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Research Article

PROTECTIVE EFFECT OF BROCCOLI AND FERULIC ACID ON IMIDACLOPRID-INDUCED NEUROTOXICITY IN RAT

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ABSTRACT

Imidacloprid may induce oxidative stress leading to generate free radicals and alternate oxygen free radical scavenging enzyme system. This study aims to investigate the neuroprotective effect of broccoli water extract and ferulic acid on imidacloprid induced oxidative stress and DNA damage in male albino rats. Rats were co-treated with broccoli water extract (200 mg/kg) or ferulic acid (20 mg/kg) with imidacloprid (80 mg/kg) orally for 28 days. The results revealed that imidacloprid induced low serum levels of total antioxidant capacity (TAC), whereas lipid peroxidation (LPO) content was increased. However, administration of broccoli improved these parameters. Broccoli and ferulic acid significantly (P<0.05) attenuated the imidacloprid-induced increases in lipid peroxidation (LPO), tumor necroses factor α (TNF-α) and nitric oxide (NO) contents and myeloperoxidase (MPO), glutathione-S-transferase (GST) and catalase (CAT) activities. Imidacloprid decreased reduced glutathione (GSH) while co-treatment with broccoli and ferulic acid significantly (P<0.05) improved the level of GSH. DNA damage as assessed by 8-hydroxydeoxyguanosine (8-OHdG) level was increased in imidacloprid-treated group. However, DNA damage was decreased in broccoli and ferulic acid treated groups. The possible mechanism of broccoli and ferulic acid extract on imidacloprid might be due to decreasing oxidative stress (LPO, NO and DNA damage) and increasing GSH content. Thus, broccoli and ferulic acid was suggested to protected rat's liver against imidacloprid-induced oxidative stress and DNA damage in brain.

Keywords: broccoli, ferulic acid, imidacloprid, DNA damage, oxidative stress, brain

INTRODUCTION

Imidacloprid is currently the most widely used insecticide in the world **[1]**. The favorable selective toxicity of imidacloprid (IMI) to insects versus mammals is attributed to differences in their binding affinity or potency in the nicotinic acetylcholine receptor (nAChR) **[2]**. Imidacloprid exposure leads to marked biochemical, histopathological and ultrastructural changes in various organs so imidacloprid was found to be a potent hepato, nephro and neurotoxic agent **[3]**. Chronic exposure to imidacloprid also induces inflammation and oxidative stress in the liver and central nervous system of rats **[4]**.

Several studies have demonstrated that broccoli might be beneficial by reducing the risk for the development of certain forms of cancer. These effects are generally attributed to glucosinolate-derived degradation products like isothiocyanates and indoles which are formed by the hydrolytic action of plant myrosinase and/or glucosidases deriving from the human microbial flora. Broccoli is a frequently consumed glucoraphanin-containing vegetable **[5,6]**. Glucoraphanin is enzymatically hydrolyzed to the bioactive isothiocyanate, sulforaphane (SFN), during crushing, chewing, or digestion of broccoli. Frequent intake of broccoli is associated with lowered risk of cancer and elevation of antioxidant enzymes, cruciferous vegetables may play an important role in decreasing the risk of premenopausal breast cancer **[7]**. Sulforaphane is one component of broccoli which increases antioxidant enzymes including NAD (P)H quinone oxidoreductase and heme oxygenase I and inhibits inflammatory cytokine**s** **[8-9].** In addition Sulforaphane could exert neuroprotective effects through increasing NF-E2-related factor-2 and heme oxygenase 1 HO-1 expression **[10]**. sulforaphane

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stimulates the transcriptional activating factor pathway of antioxidant gene expression in astrocytes and protects them from cell death in an in vitro model of ischemia/reperfusion **[11]**.

Ferulic acid (FA) is a phytochemical commonly found in fruits and vegetables such as tomatoes, sweet corn and rice bran. It exhibits a wide range of therapeutic effects against various diseases like cancer, diabetes, cardiovascular and neurodegenerative. A wide spectrum of beneficial activity for human health has been advocated for this phenolic compound, at least in part, because of its strong antioxidant activity **[12]**. Ferulic acid due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential **[13].**

Therefore, the present study was aimed to elucidate the neuroprotective role of broccoli or ferulic acid against imidacloprid-induced oxidative stress and DNA damage in rats.

