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RESEARCH ARTICLE

Modulatory Effect of Ginger Aqueous Extract against Imidacloprid-Induced Neurotoxicity in Rats

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Abstract

The current study was aimed to investigate neurotoxic impact of imidacloprid in rats and the potential modulatory role of *Zingiber officinale Roscoe* aqueous extract against such effects**.** Sixty male albino rats were randomly assigned into six groups (*n* = 10) as following: G1 is (-ve) control group (0.1 ml of distilled water for 90 days). G2 is (+ve) control group (1ml of aqueous extract of ginger (GAE) for 90 days). G3 group was administered with 0.1 ml of Imidacloprid (IMI) for 90 days. G4 group was administered with 1 ml of GAE for 2 weeks followed by administration of 0.1 ml of IMI/rat for 90 days. G5 group was administered with 0.1 ml of IMI for 90 days then 1ml of GAE for 2 weeks and the last group administered 0.1 ml of IMI and 1ml of GAE simultaneously for 90 days (G6) oral dosing of IMI and ginger aqueous extract was triple weekly. IMI exposure caused significant decrease in gamma amino butyric acid (GABA) level, significant increase in sorbitol dehydrogenase (SDH), significant depletion in glutathione (GSH), superoxide dismutase (SOD) was not affected by IMI exposure. IMI exposure upregulates toll like receptor 2 (*TLR2*) gene in the brain, intense immuno positive reactivity of *TLR2* in the brain of IMI-treated group. Histopathologically, significant alterations in the brain were observed, such as neuronal degeneration, hemorrhages, necrosis, demyelination and gliosis. In conclusion, IMI neurotoxic effect could be modulated by the use of ginger aqueous extract.

Keywords: Imidacloprid, ginger, GABA, SDH, *TLR2.*

Introduction

Neonicotinoids, systemic neurotoxic pesticides resembling nicotine are the most extensively used insecticides to protect residential plants from sucking insects found in horticulture [1]. Nowadays, this group of pesticides involves at least seven main compounds with a market share of more than 25% of total international pesticide sales [2] and is substituting older classes of pesticides like organophosphate and carbamate worldwide [3]. Imidacloprid (IMI) is one of neonicotinoid pesticide belongs to the chloronicotinyl nitroguanidine chemical family [4, 5]. IMI is used to resist sucking insects, chewing insects, termites and fleas on pet animals. Beside its local use on pets, imidacloprid may be useful to constructions, crops, soil, and as a seed treatment [5, 6]. IMI and its analogs are obviously powerful neurotoxic pesticides, which act as nicotinic acetylcholine receptor agonists (nAChRs) [7]. Although neonicotinoids make a selective agonist effect on nAChRs in insect as compared to mammalian ones, it prompts the biochemical and neurobehavioral changes in developing and mature mammals, and these deleterious effects may be related to the circulation and affinity of their metabolites [8]. Neurobehavioral changes were found in rats exposed to IMI in the uterus [9]. Exposure to IMI at concentrations more than 10 mM for <1 min in some in vitro studies could injure membrane properties of mice stellate cells [10]. IMI was also known as cytotoxic to cerebellar neurons of newborn rats. IMI caused significant excitatory Ca^{2+} influxes, which was a sign of nervous physiological action convert low-activity. Consequently, neonicotinoids may badly affect the developing brain [11]. Studies have

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demonstrated that oral exposure to imidacloprid (45 and 90 mg/ kg body weight) for 28 days cause a significant drop in the spontaneous locomotor activity and pain threshold in rats [12].

Ginger and ginger-related compounds exhibited neuroprotective action. Principally, 6-shogaol, with a concentration of 20μM suppressed prostaglandin-E2 (PGE2), induced nitric oxide (NO), COX-2 , pro-inflammatory cytokines as interleukin-1β (IL-1β), TNF-α, interleukin-6 (IL-6) and their mRNA expressions [13,14]. Furthermore, significant neuroprotection of 6-shogaol was reported in rats via the microglial inhibition [13]. Particularly, 6-shogaol (10 μM) revealed a neuroprotective effect in LPS- treated astrocytes through the up-regulation of brain-derived neurotrophic factor (BDNF) [15].

This study was designed to spot the light on the neurotoxic effect of IMI via estimation of gamma-aminobutyric acid (GABA), sorbitol dehydrogenase (SDH), oxidative stress markers in serum including: superoxide dismutase (SOD), reduced glutathione (GSH), gene expression of *TLR2* in brain, immunohistochemical staining of *TLR2* in brain and histopathological examination of brain and the modulatory effect of ginger to IMI neurotoxicity.

Materials and Methods

Tested compounds

Imidacloprid

Imidacloprid ($C_9H_{10}C_lN_5O_2$, CAS No. 138261-41-3) was purchased as cloprid plus 35% SC produced by European Community for agricultural products, Germany. IMI was orally administered to rats throughout the experimental period.

Zingiber officinale Roscoe (Ginger) aqueous extract:

Preparation of ginger aqueous extract: ginger (*Zingiber officinale* Roscoe) rhizomes were obtained from the local market at Sharkia Governorate, Egypt. One kilogram of new ginger rhizomes was cleaned, washed under faucet water, at that point cut into little portions, air dried and powdered. 125 gm of this powder were soaked in 1000 ml of distilled water for 12 h at room temperature and were then sieved. The concentration of the extract is 24 mg/ml. Every rat in the current study was given 1 ml of the absolute aqueous extract orally [16].

