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THE POTENTIAL THERAPEUTIC VALUE OF GREEN TEA AND THYME AQUEOUS EXTRACTS ON IMIDACLOPRID TOXICITY IN RATS

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ABSTRACT

Imidacloprid (IMI) is the most extensively used neonicotinoid causing many adverse health effects. The use of natural antioxidants as an alternative medicine may play a crucial role to minimize the harmful effects of insecticides. Therefore, the present study was designed to evaluate the probable ameliorative effect of green tea aqueous extract (GAE) or thyme aqueous extract (TAE) against geno-oxidative and pathological disorders induced by IMI administered at 450 ppm in the diet for 3 months to rats. Intoxicated rats exhibited significant increase in serum ALT and AST activities. Significant increase of MDA level with significant reduction in GSH content and CAT activity in liver and brain were also recorded. Moreover, IMI induced DNA damage and several pathological alterations in liver and brain tissues. On the other hand, the concurrent exposure of rats to GAE or TAE alleviated all the toxic effects evoked by IMI. In conclusion, GAE and TAE may provide a promising therapeutic value against IMI and other neonicotinoid toxic insults.

KEYWORDS: Imidacloprid, Green tea, Thyme, DNA, Oxidative.

INTRODUCTION

The indiscriminate use of insecticides in agro-vet practices results in environmental pollution and adverse health effects.[1] Among them, neonicotinoids are a recent commercially favorable class of insecticides. Imidacloprid (IMI) is the most extensively used neonicotinoid insecticide which targets nicotinic receptors to control insects. Although, IMI has low mammalian toxicity, it exerts hepatic, neurogenic, mutagenic, teratogenic and reproductive toxic effects.[2-5]

The involvement of oxidative stress as one of the main molecular mechanisms of IMI- induced toxicity necessitated the use of natural herbal medicines as an alternative therapeutic and preventive strategy for attenuation of its oxidative damages.[6,7]

Green tea, *Camellia sinensis*, belongs to *Theaceae* family and is considered as one of the most popular beverages consumed all over the world. The health benefits of green tea are attributed mainly to its high contents of polyphenol catechins which have potent antioxidative, antimutagenic, anticancer and antiproliferative activities.[8] Numerous studies have reported that green tea has potent neuroprotective[9], nephroprotective[10] and hepatoprotective[11] effects. Also, green tea extract has antimicrobial[12] and antiatherosclerosis[13] properties.

Thyme, *Thymus vulgaris*, is an aromatic herb belongs to *Lamiaceae* family, it has health-promoting and disease-preventing properties due to its nutritional, biological and pharmacological values.[14] Thyme contains a variety of vitamins and minerals, as well as its flavonoids and phenolic contents.[15] Quantitatively, thymol and carvacrol are the main phenolic constituents of thyme which are responsible for its antibacterial and antioxidant properties.[16,17] Previous studies demonstrated the neuroprotective, hepatoprotective and nephroprotective effects of thyme.

In the light of this background, the present investigation was conducted to evaluate the probable therapeutic value of GAE and TAE against the geno-oxidative and pathological alterations induced by IMI in liver and brain of rats.

MATERIALS AND METHODS

Experimental Animals

Sixty healthy male albino rats (100-120g) obtained from AL-Zyade Experimental Animals Production Center, Giza, Egypt were used in this study. All rats were maintained in natural ventilated room (22±3 °C, 40-55% RH and natural daily dark/light cycle) in polypropylene cages with mesh wire tops. Rats were provided with standard commercial diet and clean tap water *ad libitum* for 2 weeks before the beginning of the experiment. The study was ethically approved by the International Animal Care and Use Committee IACUC, Faculty of Veterinary Medicine, University of Sadat City.

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Chemicals

Imidacloprid was commercially available as IMIDAMEX 70% wg, MAC-GmbH company-Germany. Green tea and thyme dry leaves were obtained from Harraz market, EL-Azhar, Cairo, Egypt and identified in Botany department, Faculty of Science, Cairo University. Diagnostic kits for assaying serum and tissue biomarkers were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. Other utilized chemicals were of analytical grade and commercially available.

Preparation of Plant Extracts

Green tea aqueous extract (GAE) and thyme aqueous extract (TAE) were prepared according to methods adopted by Mehana et al.[21] and Shalaby and Bahr,[22] respectively. About of 15 g. of green tea or thyme dried leaves were soaked in 1 liter of boiling water in a covered flask and left for 5 min for preparation of GAE and for 30 min for preparation of TAE. The extracts were cooled, filtered and provided daily to rats as their sole source of drinking water along the experimental period.