MATERIALS AND METHODS

Chemicals

Ferulic acid, GSH and thiobarbituric acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Imidacloprid 20% suspension obtained from First Kim for fertilizer and Agricultural pesticides Co., Egypt. TNF-α and 8-OHdG ELISA kits were obtained from eBioscience and SunLong, respectively. All other chemicals and reagents used were of analytical grade.

Plant extracts

Broccoli purchased from Experimental Station of Medicinal Plants, Faculty of Pharmacy and Cairo University was dried, powdered before extraction. One hundred grams of broccoli were extracted by percolation with water. The aqueous extract was filtered then freeze-dried. Water extract was used for further study **[14]**.

Animals and experimental design

Male albino rats (160±30 g) from the laboratory stock colony of National Organization for Drug Control and Research (NODCAR) were used in the present study. The animals were kept under normal environmental conditions for two weeks before the initiation of the experiment. The animals were allowed free access to water and fed on a standard diet. The local ethics committee of NODCAR approved study protocols.

Animals were divided equally into six groups, 6 animals each.

-Group 1 served as the control.

-Group 2 was treated with imidacloprid at the dose of 80 mg/kg bodyweight **[15].**

-Group 3 was treated with ferulic acid at the dose of 20 mg/kg body weight **[16]**.

-Group 4 was treated with broccoli extract at the rate of 200 mg/kg body weight a **[17]**.

-Group 5 was treated with both imidacloprid (80 mg/kg) and ferulic acid (20 mg/kg).

-Group 6 was treated with both imidacloprid (80 mg/kg) and broccoli extracts (200 mg/kg).

These drugs were administered by oral gavage every day consequently for 28 days. At the end of the experiment, rats were sacrificed by cervical dislocation and blood was collected without any anticoagulant. The serum separated was used for studying the serum biochemical profile and TAC **[18]** and LPO **[19]** using commercially reagents. The brains were removed and washed in cold isotonic saline. The brains were homogenized in 50 mM phosphate buffer (pH 7) using an electronic homogenizer to prepare 10 % w/v homogenate. The homogenates were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was used for the estimation of LPO (measured as MDA) **[18]**, GSH **[20]**, NO **[21],** MPO **[22]**, CAT **[23]** and GST **[24]**. TNF-α and 8-OHdG levels were determined using ELISA kit. Protein content in the homogenates was estimated by the method of **[25]**.

Statistical analyses

Statistical analyses were performed with SPSS software and were calculated using one-way ANOVA followed by the least significant differences (LSD). $P<0.05$ was considered to indicate a statistically significant result **[26]**.

RESULTS

As shown in Table 1, serum analysis revealed a significant ($P<0.05$) decrease in mean values of TAC, by the administration of imidacloprid in brain when compared with control group whereas LPO increased. Significant ameliorative effect ($P<0.05$) appeared in TAC and LPO in groups treated with broccoli extracts or ferulic acid plus imidacloprid compared with the imidacloprid group. In Table 2, imidacloprid significantly increased LPO levels, GST and CAT activities in brain when compared with control group. However, GSH level was significantly decreased ($P<0.05$) in rats treated with imidacloprid only. All of these changes were ameliorated by treatment of either broccoli extract or ferulic acid when compared

with imidacloprid group. Levels of NO, MPO and TNFα were markedly increased (P<0.05) after administration of imidacloprid compared with control group. These effects were significantly opposed (P<0.05) when co-administered with either ferulic acid or broccoli extract compared with imidacloprid group (Table 3). As shown in Figure (1), 8-OHdG levels (DNA fragmentation) were significantly decreased after administration of imidacloprid in brain compared with control group. However, co-administration of broccoli extract or ferulic acid significantly (P<0.05) reversed these values when compared with imidacloprid group.

Table 1: Effect of ferulic acid and broccoli extract on serum LPO and TAC in rats treated with imidacloprid.

