response to stimuli originating from pathogens or cellular injury. Endogenous compounds designated damage-related molecular patterns are hypothesized to trigger TLRs to generate an inflammatory response. An investigation has indicated that oxidative stress-related events activate TLR4. TLR4 has also been linked to the onset and advancement of several conditions by causing endothelial dysfunction and oxidative stress. Oxidative stress has also been linked to TLR4 activation [29]. The oxidative stress and inflammation observed in the carbendazim treated group were consistent with the immunohistochemical findings of the current investigation that showed increased areas of immunostaining for NF-κB and TLR4 in brain tissues. This study was agreed with the results of Madboli and Seif [30], Salem et al [21], and Ebedy et al [23] who reported positive immunoreaction for NF-κB with an increase in inflammatory markers.

All the biochemical and histopathological changes induced by carbendazim were relieved by linalool co-administration. Linalool is an antioxidant because of its ability to eliminate ROS and activate other antioxidants from their inactive condition [31]. Several studies demonstrated the antioxidant effect of linalool in different tissues such as brain tissues. Xu et al. [32] found that linalool has a neuroprotective effect on cognitive deficits induced by Amyloid-beta (Aβ) by reduced MDA levels and elevated SOD levels. Zheng et al. [33] described the preventive impact of linalool against induced myocardial infarction in rats. The treatment with linalool significantly reduced MDA and restoring the reduced activities of SOD. Also, Oner et al [34] observed that linalool could remove doxorubicin (DOX)-induced cardiotoxicity by restoring MDA and SOD levels at different doses. Furthermore, Mohamed et al. [31] found that Linalool could defend against cisplatin-induced renal damage, as they discovered that pretreatment with linalool exhibited a substantial rise in SOD and decreases in MDA. These results were also consistent with the findings of Ola and Sofolahan [35] who found administration of linalool improved antioxidant activity evidenced by reduced MDA levels and elevated SOD levels in hepatic tissues of rats exposed to benzene. These results are parallel to the results of Zheng et al. [33] and Mohamed et al. [31] who found significant inhibition of NF-κB expression by linalool in myocardial tissues and renal tissues respectively. Periyasamy et al. [36] and Zhang et al. [8] reported that linalool can suppress

the expression of NF-κB and TLR4, which alleviates injures of liver and spinal cord through its antioxidant and anti-inflammatory activities. The results of this research coincided also with Zhang et al. [8] who recorded Linalool treatment relieved spinal cord injury through anti-inflammatory and antioxidant effects by normalizing MDA and SOD levels. Linalool generates a dose-dependent decrease of inflammation as shown by lower pro-inflammatory cytokines. Linalool effectively reduces inflammation by inhibiting inflammatory cell infiltration as well as the generation of TNF-α, IL-6, IL-1β, and IL-8. Linalool suppresses inflammation through both the NF-κB and the Nrf2/HO-1 signaling pathways. Linalool has an anti-inflammatory effect by activating Nrf-2 and acting in pathogen-mediated inflammation through the oxidative stress pathway [8,37].

Our results suggest that improvement in ACHE activity, and histopathological changes were due to reduced oxidative stress and inflammation induced by CBZ. This was achieved by increased anti-oxidant SOD and decreased inflammation caused by linalool with its antioxidant effects.

CONCLUSIONS

Carbendazim administration in a dose of 500 mg/kg/day for 8 weeks has resulted in impairment of acetylcholine esterase level with evidence of oxidative stresses in the form of elevated MDA and reduced SOD in brain tissues. These biochemical changes were associated with marked histopathological changes and an increase in immunostaining for NF-κB and TLR4 in brain tissues. Linalool administration in a dose of 50 mg/kg/day for 8 weeks caused amelioration of all these changes through its anti-oxidant and anti-inflammatory effects.

CONFLICT OF INTEREST: None.
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FIGURE LIGAND

Fig. 1. (A): Effect of carbendazim on level of TNF-α in brain tissues and role of linalool among negative control, carbendazim, and combined carbendazim and linalool treated groups, (B): Effect of carbendazim on level of IL-6 in brain tissues and role of linalool among negative control, carbendazim, and combined carbendazim and linalool treated groups.

Fig. 2. Photomicrograph of cerebral cortex showing: (A) Normal histomorphology of neuronal cells (arrow), glia cells (arrowhead), neuropil (star) and clear perivascular space in control group. (B) Photomicrograph of linalool treated group from cerebral cortex showing normal histology of neurons (arrow), glia cells (arrowhead), and neuropil (star). (C-E) Photomicrograph of carbendazim treated group from cerebral cortex showing: (C) focal area of lytic necrosis formed from central microcavitations (black arrow) surrounded by clusters of glia cells and inflammatory cells (black arrowhead). (D) neuropil vacuolations (blue arrowhead) and perivascular cuff (red arrowhead). (E) intense meningeal hemorrhages (star). (F) Photomicrograph of carbendazim and linalool treated group from cerebral cortex showing intact the majority of neurons, neuropil (star), few pyknotic neurons (arrow) surrounded by glia cell aggregations (arrowhead).

Fig. 3. Photomicrographs of immunostained brain sections for TLR4 protein showing: non-observable immunostaining in both the control group (A, B) and linalool-treated group (C, D). Moderate areas of immunostaining in carbendazim treated group (E, F). Mild immunoexpression in combined carbendazim and linalool treated group (G, H). (The positive stained cells exhibited brown color) (The arrow demonstrates the positive cells).

Fig. 4. Photomicrographs of immunostained sections from brain tissues for NF-κB marker showing: Nearly negative stained cells in both the control group (A, B) and linalool-treated group (C, D). Higher expression level in glia cell clusters in

carbendazim treated group (E, F). low immunoexpression in combined carbendazim and linalool treated group (G, H). (The positive stained cells exhibited brown color) (The arrow demonstrates the positive cells).

Citation

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