Thiamethoxam evoked neural oxido-inflammatory stress in male rats through modulation of Nrf2/NF-kB/iNOS signaling and inflammatory cytokines: neuroprotective effect of silymarin.

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Abstract

Thiamethoxam, a neonicotinoid insecticide, is a widely used insecticide with neurotoxic potential. This study explored the neuroprotective effects of silymarin on thiamethoxam-triggered cortical injury in male rats. Animals were divided into four groups and treated daily either with SM (150 mg/kg), TMX (78.15 mg/kg), or both at the aforementioned doses for 28 days. Our results revealed marked declines in cortical SOD and CAT activities with elevations in MDA, IL-1b and TNF-α levels in thiamethoxam-treated rats. Further, thiamethoxam induced down-regulation in gene expressions of *Sod, Cat, Gpx*, and *Nrf-2* genes, with up-regulation in the expression of *IL-1b, IL-6, iNOS, TNF-α and NF-kB* genes. Interestingly, pre-treatment with SM provided a notable neuroprotective action against TMX-mediated cortical damage that indicates its promising antioxidant and anti-inflammatory activities. This effect may be mediated by Nrf2/NF-kB/iNOS signalling and suppression of excess free radicals and production of inflammatory cytokines.

Practical Applications

The using of neonicotinoids as thiamethoxam is recently increased and is associated with brain damage. TMX induced excessive oxidative and inflammatory damage. Therefore, new therapeutic approaches are needed to counteract its adverse effects on the nervous system. Silymarin (SM), a flavonoid, which is extracted from the seeds and fruits of milk thistle. Due to its potent antioxidative activity, SM have been applied to mitigate oxidative stress as well as inflammatory disorders. Herein, we examined the potential therapeutic role of SM against TMX-induced brain oxidative stress and inflammation in rats through evaluating oxidative markers, inflammatory response, and histopathological changes in the brain cortical tissue.

1. Introduction

Neonicotinoids is a new major class of insecticides that have developed in the last three decades with similar structure and mode of action to nicotine (1). They are used in agriculture to protect different crops against pests as well as to protect animals from fleas, lice and flies. Besides, they are used for pest control in homes and gardens (2). They possess great environmental health concern due to their high-water solubility, long half-lives and persistence in water, soil and biota (2). Thiamethoxam (TMX) is a synthetic neonicotinoid with a potent insecticidal activity (3, 4). The insecticidal action of TMX is endorsed for its nicotinic agonist on nicotinic acetylcholine receptors (nAChRs) (5). However, TMX is highly selective for the insect nAChRs more than the mammalian, it is more selective for the mammal nAChRs after being metabolized (5).

Based on its mode of action, TMX toxicity is considered to be centrally mediated as the signs are similar to those of nicotine (1). Ford and Casida (6) found that at least 44% of TMX is metabolized after administration to mice and the brain is the location of its main metabolites. Clothianidin (CLO) is the metabolic product of TMX and persist in the brain (3). Both TMX and CLO are structurally similar, but CLO is more lipophilic than that of TMX (5). During its metabolism, the nitroguanidine moiety of TMX is cleaved enzymatically and reduced to nitrosoguanidine and aminoguanidine. The resulted metabolites are cationic

in nature and accordingly selective for the mammalian AChRs with consequent potential effect on mammalian CNS (7). Former reports have shown that TMX-induced toxicity is strongly related to oxidative stress with consequent damage of cell membrane lipids manifested by increased lipid peroxidation, as well as on DNA damage (3, 8, 9). As well, notable suppression of antioxidant enzymatic activities were also detected in investigations of TMX toxicity (3, 10).

Owing to its high levels of polyunsaturated fatty acids and relative low content of antioxidative enzymes, brain is greatly susceptible to reactive oxygen species and oxidative stress. Tremendous research attention has been devoted to investigating the possible neuroprotective impact of phytochemical agents against insecticide-induced brain damage and oxidative stress. Silymarin (SM), a flavonoid, which is extracted from the seeds and fruits of milk thistle. Mainly, it is composed of silibinin in addition to other stereoisomers, as isosilybin, dihydrosilybin, silydianin, and silychristin (11). The presence of methoxy moiety attached to the phenolic rings in its polyphenolic structure render SM a powerful antioxidant molecule (12, 13). The free radical scavenging potential of SM was reported to exceed that of vitamin E (13). Former reports have revealed the SM's antioxidant potential and ability to counteract tissue injury as hepatotoxicity (13), hepatic fibrosis (14) and skin diseases (15). The neuroprotective efficacy of SM was noticed on delayed neuronal cell death in hippocampus of rat underwent surgical ischemia (16). Further, Thakare et al. (17) found notable antioxidant effect of SM in cerebral cortex and hippocampus of mice with acute restraint stress. Significant improvement of cerebellum antioxidant status were observed in rats exposed to manganese and co-treated with SM (18).

Imbalance in redox status in neural cells was reported to be modulated by crucial transcription factors as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB) (19, 20). As a vital redox sensor, the (Nrf2) protects the cells against oxidative stress (21). Under oxidative stress conditions, it binds to the antioxidant response element (ARE) with consequent stimulation of antioxidant enzymes as well as their encoding genes (22). Numerous dietary phytochemicals exerted their cytoprotective role via stimulation of Nrf2/ARE pathway and hence averting the deleterious effects of ROS (23, 24). NF-κB, is a sensitive transcription factor which mediates the neural inflammation via regulating the transcription of proinflammatory mediators as TNF-α, IL-1β and inducible nitric oxide synthase (iNOS) (25). SM provided notable protection against docetaxel-induced neurotoxicity by reducing oxidative stress, inflammation through enhancement of the expression of Nrf2 with downregulation of Nf-kB (26). Similar findings were reported in rats with acute hepatic toxicity induced by thioacetamide and co-treated with SM (23).

