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Postnatal Exposure to A Low Dose of Imidacloprid: Oxidative Stress in Brain Without Affecting Learning and Behavior in Swiss Albino Mice

A. Sharma*†, S. Gupta* and M. Kaur*
*Department of Zoology, IIS (Deemed to be University), Jaipur 302020, India
†Corresponding author: A. Sharma; dranju1110@gmail.com

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ABSTRACT

The neurotoxic effects of exposure to low levels of the pesticide imidacloprid (IMI) and the effect of curcumin are of current interest when exposure occurs during early development. Male weanlings of Swiss albino mice (21 days old) were given 1 $mg.kg^{-1}$ body weight (1/130 of LD50 and 2 $mg.kg^{-1}$ body weight (1/65 of LD50) of imidacloprid and Curcumin (100 mg/kg body wt.) by oral gavage from postnatal day 21 to postnatal day 60. Young adult offspring were studied for behavioral parameters and learning ability using open field and Morris water maze. After completing the behavioral test, brains were processed for acetylcholine esterase activity and antioxidant enzyme estimation. The level of lipid peroxidation and activity of catalase, superoxide dismutase, and glutathione were assayed. In the present study, parameters such as locomotor activities and cognitive skills were not affected compared to lower doses of imidacloprid in the open field and Morris water test. However, activities and levels of antioxidant enzymes such as catalase and lipid peroxidation were found to be altered. In contrast, superoxide dismutase, acetylcholine esterase activity, and glutathione remained unchanged compared to the control. This suggests that subchronic exposure to low doses of IMI can lead to significant alterations in the enzymes of antioxidant protective systems such as catalase and lipid peroxidation. Co-treatment with curcumin was able to restore the activities of the affected enzymes in comparison with the control.

INTRODUCTION

Imidacloprid (neonicotinoid insecticide) is widely used to control sucking pests on a diverse range of major crops, including vegetables, rice, cotton, potato, and others (Fossen, 2006, Jeschke et al. 2011, Simon-Delso et al. 2015). Thus, non-target populations, such as mammals, especially humans, get exposed to xenobiotics via food and contaminated drinking water (US EPA 2020). Long-term acute and chronic exposure to imidacloprid and other neonicotinoids causes various health deficits in mammals (Lonare et al. 2014, Cimino et al. 2017, Katic et al. 2020).

Recent studies have also shown that imidacloprid exposure leads to biochemical changes in the central nervous system, thereby affecting the behavior of organisms. It can bind to the nicotinic cholinergic receptors in the nervous system (Tomizawa & Casida, 2002, 2003). Chronic imidacloprid exposure and adverse development can be correlated, and this has been further strengthened and confirmed by human exposure studies (Yang et al. 2014, Carmichael et al. 2014)

According to the guidelines laid down by the European Food Safety Authority (EFSA), the acceptable daily intake (ADI) and acceptable operator exposure level (AOEL) of imidacloprid for humans have been set to 0.06 $mg.kg^{-1}$ $bw.day^{-1}$ and 0.08 $mg.kg^{-1}$ $bw.day^{-1}$ respectively (EFSA 2008). A change in the previously set reference values was suggested by the EFSA 2013, lowering the AOEL to the same level as ADI to avoid developmental neurotoxicity in humans (EFSA 2014).

The most available literature on the toxicity of such chemicals is based on high doses and the adult population (Toor et al. 2013, Bagri et al. 2013, Vohra & Khera 2015, Sharma et al. 2019, 2021). Though the current limit of concern regarding imidacloprid for mice, no observed adverse effect level (NOAEL) has been found at 5 and 10 $mg.kg^{-1}$ per day (Arfat et al. 2014). However, sub-chronic low-level exposure and its effect on young and developing populations have not yet been well investigated (Katic et al. 2020).

Younger populations, such as neonates and kids, can be indirectly/directly exposed to these toxic agents.

ORCID details of the authors:

A Sharma: https://orcid.org/0000-0003-2719-6878

Even low-dose exposure during such vulnerable and sensitive development periods may cause disruption and malfunctioning of adults' brains and nervous systems, thereby affecting their behavior (Gawade et al. 2013, Burke et al. 2018).