Animals and experimental design

Sixty mature male albino rats *Rattus norvegicus* weighing (150-200 gm) were obtained from the Laboratory Animal Housing Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were clinically healthy, kept under hygienic conditions in hard wood shaving–bedded plastic cages. They were maintained on balanced ration and tap water *ad-libtum* and a photoperiod of 12/12 hrs light/dark cycle. Rats were randomly divided into six equal groups (10 rats each). Rats were kept for two weeks before starting the experiment for acclimatization. IMI and ginger aqueous extract were given orally using rat stomach tube. Groups and treatment are as following; G1 is (-ve) control group (0.1 ml of distilled water for 90 days). G2 is (+ve) control group (1ml of aqueous extract of ginger/rat for 90 days). G3 group was administered with 0.1 ml of Imidacloprid (IMI) for 90 days. G4 group was administered with 1 ml of ginger aqueous extract for 2 weeks followed by administration of 0.1 ml of IMI/rat for 90 days. G5 group was administered with 0.1 ml of IMI for 90 days then 1ml of ginger aqueous extract for ٢weeks and the last group administered 0.1 ml of IMI and 1ml of ginger aqueous extract simultaneously for 90 days (G6). Oral dosing of imidacloprid and ginger is triple weekly. The dose of imidacloprid was selected to be 1/100 LD_{50} =0.21 mg/kg BW. [17]. Concentration of imidacloprid in the commercial product (cloprid) is 35%, dose for each rat is 0.1ml.

Blood samples and tissue collection

At the end of experimental period (90 days), blood samples were collected from retro-orbital venous plexus for obtaining serum samples after anesthesia of rats by Ketamine – Xylazine by dose 0.05–0.10 ml/100 gm intraperitoneally . The blood samples were collected into clean, dry test tubes then centrifuged at 3000 rpm for 10 minutes and the sera were collected and preserved at -20° until analysis. Then, the animals were fasted overnight, sacrificed by cervical dislocation. Then, specimens from brain (cerebrum, hippocampus) were collected, and divided horizontally into 2 parts. One part was instantly preserved in liquid nitrogen for gene expression. The second part was preserved in 10% neutral buffered formalin for immunohistochemical and histopathological examination.

Serum biochemical and antioxidant parameters

Sorbitol dehydrogenase (SDH) was measured using rat ELISA Kit for SDH (Catalog No: MBS2023295) and also Gamma-Aminobutyric Acid (GABA) using rat ELISA Kit for GABA (Catalog No: MBS269152). Superoxide dismutase (SOD) and reduced glutathione (GSH) were estimated using kits. Kits were obtained from BIOMED Diagnostics. Giza, Egypt.

Gene expression of toll like receptor 2 (TLR2) in brain

RT-qPCR was used to estimate the mRNA expression levels of *TLR2* gene in the brain of rats using gene-specific primer pairs: forward primer: 5-CGCTTCCTGA ACTTGTCC-3 and reverse primer 5- GGTTGTCA CCTGCTTCCA-3 (accession number: NM_ 198769.2). RNeasy Mini Kit (Qiagen, Heidelberg, Germany) was utilized to extract total RNA which further reverse transcribed using a QuantiTect Reverse Transcription kit (Qiagen, Heidelberg, Germany) in accordance with the manufacturer's instructions. RT-qPCR was done on a Rotor-Gene Q cycler (Qiagen, Germany) using QuantiTect SYBR Green PCR kits (Qiagen, Heidelberg, Germany). Relative fold changes in the expression of target genes were accomplished using the comparative $2^{-\Delta\Delta}$Ct (Ct: cycle threshold) method [18] with the β-actin gene as an internal control to normalize target gene expression levels using forward primer: 5-CCTCTATGCCAACACAGTGC -3 and reverse primer 5- GTACTCCTGCTT GCTGATCC -3 (accession number: NM_031144).

Immunohistochemistry of TLR2

For IHC staining, avidin biotin peroxidase method was used. The sections used were fixed on charged slides, then deparaffinized in 4 changes of fresh xylene, and re-hydrated in graded ethanol (100 %, 95 % and 70 %) then washed in phosphate buffered saline (PBS) at pH 7.2 for 5 minutes. To prevent endogenous peroxidase action, the sections were dipped in 0.3% hydrogen peroxide in water. The sections were then washed in distilled water three times and then washed in PBS. Then, the sections were washed in 10% normal rabbit serum (blocking reagent) in a humid chamber for 30 minutes to reduce nonspecific binding of immunoglobulins. The Sections were incubated with antisera containing the specific primary antibody (Anti-*TLR2* antibody). The sections were incubated in a humid chamber overnight at room temperature. Excess reagent was thrown off and the slides were washed in two changes of PBS, 5 minutes each. Then, the sections were covered with biotinylated secondary anti-immunoglobulin. The slides were incubated in a humid chamber at room temperature for 30 minutes then were washed in two changes of PBS for 5 minutes. Labeling antibody (streptavidin enzyme label) was added to each section. Slides were kept in a humidified chamber at room temperature for 30 minutes and then washed in two changes of PBS, 5 minutes each. Diaminobenzidine (DAB) was used as chromogen and slides were incubated for 4 minutes at room temperature. Slides were washed in distilled water for 5 minutes. Then, the slides were counter stained with Mayer's haematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in Canada Balsam. Slides are visualized under a light microscope [19]. Anti-*TLR2* antibody, Labeling antibody and Diaminobenzidine were obtained from Abcam company, USA.

Histopahological examination

Samples from brain were collected and fixed in 10% neutral buffered formalin, dehydrated in graded ethanol (70-100%), cleared in xylene, and inserted in paraffin. Five-micron thick paraffin sections were set and then regularly stained with hematoxylin and eosin (HE) dyes [20] and then examined microscopically.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (ver. 14.0 for windows, SPSS, Inc., Chicago, IL). Duncan'sMultiple Range test was conducted to compare means value between groups. Data were expressed as mean ± SEM. A value of $p < 0.05$ was considered as statistically significant.