Experimental Design and Animal Grouping

Rats were weighed and randomly allocated into six equal groups. **Control group** was provided with free basal diet+tap water, **GAE group** was provided with free basal diet+GAE, **TAE group** was provided with free basal diet+TAE, **IMI group** was provided with diet containing IMI+tap water, **IMI+GAE group** was provided with IMI in diet+GAE and **IMI+TAE group** was provided with IMI in diet+TAE. IMI was given in the diet at 450 ppm which is equivalent to 1/10 LD50[23] for 3 months while GAE and TAE were provided to rats as their sole source of drinking water along the experimental period.

Samples Collection

At the end of the experimental period, rats were fasted overnight, anaesthetized and sacrificed for samples collection. Blood samples were collected from all animals in clean and dry glass centrifuge tubes for serum collection and then stored at -20°C for further serum biochemical analysis. Parts of liver and brain of each rat were excised and stored in ice bags at -20°C for tissue biochemical analysis, while other parts were placed in cold phosphate buffer saline (PBS) for comet assay. Additional parts of liver and brain were kept in 10% neutral buffered formalin solution for the histopathological investigation.

Biochemical Analysis

Serum ALT and AST activities were estimated according to method of Reitman and Frankel.[24] Malondialdehyde (MDA) level,[25] reduced glutathione (GSH) content [26] and catalase (CAT) activity [27] were measured in liver and brain tissue homogenates following manufacturer's instructions of their kits.

Comet Assay

DNA damage of liver and brain cells was examined using the modified method of Ellahueñe et al.[28] The prepared slides were stained with Ethidium bromide, and about 100 randomly selected cells in each slide were photographed and scanned. The data were analyzed with DNA damage analysis software (Loats Associates Inc., USA). The degree of DNA damage was determined by estimation of tail length and DNA% in tail and calculation of tail moment (tail length × DNA% in tail /100).

Histopathological Examination

The fixed liver and brain samples were prepared and stained with H&E stain according to Bancroft et al.[29]

Statistical Analysis

Values are presented as mean ± standard error (SE). Statistical significance of data was determined by one-way ANOVA (Analysis of Variance) followed by Duncan's Multiple range test for post hoc analysis. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) Version 16 released on 2007.

RESULTS

Biochemical Findings

Serum liver function markers

In control, GAE and TAE groups, no significant changes (*P<0.05*) were recorded concerning ALT and AST activities. Exposure of rats to IMI in diet for 3 months at 450 ppm induced significant elevation of serum ALT and AST activities compared to control group. Concurrent administration of either GAE or TAE with IMI restored the normal control values of ALT and AST activities (Fig. 1).

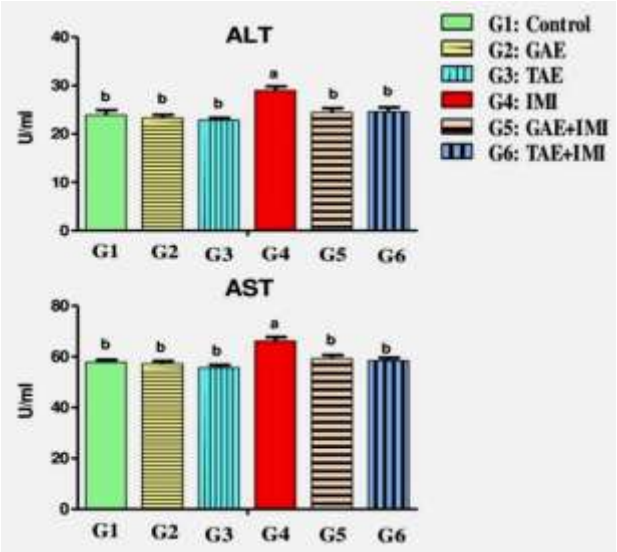

Fig. 1: Serum ALT and AST activities of the different groups. Different letters indicate significant differences at (P<0.05). GAE: green tea aqueous extract; TAE: thyme aqueous extract; IMI: imidacloprid.

Tissue Oxidant/Antioxidant Markers

Concerning the oxidant/antioxidant status, no significant variations (*P<0.05*) were observed in MDA level, GSH content and CAT activity in liver and brain of GAE and TAE groups compared to control group. Rats exposed to IMI alone showed significant increase (*P<0.05*) in liver and brain MDA level associated with significant reduction in GSH content and CAT activity, compared to control. However, these changes were completely ameliorated in GAE+IMI and TAE+IMI groups and returned to be within normal ranges (Fig. 2).