Furthermore, during the development phase, the brain is highly susceptible to neurotoxic insults as the developing blood-brain barrier (BBB) is less resistant to these toxicants than the mature adult brain, thus allowing the toxicant to reach the blood supply and brain. Therefore, exposure during the developing period is of significant interest. It has been reported by various studies that preschool children are being exposed to higher levels of pesticides than the recommended ADI. Such a high consumption rate of these pesticides indicates a possible fetal neurotoxic risk and may impair children's cognitive skills, behavior, and motor and sensory functions. Suggesting that the effect of such xenobiotics on vulnerable populations such as children and young adults should be thoroughly researched (Van Wendel de Joode et al. 2016).

Curcumin (Cur), an important ingredient of turmeric, is a potent inhibitor of free radical formation and reactive oxygen species (ROS) generation in conditions such as lung injury induced due to paraquat exposure (Venkatesan 2000), propanil-induced hepatoxicity (Otuechere et al. 2014), malathion induced testicular toxicity and oxidative damage in male mice (Ali & Ibrahim 2018). Curcumin is protective against free radical damage. Its role in improving cognitive function has recently been identified and studied thoroughly, especially in neurodegenerative diseases such as Alzheimer's and Down syndrome (Pan et al. 2015, Rueda et al. 2020).

Thus, the present study has been planned to evaluate the effects of 40-day oral exposure to two (1 $mg.kg^{-1}$ bw and 2 $mg.kg^{-1}$ bw) low doses of imidacloprid on young male Swiss albino mice. The effect of curcumin after low-dose exposure to imidacloprid with emphasis on learning behavior and biochemical endpoints was also investigated.

MATERIALS AND METHODS

Chemicals

A commercial formulation of imidacloprid (Victor Imidacloprid) 17.8%SL from the division of Insecticides India Ltd. (Delhi, India) was used to treat mice. In mice, the LD 50 value (oral) of imidacloprid was 131 $mg.kg^{-1}.bw^{-1}$ (WHO 2008). In the present study, the mice were given imidacloprid at 1 $mg.kg^{-1}.bw^{-1}$ (1/130 of LD 50) and 2 $mg.kg^{-1}.bw^{-1}$ (1/65 of LD 50). Food-grade curcumin was used.

Animals were divided into four groups (6 each) and treated daily by oral gavage from postnatal day 21 to postnatal day 60. The chosen imidacloprid doses were lesser than the documented non-observable level in mice (5-10$mg.kg^{-1}bw.day^{-1}$) (Arfat et al. 2014)

Test Animals and Treatment

Adult Swiss albino mice were obtained from the Indian Veterinary Research Institute, Izetnagar Bareli UP. The animals were housed in a well-ventilated vivarium at around 29 ± 20 C (relative humidity 33-40%) with natural light and dark cycles. The animals were housed in polypropylene cages with wood shavings spread on the floor evenly. They were maintained on standard mice feed obtained from Hindustan Level Ltd Delhi, India, and drinking water ad libitum. The Institutional Animals Ethical Committee approved the study, and all the experiments were carried out according to the guidelines of the CPCSEA (Committee for Control and Supervision of Experiments on Animals) Government of India, New Delhi.

The colony was maintained, and pregnant females were checked for parturition. The day of birth of the offspring of healthy females was noted as postnatal day 0. Healthy male weanlings (21 days old pups) weighing 93 g were selected for the study. Animals were divided into four groups (n=6 each) and treated daily via oral gavage from postnatal day 21 to postnatal day 60.

Group I served as the control and received only vehicle (Distilled Water).

Group II was exposed to imidacloprid at the dose of 1 $mg.kg^{-1}.bw^{-1}$

Group III was exposed to imidacloprid at the dose of 2 $mg.kg^{-1}.bw^{-1}$

Group IV was exposed to 2 $mg.kg^{-1}$ bw^{-1} imidacloprid and 100 $mg.kg^{-1}.bw^{-1}$ curcumin in combination

Animals were observed regularly for clinical signs of toxicity; food and water consumption were also recorded. Young animals were subjected to behavioral tests at the end of the exposure periods.