The toxic effect of TMX on the nervous system of rats, up to our knowledge, is poorly investigated. Therefore, in the current study, the neurotoxic effect of TMX on brain cortex of exposed rats and the underlying possible mechanisms were investigated as Nrf2, NF-kb. As well, the ameliorative effect of pre-treatment with SM on TMX-induced neural changes as oxidative stress, inflammation and histological alterations were also explained.

2. Materials And Methods

2.1. Animals and ethical statement

In our study, ten-week-old adult male albino rats (160 ± 20 g) were reared under controlled environmental conditions of 22–25 °C temperature with a 12-h light-dark cycle. During the acclimation period, they had free access to water and food. The experimental design and animal handling protocols were approved by research Ethics Committee, Faculty of Veterinary Medicine, Egypt (Approval number; R/28).

2.2. Experimental design

Rats were allocated into 4 equal groups (6 rats per each) and orally received the following treatments:

Control group: animals received normal saline.

TMX group: animals were administrated TMX (78.15 mg/kg bwt) (4) This dose was selected as a one-twentieth of the oral TMX LD_{50} in rats which is 1,563 mg/kg/b.wt (27).

SM group: animals were administrated SM at 150 mg/kg bwt (28).

SM + TMX group: animals were administered both the aforementioned SM and TMX doses. SM was administered 1 hour before TMX.

All treatments were administered on a daily basis for 4 weeks. At 24-h after the last treatment, rats were euthanized, by an overdose of pentobarbital (400 mg/kg bwt) via an intraperitoneal injection (3) and brain cortex was rapidly excised and separated to three portions. The first portion was immediately homogenized in ice-cold 10 mM phosphate buffer (pH 7.4) to prepare a 10% (w/v) homogenate, which was centrifuged at 4 °C for 10-min. The supernatant was used for performing the biochemical analyses. The second portion was fixed for histopathological examination of the cortical tissue. The third portion was frozen in liquid nitrogen and stored at − 80°C for RNA extraction.

2.3. Assessment of cortical oxidant/antioxidant capacity

Lipid peroxidation in brain cortex was determined in terms of malondialdehyde (MDA) according to a previously reported protocol (29). Moreover, the enzymatic antioxidant activities of superoxide dismutase (SOD) and catalase (CAT), were assessed following the methods of Mishra et al. (30) and Aebi (31), respectively.

2.4. Assessment of cortical inflammatory markers

In order to evaluate the neuroinflammation in brain tissue treated with TMX and/or SM, pro-inflammatory markers as tumor necrosis factor-α, and interleukin-1β were measured by ELISA kits obtained from Thermo Fisher Scientific, USA as mentioned by the manufacturer's instructions for TNF-α (Cat. no. BMS607-3) and IL-1β (Cat. no. BMS6002).

2.5. RNA extraction

Total RNA was extracted from brain using Trizol reagent according to the manufacturer's instructions (Direct-zol™ RNA MiniPrep, catalog No. R2050). The quantity and purity were measured by using a Nanodrop (UV-Vis spectrophotometer Q5000/USA) and the integrity was evaluated by gel electrophoresis.

2.6. Reverse transcription

The cDNA of synthesis was done following the manufacture protocol (SensiFast™ cDNA synthesis kit, Bioline, catlog No. Bio- 65053). The reaction mixture was carried out in a total volume 20 μL consisted of total RNA up to 1μg, 4 μL 5x Trans Amp buffer, 1 μL reverse transcriptase and DNase free water up to 20 μL. The final reaction mixture was placed in a thermal cycler and the following program was carried out; primer annealing at 25 C^0 for 10 min, reverse transcription at 42 C^0 for 15 min followed by inactivation at 85 C^0 for 5 min. The samples were held at 4 C^0.

2.7. q-RT-PCR

Relative quantification of mRNA levels of *IL6, IL-1β, TNF-α, iNOS, NFKB, CAT, SOD, GPX, and Nrf2* in rat brain was performed by RT-PCR using SYBR Green PCR Master Mix (2x SensiFast™ SYBR, Bioline, catlog No. Bio-98002). Primer sequences and the size of each amplified PCR product are shown in Table (1). *β-Actin* was used as housekeeping gene. The reaction mixture was carried out in a total volume 20 μL consisted of 10 μL 2x SensiFast SYBR, 3 μL cDNA, 5.4 µl H_2O (d.d water), 0.8 µl of each primer. The PCR cycling conditions were as follows: 95C^0 for 2 min followed by 40 cycles of 94 C^0 for 15 s, annealing temperatures as shown in Table (1) for 30 s, and 72 C^0 for 20 s. At the end of the amplification phase, a melting curve analysis was performed to confirm the specificity of the PCR product. The relative expression of the gene in each sample versus a control in comparison to b-actin gene and calculated according to the $2^{-\Delta\Delta Ct}$ as previously described by Pfaffl (32).

2.8. Histopathological screening

Brain cortex samples were fixed for 24 hours at room temperature in 10% neutral-buffered formalin. They were then dehydrated in alcohol, cleared in xylene, embedded in paraffin wax, and sectioned to 5-µm thick sections. The sections were stained with hematoxylin and eosin (H&E) and examined with a light microscope. Images were obtained at an initial magnification of 400× (Nikon Eclipse E200-LED, Tokyo, Japan) (33).