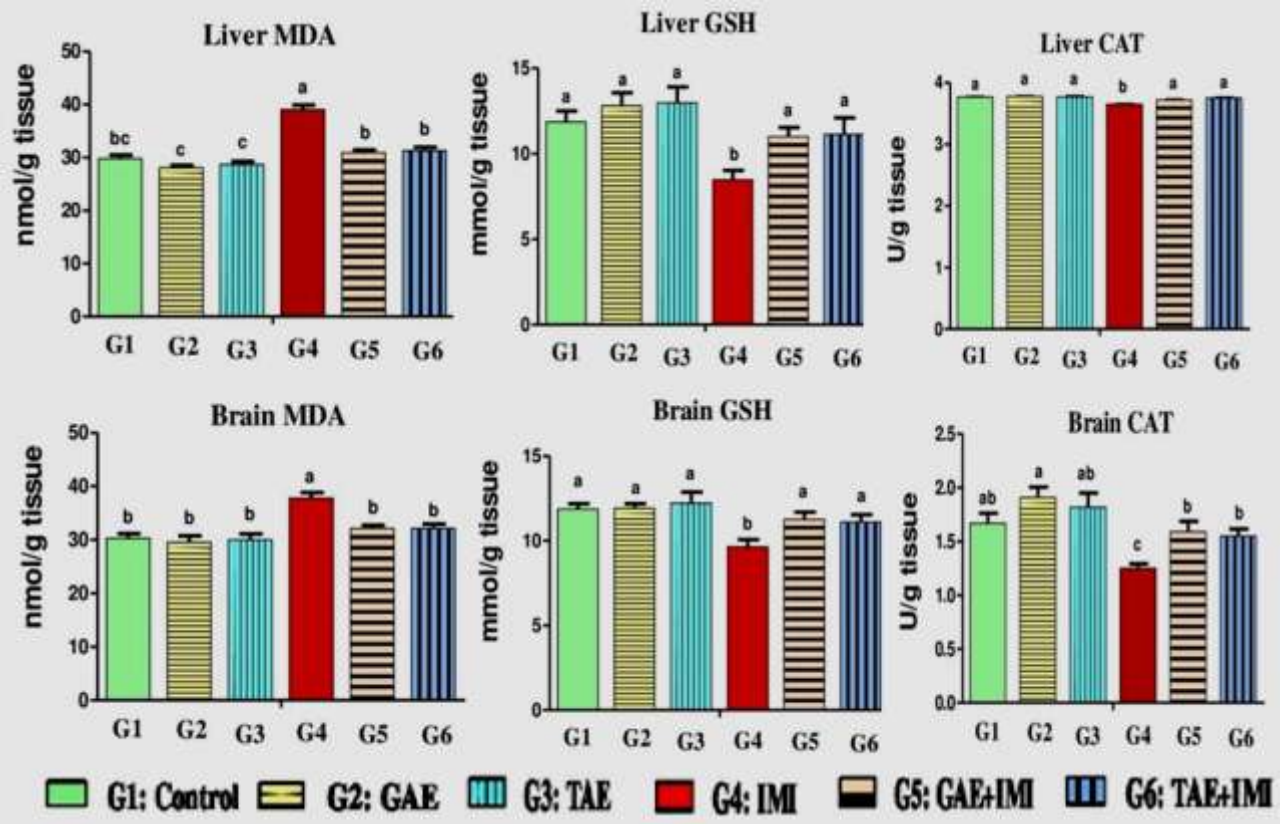

Fig. 2: Malondialdehyde level, reduced glutathione content and catalase activity in liver and brain of the different groups. Different letters indicate significant differences at (*P<0.05*). GAE: green tea aqueous extract; TAE: thyme aqueous extract; IMI: imidacloprid.

Comet Assay Findings

DNA damage in liver and brain cells are presented in Table (1) and Fig. (3), where no significant differences (*P<0.05*) in the mean values of tail length, tail DNA% and tail moment were noticed between control, GAE and TAE groups. Significant elevation of these parameters in liver and brain cells were recorded in IMI- exposed rats, compared to control group. On the other hand, significant amelioration in the elevated comet parameters in liver and brain cells were recorded in GAE+IMI and TAE+IMI groups, compared to IMI group. However, these values were still significantly higher (*P<0.05*) than normal control values.

Table 1: Mean values of comet assay parameters in liver and brain cells of the different groups.