Groups	*(TAC) (mM/L)*	*LPO (nmolMDA/ml)*
Group 1	1.92±0.15	5.63±0.07
Group 2	0.49±0.04 [a]	13.23±0.21 [a]
Group 3	2.02±0.02	8.82±0.12
Group 4	1.96±0.11	8.31±0.11
Group5	1.14±0.07 [b]	5.09±0.08 [b]
Group 6	1.04±0.02 [b]	4.97±0.05 [b]

Data are expressed as mean± S.E. of six rats per group. [a]Significant different from control group at P<0.05, [b]Significant different from imidacloprid-treated group at P<0.05.

Table 2: Effects of ferulic acid and broccoli on GSH and LPO contents and GST and CAT activities following imidacloprid administration

Groups	*GSH (µmol GSH /mg protein)*	*LPO (nmol MDA /mg protein)*	*GST (U/mg protein)*	*CAT (U/mg protein)*
Group 1	14.25±1.07	0.62±0.16	5.63±0.21	82.66±8.80
Group 2	7.93±0.72 [a]	2.36±0.23 [a]	11.34±0.14	129.38±7.86 [a]
Group 3	16.12±2.58	0.50±0.08	5.32±1.14	89.84±5.21
Group 4	15.66±1.20	0.54±0.08	5.85±0.44	79.06±6.11
Group 5	12.11±1.40 [b]	1.27±0.19 [b]	7.63±0.92 [b]	97.03±6.41 [b]
Group 6	13.25±1.01 [b]	0.77±0.23 [b]	7.97±0.81 [b]	93.44±9.46 [b]

Data are expressed as mean± S.E. of six rats per group. [a]Significant different from control group at P<0.05, [b]Significant different from imidacloprid-treated group at P<0.05.

Table 3: Effects of ferulic acid and broccoli on TNF-α and NO contents and MPO activity following imidacloprid administration

Groups	*NO (µmol/mg protein)*	*MPO (U/mg protein)*	*TNF-α (pg/mg protein)*
Group 1	5.56±1.6	5.57±0.70	7.02±1.03
Group 2	14.91±0.99[a]	13.74±1.763 [a]	23.16±1.44 [a]
Group 3	5.01±0.72	5.3±0.17	6.07±1.31
Group 4	5.52±0.56	5.27±0.57	6.91±0.48
Group 5	9.33±0.59 [b]	8.80±0.45 [b]	11.29±1.39 [b]
Group 6	9.54±0.51 [b]	8.71±0.83 [b]	10.70±2.07 [b]

Data are expressed as mean± S.E.M. of 6 rats per group. [a]Significant different from control group at P<0.05, [b]Significant different from imidacloprid-treated group at P<0.05.

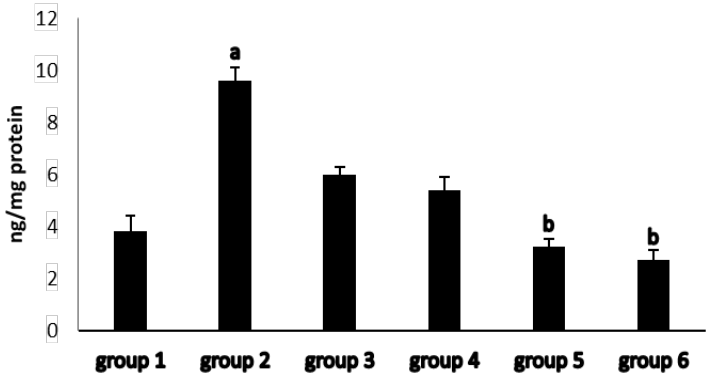

Fig 1: Effects of ferulic acid and broccoli on DNA fragmentation (8-OHdG level). Data are expressed as mean± S.E.M. of 6 rats per group. [a]Significant different from Control group at P<0.05, [b]Significant different from imidacloprid-treated group at P<0.05.

DISCUSSION

A large number of xenobiotics have capability to generate free radicals in biological system raising question whether oxidative stress is major concern for tissue damage. However, antioxidant enzymes may have effect on oxidant molecule on tissues and are active in defense against oxidative cell injury by means of their free radical scavengers pesticides mediated toxicity involves excessive production of ROS leading to alternations in the cellular antioxidant defense system and consequently affecting susceptibility to oxidative stress **[27]**. Therefore, the present study was aimed to evaluate the repeated exposure to dose of 80 mg/kg of imidaclopid on the brain enzymes, antioxidant status, nitric oxide and oxidative stress biomarkers, as well as the possible neuroprotective role of broccoli extract or ferulic acid.