2.9. Statistical analysis

Data are displayed as the means ± standard error of the mean values (SEM). Data were analyzed using one-way analysis of variance (ANOVA) and post hoc Duncan's multiple comparison test. P values < 0.05 indicated statistical significance between different groups.

3. Results

Ameliorative Effect of SM on TMX-induced oxidant/antioxidant status imbalance in brain cortex

The effect of different treatments on the enzymatic antioxidant activities in brain tissue is illustrated in Fig. 1. Non-significant difference was detected in SOD and CAT activities among SM and control groups. However, brain of TMX-exposed rats showed noteworthy declines in activities of SOD and CAT (P < 0.05) compared with control rats. Adversely, pre-treatment of rats with SM displayed notable higher activities for

both enzymes ($P < 0.05$) if compared with TMX-treated rats. Concerning the MDA levels, no observed difference was detected in its level between the control and SM groups. Nevertheless, rats in TMX group had higher level of MDA ($P < 0.05$) than those in the control group. Interestingly, SM pre-treatment lessened markedly ($P < 0.05$) MDA levels relative to TMX which indicate SM antioxidant potency.

For further understand the antioxidant potential of SM against TMX-induced neural stress, we investigated the levels of oxidative stress biomarkers on the molecular level. Gene expression results of *GPx, Nrf2* and *cat* revealed non-significant changes between SM and control groups. SM-treated rats had higher *sod* gene expression ($P < 0.05$) than those in control group. Contrastingly, rats received TMX exhibited noteworthy downregulation ($P < 0.05$) in gene expression of all tested genes compared with control rats. Moreover, the combined treatment of SM and TMX restored markedly ($P < 0.05$) the TMX-mediated suppression in the antioxidant biomarkers-related gene expression (Fig. 2).

Ameliorative Effect of SM on TMX-induced inflammation in brain cortex

Several inflammatory mediators contribute to the development of neuroinflammation and neurotoxicity. Our results revealed marked increases ($P < 0.05$) in the levels of inflammatory cytokine as IL-1β and TNF-α in rat brain tissue in respect to control rat brain tissue. Nevertheless, in group received both SM + TMX, the level of IL-1b was significantly lesser than those in group treated with TMX (Fig. 3).

Remarkably, TMX toxicity modulated the gene expression of neural inflammatory biomarkers. Significant up-regulations ($P < 0.05$) in the expression of *IL-6, IL-1β, TNF-α, iNOS*, and *NF-kB* genes were observed in the TMX-treated group, in respect to the control one. The ameliorative effect of SM was noticed in TMX + SM group, as demonstrated by marked down-regulation of the *IL-6, TNF-α* and, *iNOS* compared to TMX intoxicated group. However, SM administration did not alter the expressions of *IL-1β* and *NF-kB* in TMX-treated group. Non-significant differences were noticed in the fold change of *IL-1β, TNF-α, and iNOS* in SM group when compared to control group. SM-treated group had lower expression levels of *IL-6*, and *NF-kB* than those of control rats (Fig. 4).

Ameliorative Effect of SM on TMX-induced pathological changes in brain cortex

H&E of brain was used to detect the histological alteration induced by TMX and SM compared to control group (Fig. 5). The histological architecture of brain in the control group was normal (Fig. 5A). Numerous neuronal deaths with shrinkage of neuron and increase the space around were observed in TMX-treated group. Additionally, infarction of blood vessels with widespread edema in neuropil beside increased perivascular space and multifocal gliosis were also detected (Fig. 5B, 5C and 5D). Meanwhile, SM group showed mild pathological changes represented by mild neuropil edema (Fig. 5E). In contrast, SM treatment alleviated noticeably the pathological effect of TMX indicated by most neuron had the normal architecture with a few perivascular space (Fig. 5F).

4. Discussion

The growing evidence of neurotoxic effect of neonicotinoids, there is an urgent need for better understanding of their adverse effects on nontarget organisms. The nervous system is the main target of neonicotinoids action and alterations in antioxidant enzyme activities are implicated as a mechanism of their neurotoxicity (2, 34, 35). The brain is highly vulnerable to oxidative injury owing to its high oxygen consumption rate as well as its high polyunsaturated fatty acid content (26). In this context, we designed this study to evaluate the impact of consecutive 28-day exposure to TMX and SM on modulation of brain oxidative stress and neuroinflammation in adult male rats. The results revealed that TMX induced notable oxidative damage in brain of exposed rats demonstrated by marked decline in antioxidant enzymatic activities of SOD and CAT together with downregulation in gene expression of *Sod, Cat* and *Gpx* in respect to the control rats. SOD and CAT play a critical role in scavenging of the superoxide anion (O_2^-) by converting it into a less toxic hydrogen peroxide (H_2O_2) compound, whereas the CAT enzyme dissociates it into water and oxygen. Further, GPx contributes in the depletion of H_2O_2 by converting glutathione into glutathione disulfide (25). Induction of excess free radicals and oxidative damage to cellular components were reported formerly in liver tissue after TMX exposure (8, 9). Our results are in harmony with Lonare et al. (36) who stated that imidacloprid caused significant decrease in antioxidant enzymes activities and non-enzymes levels in rat brain. However, other studies revealed marked declines in glutathione levels in brain tissue with increases in enzymatic activities of SOD, CAT and GPx in male rats exposed to low doses of imidacloprid (2, 37). Such increases in the intracellular enzymes may be endorsed for the compensatory mechanism of brain cells against imidacloprid exposure. Similar findings were reported in rat cerebellum exposed to acetamiprid (34). In our study, suppression of antioxidant activities indicates the notable exhaustion of the endogenous antioxidant system in brain cells due to TMX exposure which was confirmed by their downregulated gene expression levels in this study.