	Liver			Brain		
	Tail Length (µm)	**Tail DNA%**	**Tail Moment**	**Tail Length (µm)**	**Tail DNA %**	**Tail Moment**
Control	3.42±0.209^{c}	3.92±0.336^{c}	0.14±0.018^{c}	5.83±0.367^{c}	4.8±0.390^{c}	0.28±0.028^{c}
GAE	4.43±0.379^{c}	2.72±0.451^{c}	0.12±0.022^{c}	5.64±0.302^{c}	3.68±0.307^{c}	0.20±0.011^{c}
TAE	4.21±0.246^{c}	2.64±0.315^{c}	0.11±0.010^{c}	5.33±0.251^{c}	4.04±0.399^{c}	0.21±0.019^{c}
IMI	13.93±0.984^{a}	8.75±0.753^{a}	1.23±0.156^{a}	18.04±2.234^{a}	24.75±2.452^{a}	4.37±0.565^{a}
GAE+IMI	7.70±0.285^{b}	5.40±0.404^{b}	0.42±0.040^{b}	11.95±0.499^{b}	9.94±1.011^{b}	1.18±0.115^{b}
TAE+IMI	6.66±0.305^{b}	5.29±0.513^{b}	0.35±0.029^{b}	9.66±0.792^{b}	11.79±0.859^{b}	1.17±0.175^{b}

Values are presented as mean ± SE (n= 5). Means with different letter superscripts in the same column are significantly different at (P<0.05).GAE= green tea aqueous extract; TAE= thyme aqueous extract; IMI= imidacloprid.

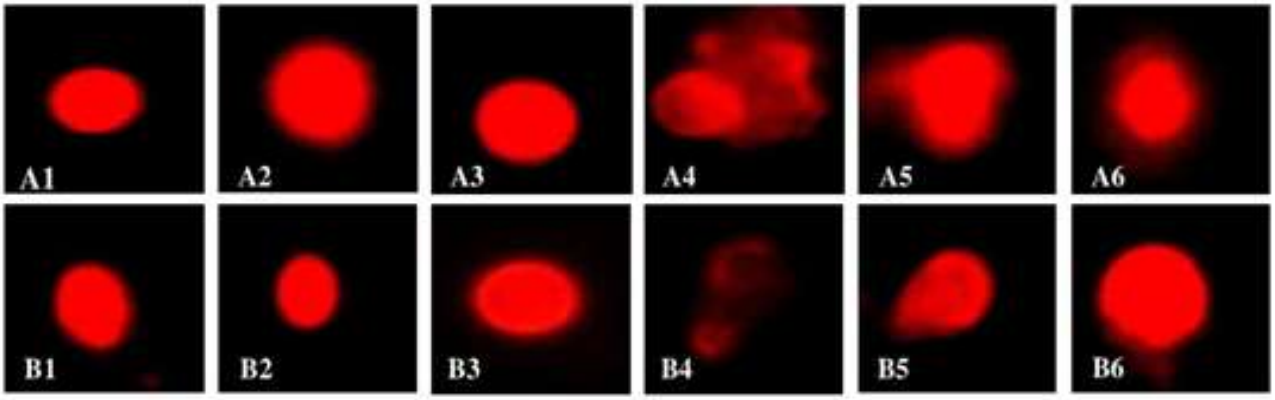

Fig. 3: Representative Comet images of liver (A) and brain (B) cells of the different groups, Ethidium bromide stain (X400). 1: control group, 2: GAE group, 3: TAE group, 4: IMI, 5: GAE+IMI and 6: TAE+IMI.

Histopathological Findings

Liver of control (Fig. 4A), GAE (Fig. 4B) and TAE (Fig. 4C) groups showed normal hepatic histological architectures. Liver sections from IMI- intoxicated rats showed degeneration of the wall of central vein (CV) with thrombus formation in CV, dilatation of blood sinusoids with disorganization of hepatic cords (Fig. 4D). Co-administration of either GAE (Fig. 4E) or TAE (Fig. 4F) with IMI protected the hepatic morphology and improved the observed degenerative changes caused by IMI.