Behavioral Tests

The open-field test (locomotor activity and anxiety) (Seibenhener & Wooten 2015): The locomotor activity and anxiety behavior were assessed using an open field (Seibenhener & Wooten 2015) with slight modifications. The open field behavior was assessed using a square wooden box measuring 50 x 50cm (side x side) and a height of 38 cm. The box was divided into 10 x10cm blocks with black lines painted into 25 similar spaces. For observations, each mouse was placed in the center arena and left free to explore for the next 5 minutes. Further, it was scored on the following

parameter: Exploration time (total time duration animals were in a mobile state), Number of blocks crossed (number of floor units crossed with four paws), and freezing duration.

The Morris water maze (learning and memory) (Vorhees & Williams 2006): Morris water maze (MWM) was used to assess spatial learning and memory in young mice following the procedure by Vorhees et al. (2006). The apparatus contained a circular tank (150cm in diameter and 50 cm in height) filled with water maintained at 29±2° C and was placed in a room with extra cues around the apparatus. The tank was divided into four equal quadrants, North-West (NW), South-East (SE), South-East (SE), and North-East (NE), with extra maze cues. A hidden platform (10 cm^2) was located in one of the quadrants. This experiment was performed during postnatal days 56-60. A video system was used to record the movement of each mouse within the maze. Mice were given four training sessions. For each trial, mice were placed in each quadrant, and the time taken to reach the platform was noted. The animals use the extra cues for reference to reach the platform. After training sessions, the platform was completely submerged in the test session. Each mouse was placed in the test paradigm in all the quadrants, and latency to reach the platform was noted.

On postnatal day 60, all the animals were weighed and euthanized by cervical dislocation. The brain was removed and stored in cold isotonic saline after being weighed. They were further homogenized in the appropriate buffer for biochemical estimation.

Biochemical Estimation

Acetylcholine esterase (Ellman et al. 1961): The acetylcholinesterase enzyme activity (AchE) was estimated in brain tissue according to the method described by Ellman et al. (1961) using acetylcholine iodide as a substrate and 5,5 dithibis-2 nitrobenzoic acid (DTNB) as the coloring agent. The degradation of acetylcholine iodide was measured at 412 nm.

Lipid peroxidation (Ohkawa et al. 1979): Malonaldehyde (MDA) was measured according to the standard method. The level was determined by thiobarbituric acid (TBA) reactive substance (TBARS) in brain tissue, based on the reaction between MDA and TBA. The absorbance of the organic layer (upper layer) was read by the UV-Vis spectrophotometer at 532 nm against blank using distilled water. TBA was allowed to react with MDA and formed a colored complex [MDA-(TBA)2 complex], which was measured by the spectrophotometer (Systronics UV-Vis Double Beam Spectrophotometer 2201)

Catalase activity (Luck et al. 1965): Catalase is an enzyme that scavenges hydrogen peroxide converting it to water and oxygen molecule. The activity of this enzyme depends on the ultraviolet absorption of the hydrogen peroxide solution, which can be measured at 240 nm (Luck et al. 1965. The activity of catalase is expressed as a unit/mg protein.

Superoxide dismutase (Marklund & Marklund 1974): It catalyzes the dismutation of superoxide radicals. Measurement of superoxide dismutase activity was based on the inhibition of pyrogallol autoxidation caused by superoxide dismutase as described by (Marklund & Marklund 1974).

Glutathione (Moron et al. 1979): Reduced GSH as a non-enzymatic antioxidant was measured according to the method described. GSH is determined based on the reaction 5,5- dithiobis-2-nitrobenzoic acid (DTNB) with GSH, which is yellow colored chromophore with maximum absorbance at 412 nm. The amount of reduced glutathione in brain tissue was calculated at l $g.g^{-1}$ tissue.

STATISTICAL ANALYSIS

GraphPad Prism 8 was used to analyze the data. One-way analysis of variance (ANOVA) and post hoc test (Tukey's multiple comparisons test) were applied to the data set. The data are represented as mean SEM, and differences were considered significant when $p<0.05$ and highly significant $p<0.01$.