As a result, overgeneration of free radicals resulted in peroxidative damage of cell membrane lipids that can be measured by assessment of MDA levels (2, 25). In consistence with previous studies (3, 38), our study unveiled that TMX evoked marked lipid peroxidation in rats brain that refers to the injurious effect of ROS on lipid constituent of the cell membrane. These results were validated by brain histopathological screening. Noteworthy elevations in plasma and brain levels of MDA after sub-chronic oral exposure of rats to low doses of imidacloprid in a dose-dependent manner (2). Former authors reported similar results in imidacloprid-exposed rats (36, 37). Further, marked higher levels of MDA were detected in the cerebellum of rats exposed to acetamiprid in respect to control rats (34).

Nrf2 is a redox-sensitive factor that can be considered as a master regulator of the cellular defence mechanism under oxidative damage (39). Its activation initiates downstream antioxidant proteins and phase II detoxifying enzymes (20). Our results revealed that TMX exposure reduced the expression of Nrf2 in brain tissue in comparison with the control. In support, imidacloprid was reported to induce downregulation of Nrf2 expression in quails with liver fibrosis (40). In addition, acetamiprid induced marked decline in the expression of Nrf2 in renal tissue of male rats (41). Hence, our results demonstrate that TMX exposure significantly evoked oxidative stress in rat brain and that was mediated through inhibition of the Nrf2 and its downstream genes.

SM, a polyphenolic flavonoid of *Silybum marianum*, has powerful antioxidant properties that has been used for management of toxins-induced hepatic damage (23). Recently, its putative neuroprotective action against the progress of neurodegenerative diseases has gained much attention owing to its excellent free radical scavenging activity (26, 42). In our study, SM restored the antioxidant defense of SOD and CAT in brain tissue of TMX-exposed rats. This may be explained by its ROS scavenging ability and boosting the antioxidant enzymes. Marked neuroprotective action of SM was reported previously against brain injury induced by docetaxel (26), aspartame (42) and aluminium (43) toxicities. Furthermore, significant antioxidant activity was reported for SM in animal models of depression (44), dementia (11) and Alzheimer disease (45). Zhou et al. (46) also reported noteworthy antioxidant activities of isosilybin, an isomer of SM, against $A\beta_{25-35}$-induced oxidative stress in HT-22 hippocampal cells. Similarly, SM has been reported to have protective effects in PC12 cells exposed to acrylamide (47). The antioxidant property of SM may be endorsed for stopping the generation of free-radical generation, suppressing specific ROS-producing enzymes, activating the antioxidant enzymes, and genes that contribute to synthetize specific protective molecules (13). Our gene expression results also validated these results as witnessed by marked upregulation in SOD, CAT and GPx in brain tissue of TMX-treated rats. Formerly, SM have been proved to react with and alter the physicochemical properties of cell membranes with consequent increase in their resistance (48). We found marked decrease in MDA levels in brain tissue of rats exposed to SM + TMX. As well, the histopathological finding confirmed the protective action of SM on cellular membrane. In agreement with previous results (23, 26, 47), SM enhanced Nrf2 gene expression in brain tissue subjected to TMX toxicity. Former *in vitro* studies have also reported the modulating effect of SM on the expression of Nrf-2 (46, 49). The induction of Nrf2 gene in TMX + SM group strongly explains the antioxidant effect of SM and supports the histopathological findings.

Our results also revealed noteworthy elevations in inflammatory cytokines as well as upregulations in the mRNA transcriptions of IL-1b, TNF-α, IL-6 and iNOS in TMX-exposed rats in respect to the controls. Oral administration of rats to imidacloprid for 30 days was reported to increase the mRNA transcriptions of TNF-a, IL-6, IL-1b and IFN-c and decrease that of IL-12 in brain tissue (37). Further, in another study, imidacloprid enhanced the gene and protein expressions of IKKα, IKKβ, IL-1β, IL-6, and TNF-α in the liver of quails in a dosage-dependent manner (40). TMX-mediated neuroinflammation in rats' brain may be attributed for over generated free radicals which triggers the NF-kB signalling pathway. NF-kB enhances the expression of iNOS and pro-inflammatory cytokines (TNF-α and IL-6) as well as markers associated with oxidative stress (50). Gargouri et al. (51) found marked increase in protein synthesis and mRNA expression of NF-kB in human neuroblastoma cells exposed to bifenthrin at different concentrations for 24 hours. The histopathological findings of our results validated the neuroinflammation induced by TMX in brain tissue in rats.

Administration of SM had ameliorative effects on brain functions in TMX-treated rats as shown by lowered levels of inflammatory cytokines and down-regulated expression pattern of inflammatory biomarkers compared to TMX intoxicated group. These are in harmony with the histopathological results and in accordance with former authors (26, 43, 44). SM alleviated the neuroinflammation in docetaxel-induced neurotoxicity by decreasing the level of brain proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in

rats (26). Also, significant suppression of IL-1β and TNF-α in rats hippocampus after treatment with aluminum chloride and SM (45). The anti-inflammatory activity of SM has been attributed for suppression of inflammation-related pathways as NF-κB pathway (52). However, our gene expression results declared no effect for SM on NF-κB in brain tissue of TMX-treated rats. Further, SM decreased the expression of iNOS in brain tissue and this is in line with former studies (53, 54). Notable *in vitro* anti-inflammatory effect of silibinin was reported in human retinal pigment epithelial cells exposed to lipopolysaccharide as indicated by lowered gene expression of iNOS, (55).