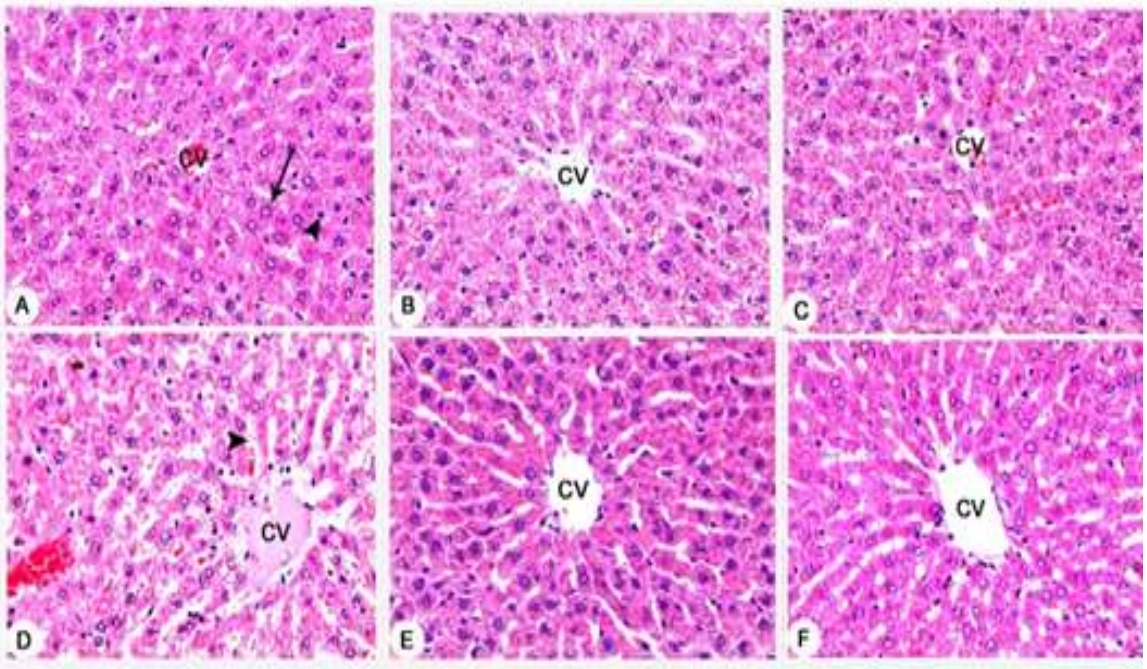

Fig. 4: Photomicrograph of liver of the different groups (H&E, X20). A (control), B (GAE) and C (TAE) groups: showing normal histological architectures, CV (central vein); hepatocytes (arrow); Kupffer cell (arrowheads). D (IMI group): revealing degeneration of CV wall with thrombus formation, and dilatation of blood sinusoids (arrowhead) with disorganization of hepatic cords. E (GAT+IMI group) and F (TAE+IMI group): showing normal hepatic morphology.

Sections of cerebellum obtained from control (Fig. 5A) and GAE- (Figs. 5B) and TAE- (Fig. 5C) treated rats showed normal morphology. Degeneration of neurocytes of granular layer (GL) with degeneration and loss of Purkinje cell layer (PL) were observed in IMI- exposed rats (Fig. 5D). Cerebellum showed only degeneration and necrosis of PL in GAE+IMI group (Fig.5E). In TAE+IMI group, cerebellum was completely protected from the degenerative effects of IMI and kept its normal histological architectures (Fig. 5F).