Results

Signs of intoxication

Signs of intoxication were mainly decreasing of feeding behavior in IMI- intoxicated rats accompanied with decreased body weight while there was no change in feeding behavior of control rats. There were no significant changes in mortality percentage among all experimental groups.

Effects of imidacloprid (IMI), ginger aqueous extract and their co-administration on brain function parameters

The effects of IMI, ginger aqueous extract and their co-exposure on biochemical parameters related to brain functions of treated rats have been summarized in Table (1).

Table 1: Effects of imidacloprid (IMI), ginger aqueous extract (GAE) and their co-administration on serum activities of SDH, GABA and SOD, GSH.

Item	treatment	SDH (U/L)	GABA (pg/ml)	SOD (U/ml)	GSH (mmol/ml)
G1	0.1 ml of distilled water/rat for 90 days	37.62 ± 1.30^{d}	149.20 ± 3.72^{a}	0.25±0.02	0.65 ± 0.02^{b}
G2	1ml of GAE /rat for 90 days	38.40 ± 0.55^{d}	156.20 ± 1.09^{a}	0.28±0.06	0.72 ± 0.03^{a}
G3	0.1 ml of IMI/rat for 90 days	109.27 ± 4.49^{a}	119.59 ± 4.30^{c}	0.24±0.05	0.41 ± 0.01^{d}
G4	1 ml of GAE/rat for 2 weeks then 0.1 ml of IMI/rat for 90 days	52.19 ± 2.31^{c}	131.34 ± 1.26^{b}	0.25±0.02	0.51 ± 0.01^{c}
G5	0.1 ml of IMI/rat for 90 days then 1 ml of GAE/rat for 2 weeks	81.14 ± 0.57^{b}	$122.63\pm.926^{c}$	0.27±0.06	0.50 ± 0.01^{c}
G6	0.1 ml of IMI and 1ml of GAE simultaneously for 90 days	43.75 ± 1.91^{d}	148.73 ± 1.53^{a}	0.26±0.03	0.64 ± 0.02^{b}
p-*value*		0.001	0.001	0.213	0.009

Means within each column carrying different superscript are significant at ($P \leq 0.05$).
SDH: Sorbitol dehydrogenase, GABA: Gamma amino byutric acid, SOD: Super oxide dismutase, GSH: Reduced glutathione.

Regarding to sorbitol dehydrogenase (SDH) values, it shows a significant increase in (G3) group than (G1) followed by the (G5) group then the (G4) group while the prophylactic use of ginger aqueous extract (G6) restored the SDH to control values.

The results of GABA revealed that administration of IMI (G3) caused a significant decrease in GABA than (G1). The same result was obtained in (G5) group. While, improvement of the GABA level was noticed in (G4) than (G3) group but did not reach to control level (G1). On the other hand, administration of ginger aqueous extract alone (G2) or simultaneously with IMI (G6) could modulate the GABA level to control values.

Effects of IMI, ginger aqueous extract and their co-administration on serum antioxidants

Effects of IMI, ginger aqueous extract and their co-exposure on antioxidant indices have been shown in Table (1). The results showed that SOD activity did not be affected by any treatment compared to control. Rats exposed to IMI (G3) revealed a significant reduction in GSH content. While GSH content was significantly increased in (G4) and (G5) groups than IMI alone group (G3) however did not reach the control level (G1). The prophylactic use of ginger aqueous extract (G6) could normalize the GSH content to the control value.

Effects of IMI, GAE and their co-administration on relative expression of TLR2 gene in brain

The obtained qPCR data revealed significant (P≤0.05) upregulation of *TLR2* gene expression in the brain of IMI-intoxicated rats (G3) (8.69±0.36) as compared to the control group (G1) (1.00±0.08).This increased expression of *TLR2* obtained by IMI was significantly downregulated following treatments with ginger, with lowest expression in the protective group (G4) (4.72±0.26) followed by the treatment group (G5) (5.82±0.3) and then prophylactic group (G6) (6.41±0.32). However, none of these treatments returned the expression to the normal levels On the other hand, no significant difference was noticed between the control (G1) and (G2) (1.68±0.08) as shown in Figure (1).