5. Conclusion

To sum up, the results of the current study indicated that SM, hepatoprotective agent, exerted notable neuroprotection against TMX-induced neural injury in male rats. Significant enhancement of endogenous antioxidant defenses in brain tissue alongside with noticeable declines in inflammatory biomarkers were noticed in SM-pretreated rats. On the molecular level, SM triggered the gene expression of antioxidant enzymes, with marked suppression of gene expression of inflammatory cytokines. Fascinatingly, our results provided new insights on the modulating effect of TMX and SM on Nrf2 NF-kB and iNOS signaling pathways in rat brain tissue.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate: The experimental design and animal handling protocols were in accordance with ARRIVE guidelines and approved by research Ethics Committee, Faculty of Veterinary Medicine, Egypt (Approval number; R/28).

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Authors' contributions

Ola A. Habotta: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Supervision, Writing -original draft, Writing-review, editing & Final revision.

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Rasha Saleh: Methodology, Formal analysis, Investigation, Validation, Writing -original draft, Writing-review & editing.

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Tables

Table 1. Primers used for real-time PCR amplifications.

Gene	GenBank accession number	Oligonucleotide sequence	Annealing temperature (C^0)	Size (bp)	Reference
IL6	NM_012589.2	f5,- AGCGATGATGCACTGTCAGA-3, r5,- GGAACTCCAGAAGACCAGAGC-3,	60	127	Current study
IL-1β	NM_031512.2	f5′- GACTTCACCATGGAACCCGT -3, r5,- GGAGACTGCCCATTCTCGAC -3,	58	104	Current study
TNF-α	NM_012675.3	f5,- CTGTGCCTCAGCCTCTTCTC -3, r5,- ACTGATGAGAGGGAGCCCAT -3,	60	126	Current study
iNOS	NM_012611.3	f5,- TGGGTGAAAGCGGTGTTCTT -3, r5,- TAGCGCTTCCGACTTCCTTG -3,	60	108	Current study
NFKB	AF079314.2	f5,- TGGACGATCTGTTTCCCCTC -3, r5,- CCCTCGCACTTGTAACGGAA-3,	56	118	Current study
CAT	NM_012520.2	f5,- GGAGAGGCAGTGTACTGCAA-3, r5,- TTGCCACTGGCGATGGCATT-3,	58	140	Current study
SOD	X05634.1	f5,- GAAGGCCGTGTGCGTGCTGA -3, f5′- CCTTCAGTTAATCCTGTAATC-3′	58	117	Current study
Gpx	NM_030826.4	f5,- CCGTGTATGCCTTCTCCGCG-3, r5,- TGCCTCAGAGGGACGCGACA -3,	56	108	Current study
Nrf2	NM_031144.3	f5, - GTCCACCCGCGAGTACAACCT-3, r5, - GGAGCCGTTGTCGACGACGA-3,	60	119	Current study
β-Actin	NM_031144.3	f5′- GGCATGTGCAAGGCCGGCTT -3, r5′- TAGGAGTCCTTCTGACCCATA -3′	58	116	Current study