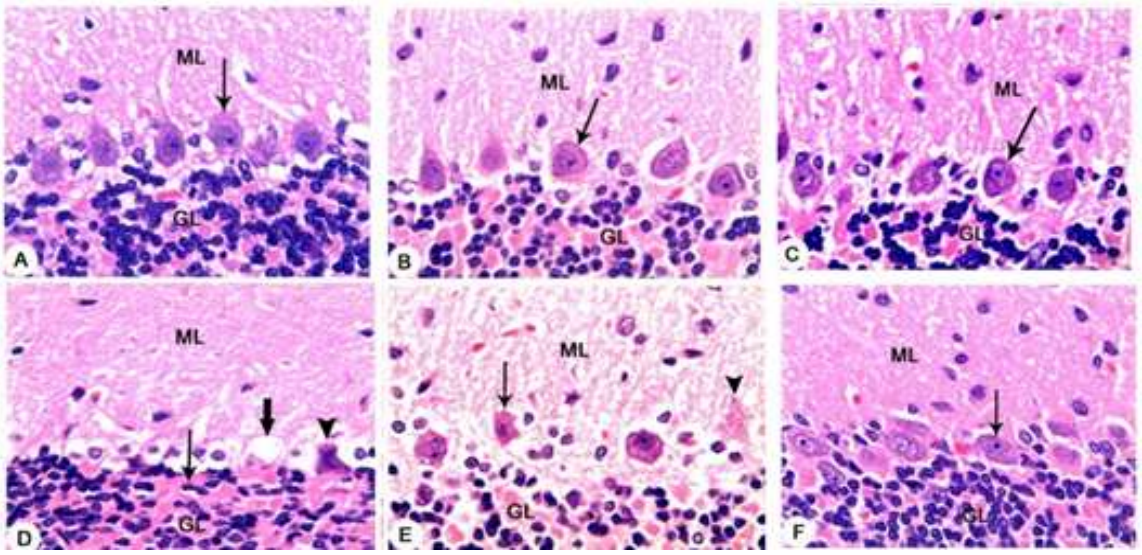

Fig. 5: Photomicrograph of brain cerebellum of the different groups (H&E, X40). A (control), B (GAE) and C (TAE) groups showing normal histological architectures: ML, molecular layer; GL, granular layer; arrow indicating Purkinje cell layer (PL). D (IMI group): showing degeneration of neurocytes of GL (thin arrow), degeneration (arrowhead) and loss (thick arrow) of PL. E (GAE+IMI group): showing degeneration (arrow) and necrosis (arrow head) of PL. F (TAE+IMI group): showing normal histological architectures.

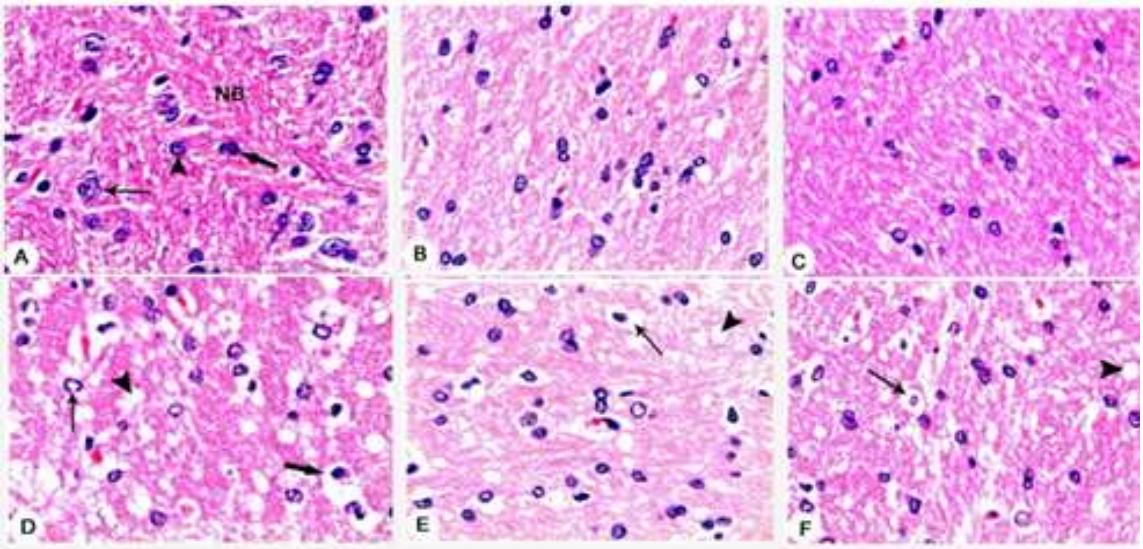

Fig. 6: Photomicrograph of brain cerebrum of the different groups (H&E, X40). A (control), B (GAE) and C (TAE) groups: showing normal histological architectures: NB, nerve bundles; neuron (thin arrow); oligodendrocyte (thick arrow); astrocyte (arrow head). D (IMI group): showing necrosis of astrocyte (thin arrow), hydropic degeneration of oligodendrocyte (thick arrow) and vacuolar degeneration of nerve bundles (arrowhead). E (GAE+IMI group) and F (TAE+IMI group): showing hydropic degeneration of oligodendrocyte (arrow) and vacuolar degeneration of nerve bundles (arrowhead).

The obtained cereberum photomicrographs of control (Fig.6A), GAE (Fig.6B) and TAE (Fig.6C) groups showed normal histological architecture. Rats intoxicated with IMI revealed degenerative changes in the cerebrum in the form of necrosis of astrocytes, hydropic degeneration of oligodendrocytes and vacuolar degeneration of nerve bundles (Fig.6D). Slight improvement was observed in GAE+IMI (Fig.6E) and TAE+IMI (Fig.6F) groups where hydropic degeneration of oligodendrocytes and vacuolar degeneration of nerve bundles were noticed.