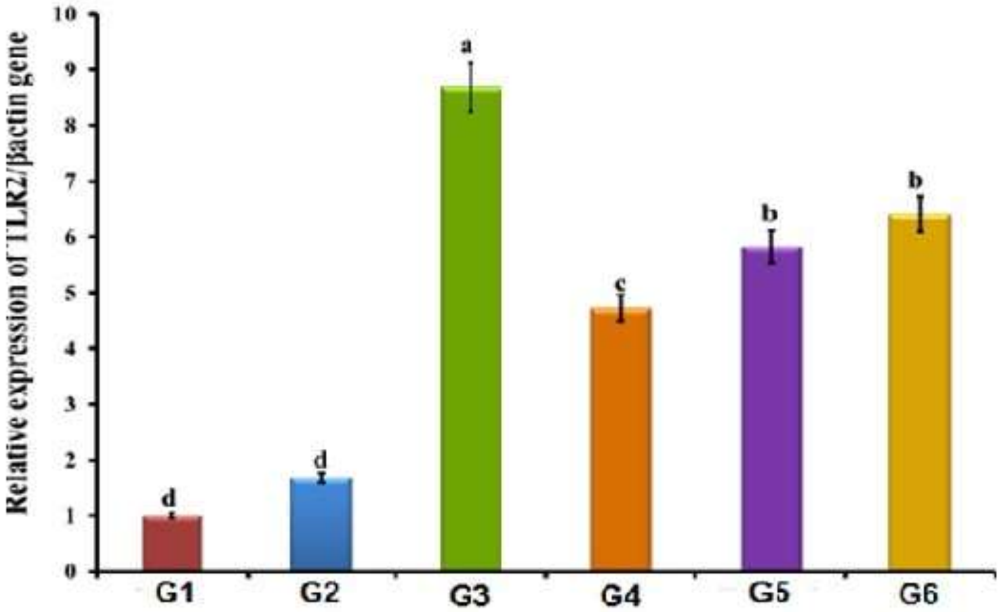

Figure 1: Graphical presentation of real-time quantitative PCR analysis of the expression of *TLR2* gene in brain of imidacloprid-intoxicated rats following treatment with ginger. Means of columns carrying different superscript letters are significantly different at P≤ 0.05. B actin gene was used as internal reference (housekeeping gene). G1: 0.1 ml of distilled water/rat for 90 days, G2: 1ml of GAE /rat for 90/days, G3: 0.1 ml of IMI/rat for 90 days, G4: 1 ml of GAE/rat for 2 weeks then 0.1 ml of IMI/rat for 90 days, G5: 0.1 ml of IMI/rat for 90 days then 1 ml of GAE/rat for 2 weeks, G6: 0.1 ml of IMI and 1ml of GAE simultaneously for 90 days.

Effects of IMI, GAE and their co-administration on immunohistochemical staining reaction of TLR2 in brain of rats

TLR2-IHC stained sections of brain cerebral cortex from control rats (G1) showed negative immuno- reactive staining (A). TLR2-IHC stained sections of brain cerebral cortex from (G2) group showing slight immuno- reactive staining in a few cells (B). TLR2-IHC stained sections of brain cerebral cortex from (G3) group showing intense positive reactivity staining (C). TLR2-IHC stained sections of brain cerebral cortex from (G4) had moderate to intense positive reactivity (D). TLR2-IHC stained sections of brain cerebral cortex from (G5) showing mild to moderate immuno-reactive staining of a few neurons (E). TLR2-IHC stained sections of brain cerebral cortex from (G6) showed faint positive reactivity (Figure 2).