Figures

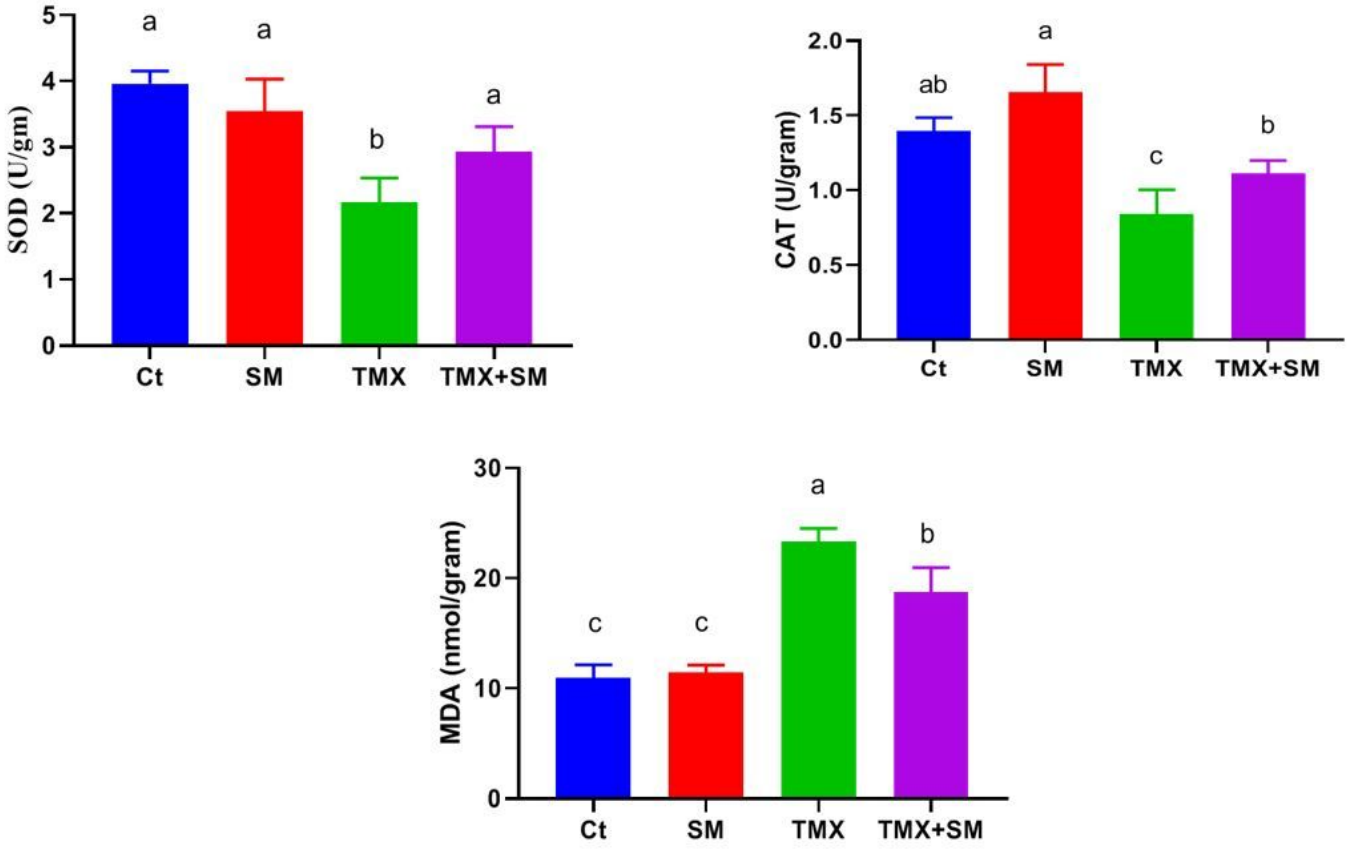

Figure 1

Mitigating effect of silymarin (SM) on oxidative stress biomarkers [superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA)] in brain cortex after exposure to thiamethoxam (TMX) toxicity in rats. The data are expressed as the mean ± SEM (n = 6) for each experimental group. Different superscript letters indicate statistical significance at $P < 0.05$.

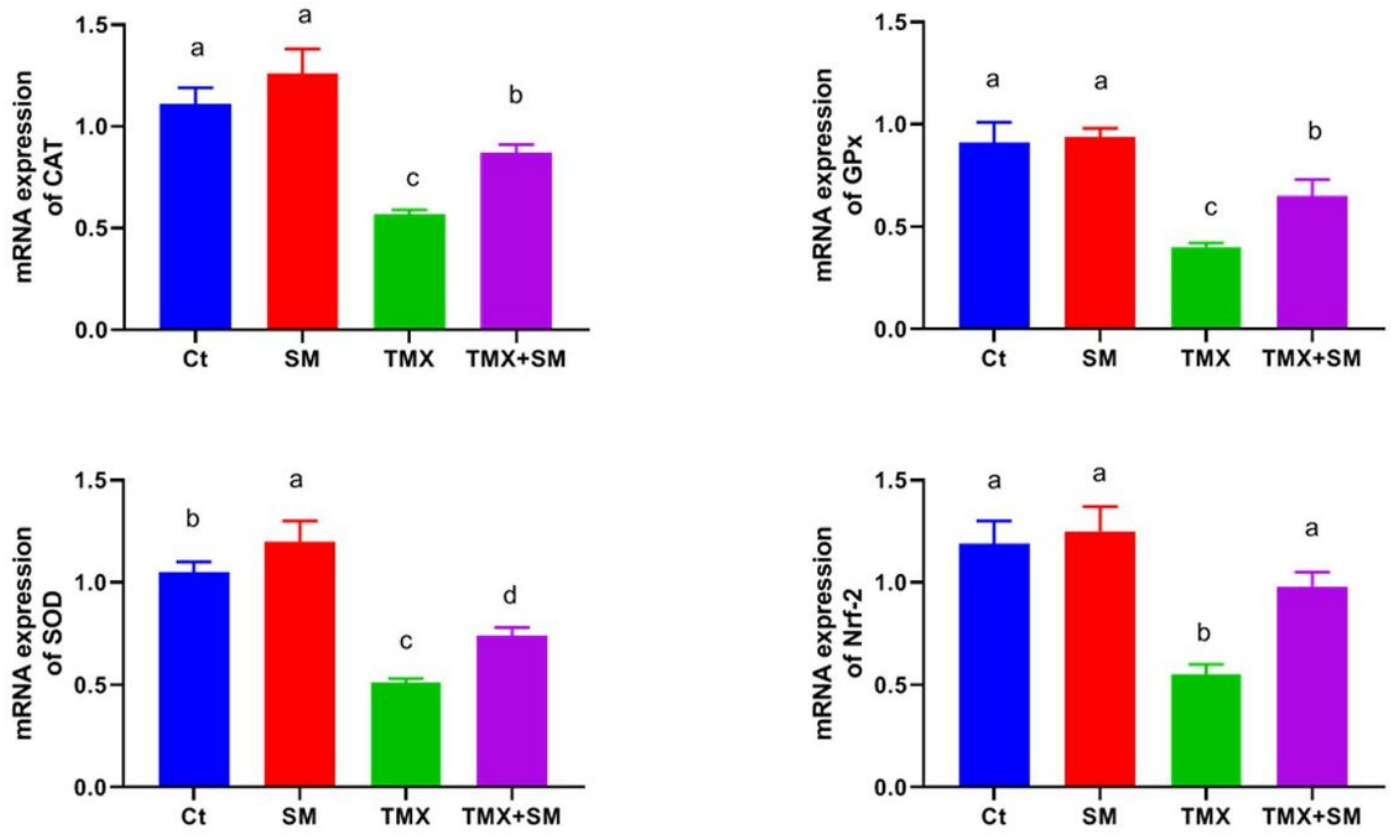

Figure 2

Mitigating effect of silymarin on relative gene expressions of *sod, cat, Gpx* and *Nrf2* in brain cortex after exposure to TMX. The data are expressed as the mean ± SEM (*n* = 6) for each experimental group. Different superscript letters indicate statistical significance at P < 0.05.