-Serum LPO and TAC contents

The data depicted in Table 1 revealed a significant (P<0.05) decrease in TCA along with significantly (P<0.05) increase in LPO after treatment of imidacloprid. These results are in agreement with **Lonare [28]**, whereas co-administration of broccoli extract for 4 weeks significantly preserves these aforementioned changes as compared with that of imidacloprid intoxicated rats. **Kapoor [29]** investigated the effects of imidacloprid on antioxidant enzymes and lipid peroxidation and made similar observations. They suggested that oxidative metabolites and/or free radicals are produced during imidacloprid metabolism. **Turkez [30]** reported that permethrin (PM) caused increases of total oxidant status level and decrease of TAC level as compared to control group on blood cells in rats.

-GSH and LPO contents and GST and CAT activities in brain

Brain glutathione and other thiol containing proteins play a crucial role in cellular defense against toxicity of pesticides **[31]**. GSH acts as a direct free radical scavenger as well as co-substrate for glutathione peroxidase (GPx) and GST to react with the highly reactive free radicals and organic peroxides. In the current study, GSH content significantly (P<0.05) was decreased in imidacloprid-treated group, This results are in agreement with **Duzguner and Erdogan [32]** who suggested that imidacloprid cause oxidative stress and inflammation in central nervous system and liver in rats. The decreased brain GSH content observed in our current study may reflect, at least partially, GSH conjugation or oxidation of GSH to glutathione disulfide (GSSG) due to the pesticide-induced generation of oxygen free radicals and their byproducts **[32]**.

The brain tissue is highly susceptible to LPO because of its high rate of oxygen utilization, an abundant supply of polyunsaturated fatty acids, a deficient antioxidant defense and a high content of transition metals like copper and iron **[33]**. Table 2 illustrated that the brain marker LPO was significantly increased (P<0.05) in imidacloprid-intoxicated rats as compared with control rats, confirmed the susceptibility of brain tissues to oxidative stress. This result is in agreement with **Kappor et al. [29]** who recorded significant increase of LPO of rat which take a dose of imidacloprid equal 20 mg/kg. However, co-administration of imidacloprid for 4 weeks with broccoli or ferulic acid was significantly reinforcing the resistance of the brain towards imidacloprid toxicity by direct scavenging ROS. GST in Table 2

increase significantly because of imidacloprid toxicity. This result is in agreement with **EL-Gendy et al. [34]**. The detoxification of ROS in brain involves the co-operative action of all intracellular antioxidant enzymes. Superoxide dismutase (SOD) is the first line of antioxidant enzymatic defense catalyzes the conversion of superoxide radicals to less toxic H_2O_2. Catalase (CAT) metabolizes H_2O_2 to water. When this mechanism is saturated, the second line of antioxidant enzymatic defense mainly GPx that regulated by selenium availability is activated **[35].** Antioxidant enzyme as CAT may have effect in oxidant molecules on tissues and active in defense against oxidative injury by means of their being free radical scavengers. Pesticides mediated toxicity involves excessive production of ROS leading to alternation in cellular antioxidant defense system consequently and affecting susceptibility to oxidative stress **[27]**. Imidacloprid for 4 weeks were significantly ($P<0.05$) increased the activity of CAT (the antioxidant defense enzymes) as compared with that of the normal rats. Our results are in agreement with **Duzguner and Erdogan [32]** who showed that catalase activity was elevated almost 2-fold in brain because of exposed to 1mg/Kg for 30 days. Some studies show an increase in intracellular antioxidants to compensate for the generation of free radicals induced by pesticide exposure **[36]**.

-Brain TNF-α and NO contents and MPO activity

Nitric oxide is produced from the amino acid L-arginine by the enzymatic action of Nitric oxide synthase (NOS). While it has physiological actions, abnormal production of NO can damage numerous molecules (including lipids, proteins and DNA) causing alterations in the functioning of target cells potentially leading to cell death **[32]**. In the present study, we found that long-term exposure to imidacloprid significantly increased NO production by approximately 57% in brain ($P<0.05$) as a result of imidacloprid neurotoxicity. The result in agreement with **Duzguner and Erdogan [32]** who reported that imidacloprid application (1 mg/kg/day for 30 days) caused a significant increase in nitric oxide production in brain. However, simultaneous administration of broccoli or ferulic acid with imidacloprid for 4 weeks was significantly maintained level of NO in brain to near the normal level of control rats.