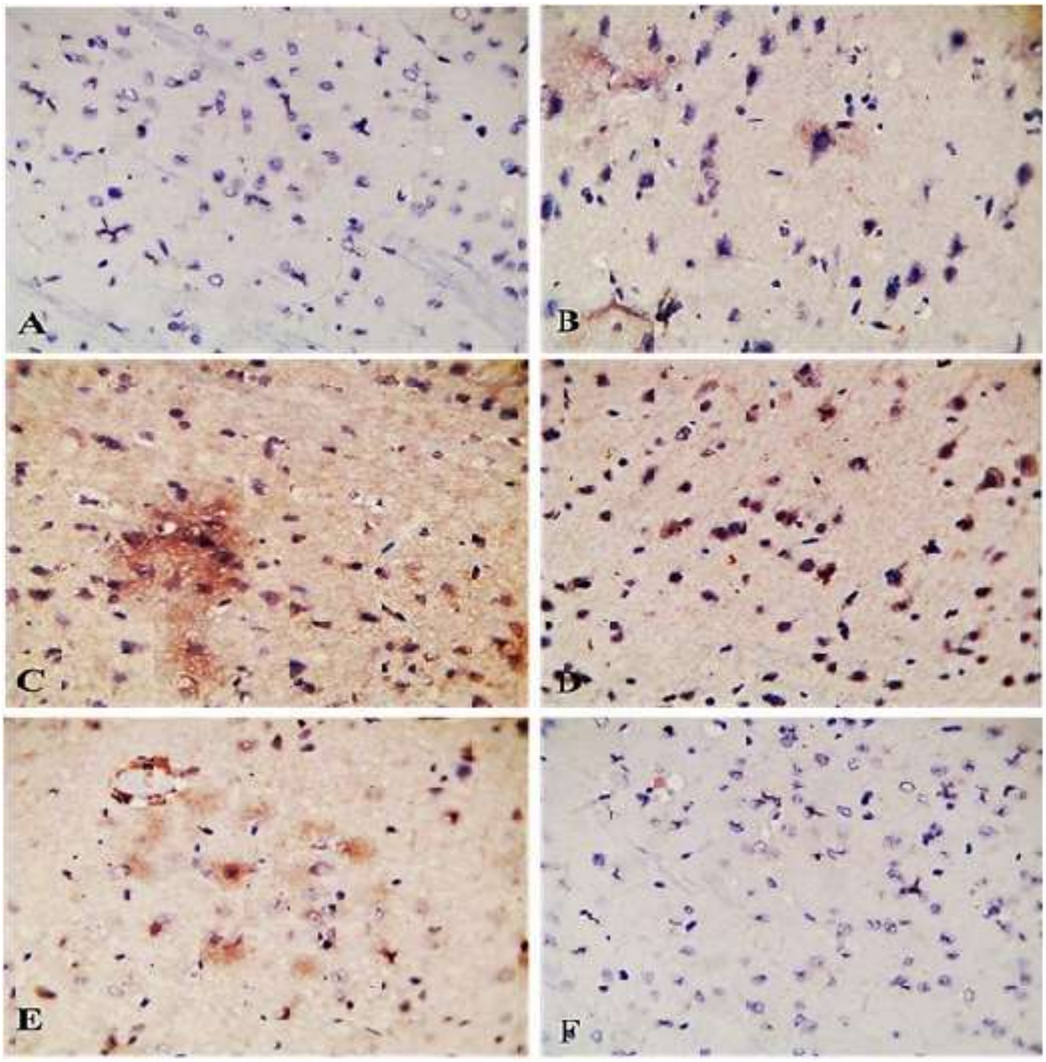

Figure 2: Photomicrograph of toll like receptor 2-immunohistochemically (TLR2-IHC) stained sections of brain cerebral cortex from G1 rats showed negative immuno reactive staining (A). TLR2-IHC stained sections of brain cerebral cortex from G2(1ml of GAE /rat for 90/days)showed slight immuno reactive staining in a few cells (B). TLR2-IHC stained sections of brain cerebral cortex from G3(0.1 ml of IMI/rat for 90 days)viewed intense immuno positive reactivity staining (C). TLR2-IHC stained sections of brain cerebral cortex from G4(1 ml of GAE/rat for 2 weeks then 0.1 ml of IMI/rat for 90 days) had moderate to intense immuno positive reactivity (D). TLR2-IHC stained sections of brain cerebral cortex from G5(0.1 ml of IMI/rat for 90 days then 1 ml of GAE/rat for 2 weeks) showed mild to moderate immuno- reactive staining of a few neurons (E). TLR2-IHC stained sections of brain cerebral cortex from G6(0.1 ml of IMI and 1ml of GAE simultaneously for 90 days) presented faint immuno -positive reactivity. X400. H&E.

TLR2-IHC stained sections of brain hippocampus from control rats (G1) and rats from (G2) group had no immuno- reactive staining (Figure 2 A and B). TLR2-IHC stained sections of brain hippocampus from (G3) group showed intense immuno-positive staining reaction (Figure 2 C).

TLR2-IHC stained sections of brain hippocampus from (G4) viewed moderate positive reactivity (Figure 2 D). TLR2-IHC stained sections of brain hippocampus from (G5) presented mild to moderate immune reaction (Figure 2 E). TLR2-IHC stained sections of brain hippocampus from (G6) group showed mild immuno- positive reactivity (Figure 3).

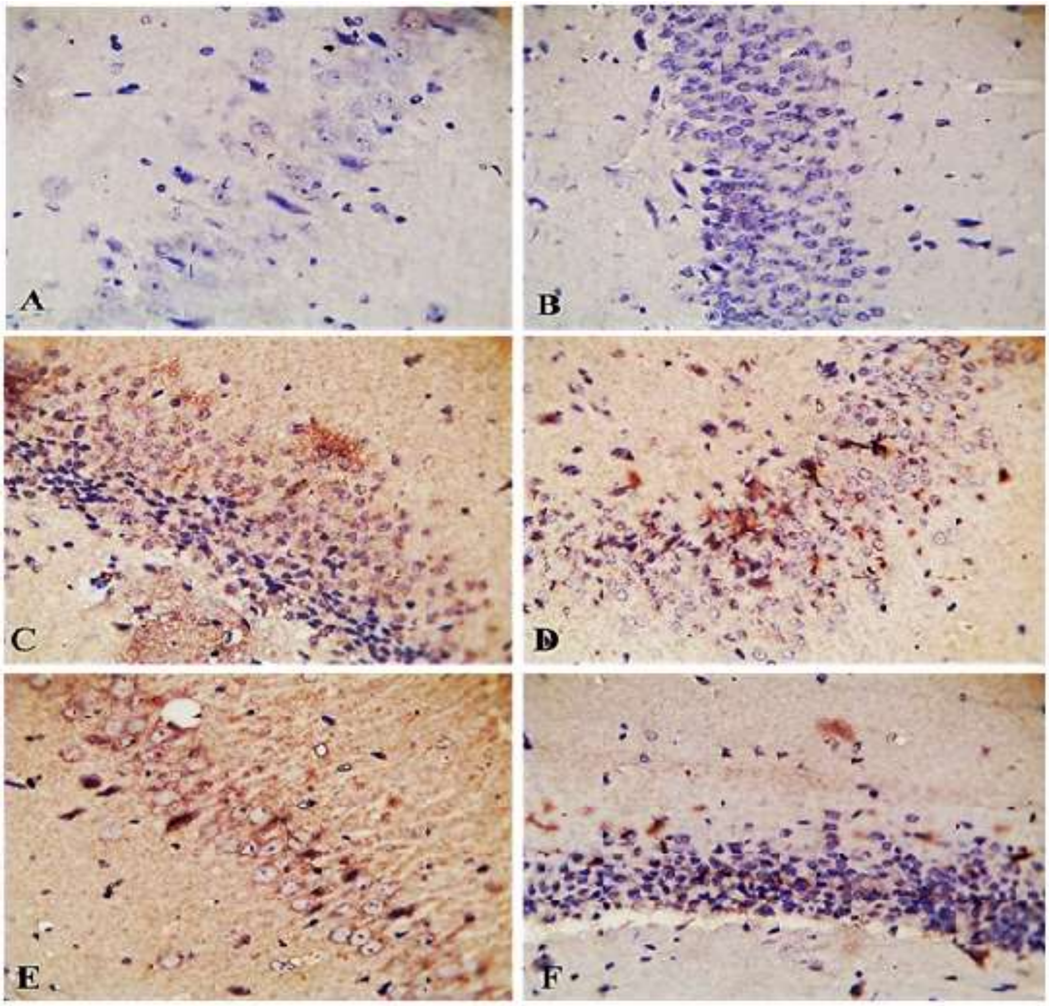

Figure 3: Photomicrograph of TLR2-IHC stained sections of brain hippocampus from G1 rats and rats from G2 group showed negative immuno reactive staining (A) and (B) respectively. TLR2-IHC stained sections of brain hippocampus from G3 group showed intense immunopositive reactivity staining (C). TLR2-IHC stained sections of brain hippocampus from G4 showed moderate immuno positive reactivity (D). TLR2-IHC stained sections of brain hippocampus from G5 group showed mild to moderate immuno reactive staining (E). TLR2-IHC stained sections of brain hippocampus from G6 group showed mild immuno positive reactivity. X400.H&E.