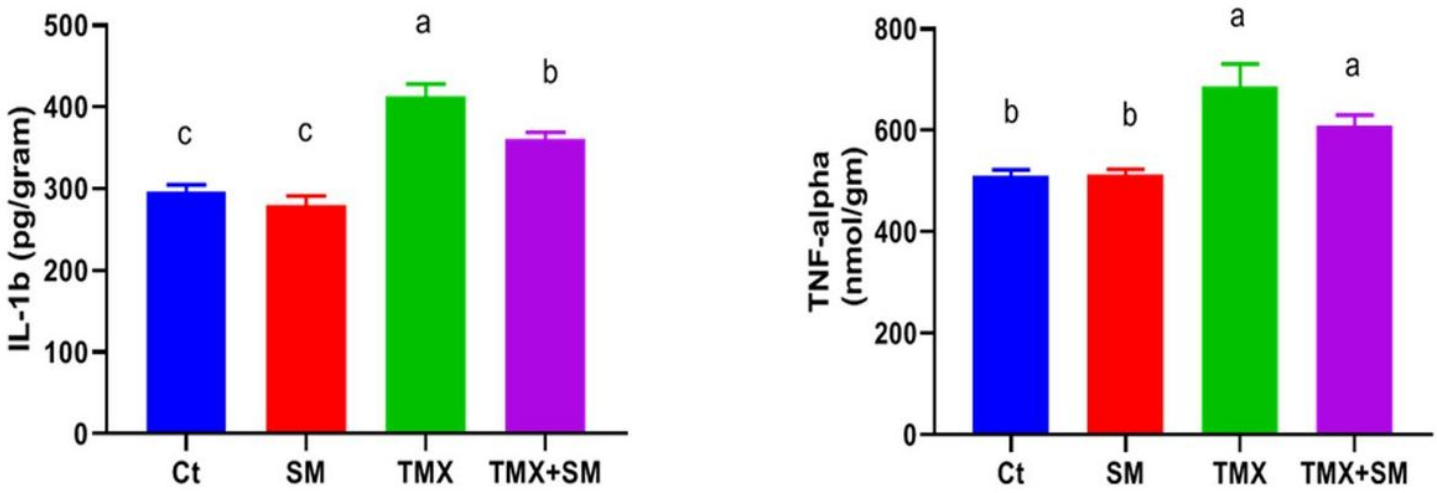

Figure 3

Mitigating effect of silymarin (SM) on inflammatory biomarkers [IL-1b, and TNF-α] in brain cortex after exposure to thiamethoxam (TMX) toxicity in rats. The data are expressed as the mean ± SEM (n = 6) for each experimental group. Different superscript letters indicate statistical significance at $P < 0.05$.