Normal histological architecture of medulla oblongata was noticed in the control (Fig.7A), GAE (Figs.7B) and TAE (Figs.7C) groups. Medulla oblongata of IMI-exposed rats revealed neuronal necrosis and perineuronal edema around dead neuron (Fig.7D). The IMI+GAE group showed only perineuronal edema around necrotized neurons (Fig.7E), while TAE prevented the neurodegenerative effect of IMI and the medulla oblongata appeared normal (Fig.7F).

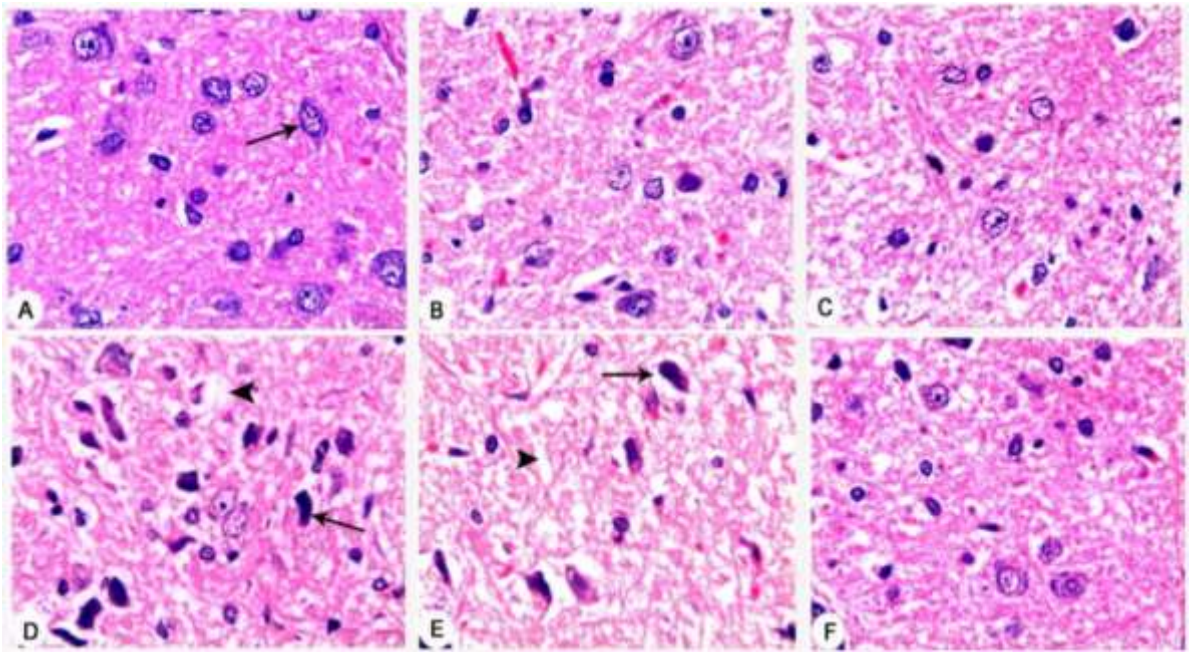

Fig. 7: Photomicrograph of brain Medulla Oblongata of the different groups (H&E, X40). A (control), B (GAE) and C (TAE) groups: showing normal histological architectures: Neuron (arrow). D (IMI group): showing neuronal necrosis (arrow) and perineuronal edema around dead neuron (arrowhead). E (GAE+IMI group) showing perineuronal edema around necrotized neuron (arrow). F (TAE+IMI group): showing normal histological architectures.

DISCUSSION

Neonicotinoids are a recent class of insecticides, which had been developed since the early 1990s to replace the other more harmful insecticides such as organochlorines, organophosphates and carbamates.[30] Imidacloprid (IMI), a broad-spectrum neonicotinoid insecticide, is one of the top selling insecticides worldwide resulting in continuous animal and human exposure and hence, induces toxic health hazards.[31]

Several investigations have plainly proved the implication of oxidative stress in IMI- induced toxicity[6,7] suggesting the prospective role of natural plants with antioxidant properties to protect against IMI-induced toxic effects. The flavonoids and polyphenolic contents of green tea and thyme extracts[32,33] raised the attention for their possible use as natural antioxidants against pesticide- induced oxidative damage.[15,34] Depending upon this background, the present study aimed to evaluate the probable therapeutic value of green tea and thyme aqueous extracts against the geno-oxidative and pathological alterations induced by IMI in liver and brain of rats.