Effects of imidacloprid (IMI), ginger aqueous extract and their co-administration on histopathological structure of brain

Brain of control group (G1) showed normal brain structure (Figure 4 A). Brain of rats from (G2) showed normal cerebral neuronal and glial cells (Figure 4B). Brain of rats from (G3) group revealed characteristic lesions represented by focal neuronal degeneration and neuronophagia beside hemorrhages (Figure 4C). Brain of rats from (G4) group revealed most parts of the brain parenchyma including cerebral and cerebellum structures were apparently normal. Moreover, focal cerebral neuronal degeneration at the cortex and the hippocampal cells was noticed. Mild congested choroid plexus could be seen (Figure 4D). Brain of rats from (G5) group showed most of the structures of the cerebrum and cerebellar were normal whereas, minute focal neuronal degeneration and demyelination were seen. Moreover, apparently normal hippocampal with mild congested choired plexus (Figure 4 E). Brain of rats from (G6) group showed the most brain tissue is within the normal (Figure 4 F).

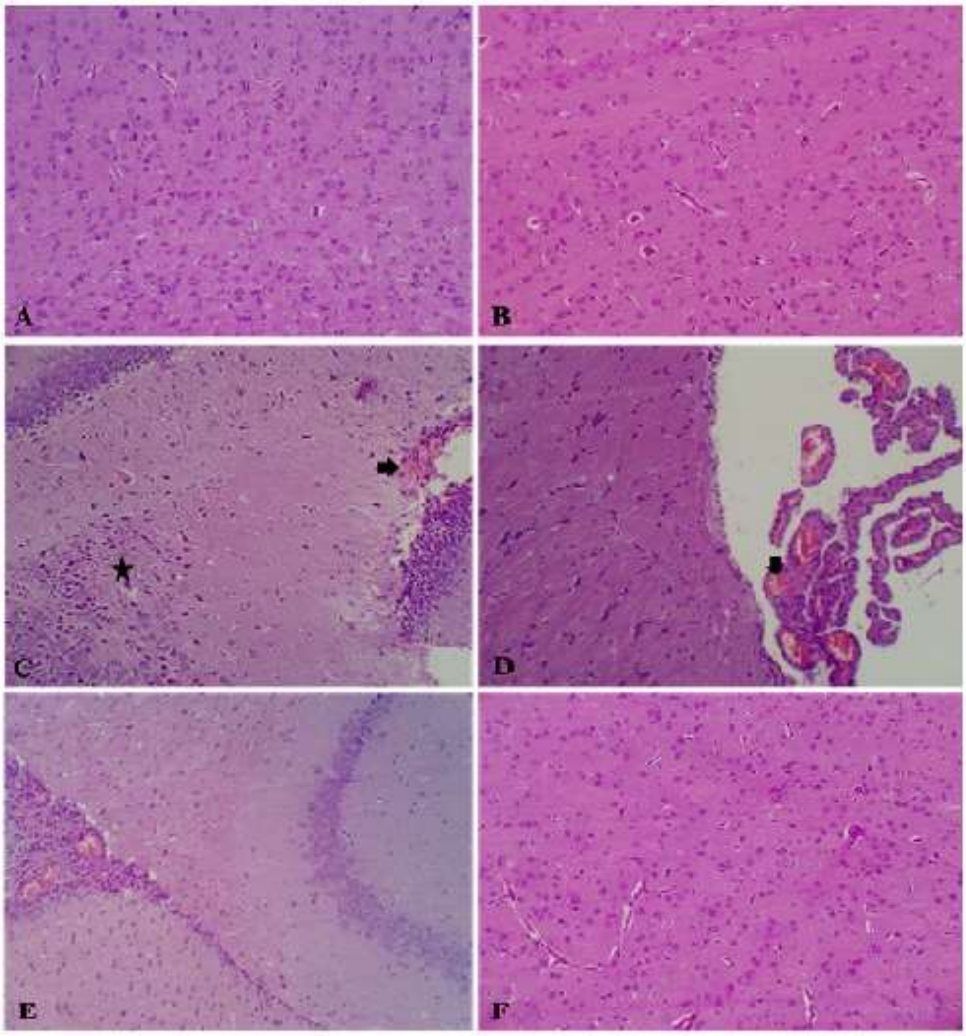

Figure 4: Photomicrograph of H&E stained brain from G1 rats showed normal brain (cerebral cortex) structures (A). Brain of rats from G2 (1ml of GAE /rat for 90/days) showed normal cerebral neuronal and glial cells (B). Brain of rats from G3 (0.1 ml of IMI/rat for 90 days) showed focal neuronal degeneration (star) beside hemorrhages (arrow) (C). Brain of rats from G4(1 ml of GAE/rat for 2 weeks then 0.1 ml of IMI/rat for 90 days) showed mild congested choroid plexus (arrow) (D). Brain of rats from G5(0.1 ml of IMI/rat for 90 days then 1 ml of GAE/rat for 2 weeks) showed normal hippocampal with mild congested choroid plexus (E). Brain of rats from G6 (0.1 ml of IMI and 1ml of GAE simultaneously for 90 days) showed normal brain tissues (F). H&E stain, (X 200).