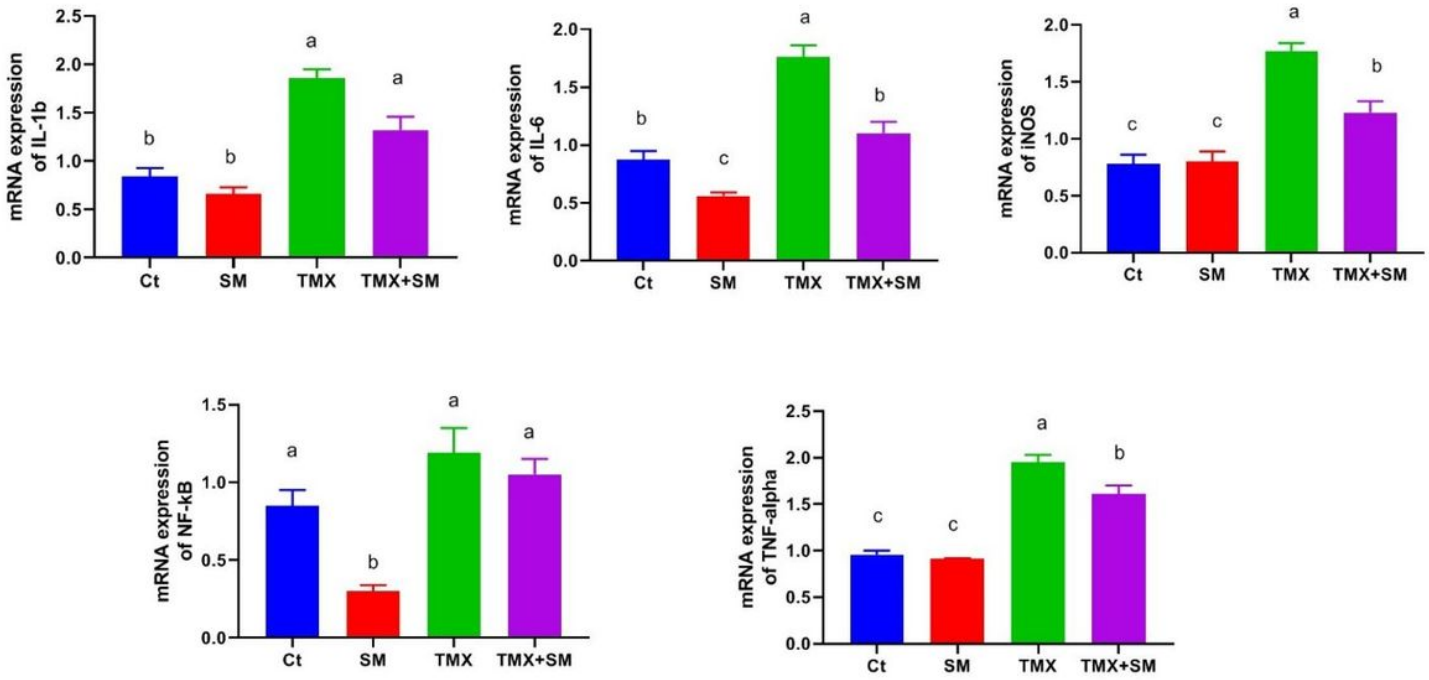

Figure 4

Mitigating effect of silymarin on relative gene expressions of *IL-1b, TNF-α, IL-6, iNOS* and *NF-kB* in brain cortex after exposure to TMX. The data are expressed as the mean ± SEM (*n* = 6) for each experimental group. Different superscript letters indicate statistical significance at P < 0.05.

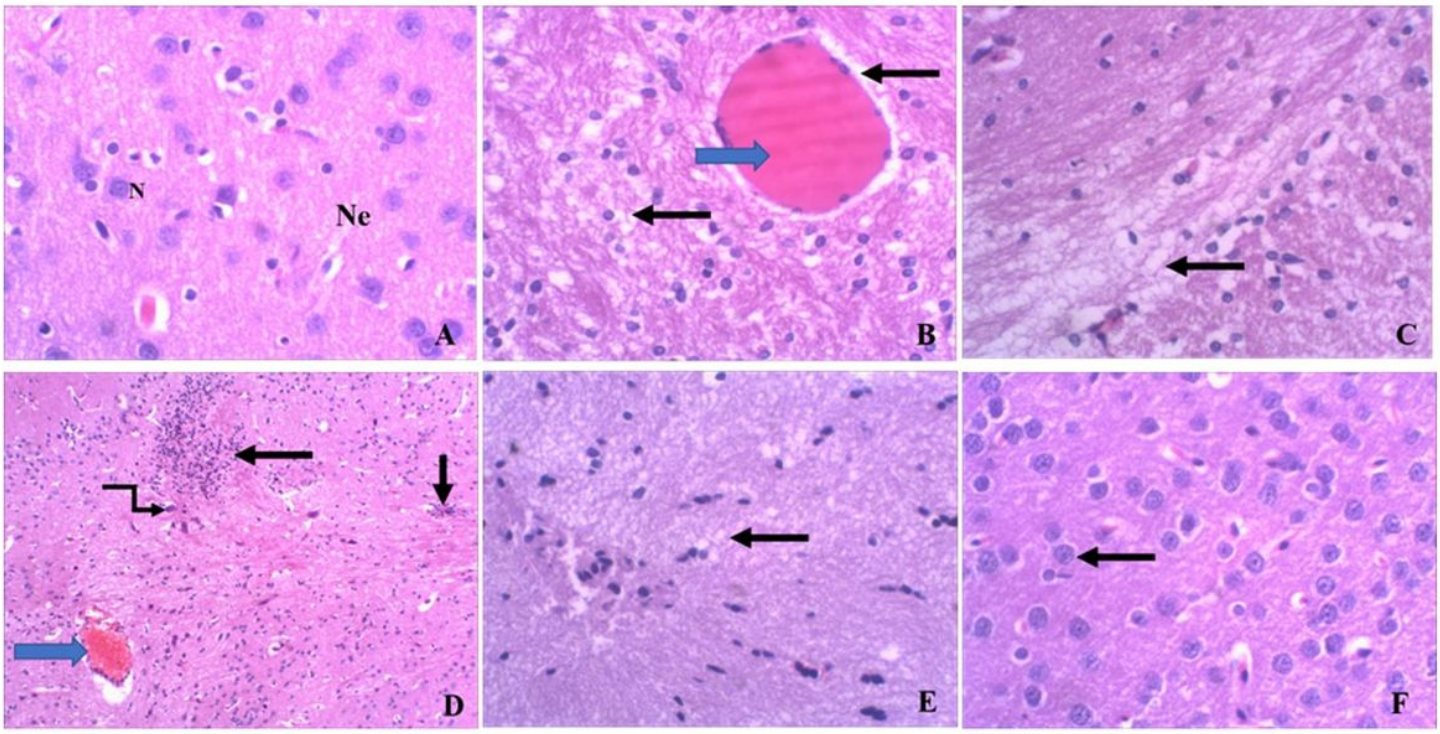

Figure 5

Photomicrograph of brain cortical tissue A) control group showing normal architecture of brain tissue with normal neuron (n) and neurophil (ne), H&E, 400X. B,C,D) Brain from group exposed to thiamethoxam, B) showing hyalinization of blood vessels (thick arrow) with diffuse edema either perivascular or in surrounding neuropil (thin arrows, H&E, 400X). C) diffuse, severe edema widely separated the white matter neuropil (arrow), H&E, 400X. D) multifocal gliosis (thin arrows) surrounding shrunken, necrotic neuron (thin twisted arrow) with infarcted blood vessel and perivascular edema (thick arrow), H&E, 100X. E) Silymarin brain exposed group showing mild spongiosis in white matter neuropil (arrow), H&E, 400x. F) thiamethoxam and silymarin brain exposed group showing normal neuron structure with normal neuropil (Arrow). H&E, 400x.