Myeloperoxidase, present in the granules of neutrophils, catalysis the production of hypochlorous acid which has bacteriocidal properties **[37]**. It is reported that chronic exposure to endosulphane causes an increase in MPO activity because of neutrophil activation **[38]**. Based on the observations it is suggested that the increased activity of MPO following chronic imidacloprid exposure may evidence the intensity of the oxidative process and the presence of inflammation.

Tumor necrosis factor (TNFα) is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as NK cells, neutrophils, mast cells, eosinophils, and neurons. TNF alpha is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis. The TNF is also important for resistance to infection and cancers **[39]**. Our results are in agreement with **Duzguner and Erdogan [32]** who reported that imidacloprid treatment up-regulated inflammatory cytokines TNF-α, IL-6 and IL-1β mRNA transcriptions by 2.5- to 5.2-fold increases in both brain and liver.

Brain DNA damage

In present investigation, treatment of rats with imidacloprid increased DNA damage in brain rats. Imidacloprid was found to induce DNA damage in a dose-related manner in earthworms as well as to increase the frequency of adducts in pesticide-treated calf thymus DNA, indicating agent-induced genotoxicity **[40]. Bal1 et al. [41]** observed that apoptosis and fragmentation of seminal DNA were higher in rats treated with imidacloprid by two doses (2- and 8-mg/kg). Toxicity may cause DNA damage by secondary mechanisms **[42]**. Excessive production of oxidants can result in oxidative damage, due to the oxidation of lipids, proteins and DNA in the cells **[43]**. The present study revealed that co-treatment of either broccoli extract or ferulic acid with imidacloprid caused a significantly improved LPO and TAC levels in serum, also they significantly ameliorated GST, CAT and MPO activities, NO, TNF-α and 8-OHdG contents in brain.

Sulforaphane is a natural dietary isothiocyanate found in broccoli. sulforaphane is a promising antioxidant agent that is effective to attenuate oxidative stress and tissue/cell damage in different in vivo and in vitro experiments **[44]**. In addition, sulforaphane (one of broccoli component) has neuroprotective effects in several experimental **[45]**. Broccoli has anti-inflammatory effect due to its high content of polyphenols and flavonoids **[46]**. **Ahmed and Nasr [47]** reported that broccoli extract had markedly contents of total phenolics, total flavonoids and total isothiocyanate. In addition, they showed that broccoli and ferulic acid had hepatoprotective effect on imidacloprid. Therefore**,** neuroprotective role of broccoli in the present study which may result from its antioxidant properties due to its bioactive content such as isothiocyanate, polyphenols and flavonoids.

FA (25–50μM) significantly attenuated peroxyl radical-induced cell death in hippocampal neuronal cells **[48]**. Moreover, long-term administration of FA or FA ethyl ester inhibited the expression of endothelial and inducible nitric oxide synthase in mouse hippocampus **[49]**. **Yan et al. [50]** reported that ferulic acid is an antioxidant and anti-inflammatory agent derived from plants and suggest that ferulic acid may be a useful chemopreventive agent against Alzheimer's disease. Because ferulic acid has both antioxidant **[51]** and anti-inflammatory activities **[52]**.

The production of free radicals and neuroinflammation contribute to the destruction of some brain regions such as the cortex **[53].** Chronic exposure to imidacloprid also induces inflammation and oxidative stress in the liver and central nervous system of rats [**32].** Thus, broccoli and FA can have a favorable effect on imidacloprid neurotoxicity due to their anti-inflammatory and antioxidant properties.

In conclusion, the present study concluded that the water extract of broccoli and ferulic acid had neuroprotective effects against imidaclopride-induced oxidative stress and DNA damage in rats. The high protective effect of the broccoli extract on imidacloprid appeared to be due to its high total phenolics, total flavonoids and total isothiocyanate contents.

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