Discussion

The wide utilization of pesticides in agro-vet practices for the managing of diversity of pests resulted into environmental pollution [21], and their residues may persist in food stuff in a considerable amount causing possible health effects such as neurological dysfunctions, respiratory diseases, reproductive disorders and cancers [22,23].

There is proof that dietary supplementation with nutritional antioxidants could improve brain injury and cognitive function [24].

Ginger, the rhizome of *Zingiber officinale Roscoe* (a member belonging to the *Zingiberaceae* family) are commonly used as a spice or dietary supplement with a long history of utilization in the traditional medicine [25]. About 400 kinds of ingredients have been identified in the ginger, however, the pharmacological effects of ginger are largely attributed to the gingerols, shogaols, zingerone and paradols [26].

GABA is one of the typical neurotransmitters in the CNS, where it has a primarily inhibitory function. It is implicated in a variety of biological functions as learning, locomotor activity, circadian rhythms and reproduction [27].

The results of GABA showed that IMI exposure caused a significant decrease in GABA level. These results agree with that obtained by Abd-Elhakim *et al.* [28]. Administration of GAE simultaneously with IMI modifies the release of some neurotransmitters particularly GABA [29]. [30] Riyazi *et al.* has previously demonstrated that extract of ginger and its fractions have anti 5 hydroxytryptamine 3- receptor (anti-5HT3-receptor) effects. 5-HT3-receptor stimulation modulates the secretion of several neurotransmitters including GABA, the exocytosis of which is improved by direct Ca2+ influx through the ionophore of presynaptic 5-HT3-receptors [31, 29].

SDH is widely distributed in nearly all mammalian tissues, including the brain, liver, eye lenses and erythrocytes [32-33-34]**.**

Regarding to sorbitol dehydrogenase (SDH) values, it shows a significant increase in IMI-treated group than control. This result is in accordance with the result obtained by Lonare *et al.* [12] who observed that IMI induced damage to brain and some other organs. The simultaneous use of GAE with IMI restored the SDH to control values.

Ginger extract dropped serum SDH level in acetaminophen and carbon tetrachloride - induced hepatotoxicity [35].

Glutathione (GSH) is a chief antioxidant that inhibits oxidative injury and aids detoxification, It acts as a crucial co-factor for anti-oxidant enzymes like glutathione S-transferase (GST) and glutathione peroxidase (GPx) [36]. GSH is consumed by GSH dependent enzymes under oxidative stress to remove peroxides resulted from lipid peroxidation burden [37].

Regarding oxidative stress markers, there is a significant depletion in GSH content in rats exposed to IMI compared to control group. These results agree with that obtained by Duzguner and Erdogan [38] who reported decrease in GSH level in IMI-intoxicated rats due to consumption of GSH to overcome the oxidative stress induced by IMI Iintoxication. and also suggested that the decreased GSH level noticed in our current study may reflect GSH conjugation or oxidation of GSH to glutathione disulfide (GSSG) due to the IMI-induced generation of oxygen free radicals and their byproducts. This may be evidence for depressed antioxidant activity by imidacloprid. Many studies reported that pesticide exposure for long period's results in a significant reduction in GSH [39, 40].

The prophylactic use of GAE could normalize the GSH content to the control value. Ginger has antioxidant actions, which are attributed to gingerols, shogaols and other ketone-phenolic byproducts which have favorable results in fading ROS-induced CNS damage. Ginger administration diminishes monosodium glutamate-mediated neurotoxicity by numerous methods like restoration of the antioxidant enzymes as catalase (CAT) and superoxide dismutase (SOD), prevention of lipid peroxidation, improvement of GSH levels, scavenging of hydroxyl radical and inhibition of the NO production [41].

6-shogaol, one of active principals of ginger, inhibits the ROS production, but elevates the release of certain anti-oxidant components as GSH via the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) stimulation [42]. Further, 6-gingerol-rich element increase GSH production in the chlorpyrifos-exposed rats [43].

Superoxide dismutase (SOD) is an antioxidant enzyme, which act as preventative antioxidant and plays a vital role in the prevention of injurious effects of lipid peroxidation [44].

Our results indicated that SOD activity did not be affected by any treatment compared to control. These results agree with the results obtained by [38] who reported that SOD activity wasn’t affected after exposure to 1mg/kg b.wt. of 1/15 LD_{50} of IMI for 30 days. This could be explained by the increase in oxidant molecule production due to pesticide exposure inhibits antioxidant enzyme activity [45].

Although several studies prove elevation in intracellular antioxidants to compensate for the production of free radicals provoked by pesticide exposure [46], others have stated that the rise in oxidant molecule release hinders antioxidant enzyme action [47, 48].

Toll-like receptors (*TLRs*) are belonging to the membrane-related pathogen recognition receptors (PRRs) that are predominantly expressed by microglial cells in injured brain [49]**.** *TLRs* perform important functions in recognition of exogenous pathogen-associated molecular patterns (*PAMP*) and endogenous damage-associated molecular patterns (*DAMP*) [50]. Stimulation of *TLRs* by *PAMP* or *DAMP* lead to secretion of chemokines and cytokines which exaggerate the inflammatory responses [51].

The obtained qPCR data revealed significant upregulation of *TLR2* gene expression in the brain of rats administered IMI for 90 days as compared to the control groups. This upregulation can be resulted from gliosis induced by IMI as shown in histopathological findings of this study.

Regarding immunohistochemical activity of *TLR2*, cerebral cortex and hippocampus sections of rats administered IMI for 90 days showed intense immunopositive reactivity of *TLR2* and this is correlated to upregulation of *TLR2* gene by IMI treatment in our study. This could be explained by the fact that Chronic exposure to IMI induces inflammation [38] and it is well known that *TLR2* is included in innate immunity & inflammation pathways [52].