ALT and AST are the most widely used liver biomarkers. Our findings revealed that exposure of rats to IMI produced significant elevations in serum ALT and AST activities. In accordance with our results, Mehmood et al.[5] and Chakroun et al.[35] reported that exposure of rats to IMI resulted in significant increase in the activities of liver enzymes. The recorded elevation in serum ALT and AST activities could be attributed to the recorded oxidative damage of hepatocellular membrane and disturbance in its permeability resulting in their release into blood stream.[36]

Oxidative biomarkers assessment and comet assay along with histopathological investigation of liver and brain tissues can be used to evaluate the potential hepatotoxic and neurotoxic effects of insecticides and may help to estimate the potential therapeutic and protective role of natural antioxidants against such toxic effects.

Oxidative stress is the disturbance of the oxidant/antioxidant balance resulting in oxidation of lipids, proteins and DNA in the cells and finally, tissue damage.[37] It usually occurs due to free radical overproduction and reduction of the intracellular enzymatic and non-enzymatic antioxidants[38] leading to lipid peroxidation, alternations in membrane permeability, DNA damage and different pathological alterations.

MDA, a lipid peroxidation product together with GSH and CAT, endogenous antioxidants, are valuable indicators of oxidative tissue damage. Our results revealed significant elevation of MDA level in liver and brain of IMI- intoxicated rats concurrently with reductions of GSH content and CAT activity. Our findings are consistent with previous studies of Ahmed and Nasr[4] and Mehmood et al.[5] Also, Kapoor et al.[39] demonstrated oxidative stress in rats exposed to IMI at 20 mg/kg for 90 days evidenced by elevation of MDA level of liver and brain with reduction of GSH content and CAT activity.

The recorded increase of MDA level concurrently with reduction of GSH content and CAT activity in liver and brain of IMI- exposed rats may be attributed to the ability of IMI to increase the generation of free radicals causing peroxidation of polyunsaturated fatty acids resulting in degradation of phospholipids, cellular deterioration as well as reduction of endogenous antioxidants and consequently, oxidative tissue damage.[39] These findings may help to explain the recorded elevation of serum ALT and AST activities and the observed DNA damage and pathological changes in liver and brain of IMI- intoxicated rats.

Comet assay is a simple, sensitive and valuable test used for measuring the degree of DNA damage in tissues exposed to genotoxins. Our results demonstrated the genotoxicity of IMI evidenced by significant elevation of tail length, tail DNA% and tail moment in liver and brain cells. Previous *in vivo* and *in vitro* studies confirmed the genotoxic effect of IMI.[4,40] It is possible that IMI produces its genotoxic effects through an oxidative stress mechanism. These findings are entirely consistent with the histopathological alterations observed in liver and brain of IMI- exposed rats that also recorded in previous studies.[5,6]

Concerning the protective potential of green tea and thyme aqueous extracts, exposure of rats to either GAE or TAE ameliorated the toxic effects of IMI as evidenced by reduction in serum ALT and AST activities as well as reduction of MDA level concomitantly with elevation of GSH content and CAT activity in liver and brain. Moreover, GAE or TAE ameliorated the observed DNA damage and histopathological changes in liver and brain tissues.

Several studies confirmed the hepato-protective[34,41] and neuro-protective[9,42] effects of green tea. Furthermore, GAE possesses geno-protective and anti-apoptotic properties.[43] The protective effect of green tea could be attributed to its high content of the antioxidant polyphenol catechins,[44] in addition to the antioxidant vitamins and minerals. These can scavenge ROS and activate the antioxidant enzymes and inhibit the lipid peroxidation.[32,45]

Also, many investigations have demonstrated the antioxidant and protective effect of thyme against toxicants such as acetaminophen-, aflatoxin- and carbon tetrachloride- induced hepatotoxicity [19,46,47] and against alcohol- induced neurotoxicity.[48] Also, the anti-genotoxic effect of thyme extract was recorded by El-Sayed and Ramadan.[49] The protective antioxidant activity of thyme is mainly attributed to the presence of phenolic monoterpenes, thymol and carvacrol, as the major components of essential oil of thyme. Also, phenolic acid (rosmarinic acid) and flavonoids are suggested to be the polyphenolic compounds that responsible for the antioxidant properties of thyme aqueous and ethanolic extracts.[33]

CONCLUSION

On the basis of our findings it can be concluded that, exposure to IMI induced geno-oxidative damages together with histopathological alterations in liver and brain of intoxicated rats. All these toxic effects were abolished by coadministration of GAE or TAE. Therefore, GAE and TAE may be used as a new therapeutic and preventive strategy against IMI and other neonicotinoids insults.

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