This increased expression was significantly downregulated by treatment with ginger with the lowest expression in G4 group. This could be explained by reduction the expression of *TLR2* by zingerone (one of ginger active constituents) [53].

Cerebral cortex sections of G4 group showed faint immunopositive reactivity while hippocampus sections showed mild immunopositive reactivity of *TLR2* as a result of protective effect of ginger aqueous extract.

Regarding histopathological findings in the brain of IMI-treated albino rats for 90 days, the brain revealed focal neuronal degeneration and neuronophagia beside hemorrhages, necrosis, Focal malacia and demyelination. Moderate to mild congestion of cerebral and meningeal blood vessels were noticed. Moreover, congestion and hyperplasia and degeneration in area of hippocampus, ependymal and choroid hyperplasia could be seen. Multifocal and/or diffuse gliosis with oligodendroglia aggregations, which partially replace the brain tissue. The microglial cells can secrete cytokines such as *TNF-α* [54] this explains intense immunoreactivity of *TNF-α* in IMI-intoxicated group resulted from gliosis, which noticed in our histopathological results.

These results are in accordance with that of Nellore *et al.* [55] who reported that oral administration of the IMI leads to histopathological changes in the brain regions and [56] who revealed vacuolation around neuronal cell body, chromatolysis and marked congestion after 28 days of administration of 80 mg/kg BW of IMI. Exposure of high dose of IMI for 60 days produced dead purkinji cells with loss of dendrites in brain of female rats [57]. Japanese quail exposed to IMI for 6 weeks showed similar histopathological lesions in brain [58] and layer chickens exposed to 139 mg/kg IMI [59]. There was focal gliosis in female and male mice brains [60]. Chronic exposure to IMI also prompts inflammation and oxidative injury in CNS of rats [38]. So we can also relate these pathological changes to oxidative damage caused by IMI. The oxidative stress disturb typical functions of the cell and interfere with the neural homeostatic condition, finally result in apoptosis [61]. Moreover, oxidative stress injures the mitochondria, which interrupt transportation of adenosine triphosphate through the axon, finally results in neuro-degeneration [62]. ROS also increase myelin breakdown, neuronal and axonal damage, and oligodendroglial injury [63].

The brain of all ginger-supplemented groups showed restoration in the structure in comparison to IMI-treated group especially the group administered ginger aqueous extract simultaneously with IMI because ginger has neuroprotective effect [64] and also decrease cell death and repair motor function in a rat spinal cord injury [65].

The rise in chemokines and cytokines is closely related with microglial activation, astrogliosis and axonal dysfunction [66], which provides proof for the relationship between activated immune response and brain pathology [67].

Conclusion

It could be concluded from the present study that oral exposure of rats to IMI has caused brain toxicity and changes in brain architecture. However, ginger aqueous extract succeeded to modulate the neurotoxic effect especially when administered simultaneously with IMI.

Conflict of interest

The authors have no conflict of interests to declare.

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الملخص العربي

التأثير العلاجي لمستخلص الزنجبيل المائي على التاثير العصبي السام للايميداكلوبريد علي الجرذان

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تم إعداد هذه الدراسة لتقييم التأثير السام للايميداكلوبريد على الجرذان وتخفيف الزنجبيل لهذا التأثير. تم استخدام ٦٠ ذكر ناضج من الجرذان البيضاء تم تقسيمهم إلي ٦ مجموعات (١٠ جرذان بكل مجموعة) كالآتي: المجموعة الضابطة السالبة (١ مل من الماء المقطر لمدة ٩٠ يوما)، المجموعة الضابطة الموجبة (١ مل من مستخلص الزنجبيل المائي لمدة ٩٠ يوما)، مجموعة تجرعت ب ٠.١ مل من الايميداكلوبريد لمدة ٩٠ يوما، المجموعة التي تم تجريعها ب ١ مل من مستخلص الزنجبيل المائي لمدة اسبوعين ثم تجريعها ب ٠.١ مل من الايميداكلوبريد لمدة ٩٠ يوما، المجموعة التي تم تجريعها ب ٠.١ مل من الايميداكلوبريد لمدة ٩٠ يوما ثم تجريعها ١ مل من مستخلص الزنجبيل المائي لمدة اسبوعين، و المجموعة الاخيرة تم تجريعها ب ٠.١ مل من الايميداكلوبريد و ١ مل من مستخلص الزنجبيل المائي معا لمدة ٩٠ يوما، التجريع عن طريق الفم لكلا من الايميداكلوبريد و الزنجبيل ثلاث مرات اسبوعيا. التعرض للايميداكلوبريد تسبب في نقص معنوي في مستوي الجاما امينو بيوتريك أسيد، زيادة معنوية في السوربيتول ديهيدروجينيز، نقص معنوي في الجلوتاثيون، لم يتأثر السوبر اوكسيد ديهيدروجينيز بالتعرض للايميداكلوبريد. التعرض للايميداكلوبريد زاد من التعبير الجيني لتول لايك ريسيبتور2 *TLR* ٢ في المخ، تفاعل مناعي موجب لكلا من التول لايك ريسيبتور في مخ الجرذان المعرضة للايميداكلوبريد. الدراسة الباثولوجية أظهرت تغيرات معنوية في نسيج المخ مثل التنكس في الخلايا العصبية، أنزفة، نخر، إزالة الميالين ووجود عدد كبير من الخلايا المدعمة. الزنجبيل يحسن من التأثير السام للايميداكلوبريد على الجهاز العصبي.