RESULTS

No adverse signs of clinical toxicity were observed in control and treated animals. The gain in the body weight of mice has exhibited significant change ($p < 0.05$) at both the dose levels 1$mg.kg^{-1}$ and 2$mg.kg^{-1}.bw^{-1}$ as compared to the control (Table 1). At the same time, co-treatment with curcumin resulted in a decrease in body weight gain compared to the control (Table 1). A similarly highly significant decrease was observed in the neurosomatic index of treated animals at 1mg/kg bw (1.74± 0.053, $p < 0.01$) and 2mg/kg bw (1.465 ± 0.078, $p < 0.01$) dose levels as compared to control (2.207 ± 0.036). Co-treatment with curcumin resulted in a nonsignificant increase in the neurosomatic index compared to the treated group (Table 1).

Parameters

Open Field Exploration Test: The effects of imidacloprid in the open field behavior are summarized in Table 2

The locomotor activity of animals was assessed by the number of squares crossed during 300 seconds. The animals in the control group could cross an average of 267 ± 22.36 squares. While the average number of squares crossed by 1$mg.kg^{-1}.bw^{-1}$ IMI and 2 $mg.kg.bw^{-1}$ IMI group animals

Table 1: Effect of IMI and Cur exposure in Swiss albino mice on Body Weight Gain and Percent neurosomatic index following exposure to IMI, Cur, and their combination in young male weanlings. IMI: Imidacloprid; Cur: Curcumin.

Parameter	Group I Control	Group II 1mg.kg.bw^{-1} IMI	Group III 2mg.kg.bw^{-1} IMI	Group IV 2mg.kg.bw^{-1} IMI + 100mg.kg.bw^{-1} Cur
Weight Gain (gm) (Mean ±SEM)	9.2 ± 0.2	13.37 ± 0.23*	15.25 ± 1.26**	13.25 ± 0.42*
Brain Weight	0.465 ± 0.0078	0.440 ± 0.0176	0.451 ± 0.0034	0.487 ± 0.0145
% Neurosomatic Index	2.207 ± 0.036	1.74 ± 0.053**	1.465 ± 0.078**	1.802 ± 0.054*

Values represent Mean ± SEM bearing different superscripts in the same rows differ significantly * indicates ($p<0.05$) and ** indicates ($p<0.01$). One-way analysis of variance followed by Tukey's multiple comparison test.

Table 2: Effect of IMI and Cur exposure in Swiss albino mice on Exploration percent time, Number of Blocks Crossed, and Freezing percent time in Open Field Test following exposure to IMI, Cur, and their combination in young male weanlings. IMI: Imidacloprid; Cur: Curcumin

Parameters	Group I Control	Group II 1mg.kg.bw^{-1} IMI	Group III 2mg.kg.bw^{-1} IMI	Group IV 2mg.kg.bw^{-1} IMI + 100mg.kg.bw^{-1} Cur
Number of Blocks Crossed	267 ± 22.36	239.33 ± 34.49	194.75 ± 32.28	207.33 ± 63.63
Exploration % time	86.13 ± 1.78	87.19 ± 4.44	74.86 ± 4.96	89.88 ± 0.80
Freezing % time	4.518 ± 1.82	5.93 ± 2.49	11.7767 ± 4.91	4.7733 ± 0.98

Values represent Mean ± SEM bearing different superscripts in the same rows differ significantly ($p<0.05$). One-way analysis of variance followed by Tukey's multiple comparison test.

were recorded to be 239.33 ± 34.49 and 194.75 ± 32.28, respectively. The number is less in comparison to the control. However, not significant. Similarly, a non-significant decrease in the percent exploration time of 74.86 ± 4.96 was noted in animals exposed to 2 mg.kg^{-1}.bw^{-1} IMI. At the same time, no change was observed in the 1 mg.kg^{-1}.bw^{-1} (87.19 ± 4.44) animals compared to the control (86.13 ± 1.78). Further, a non-significant increase in the freezing percent time (11.7767 ± 4.914) was observed in animals exposed to 2 mg.kg^{-1}.bw^{-1} of IMI, while no change was observed at the 1 mg.kg^{-1}.bw^{-1} (5.93 ± 2.49) dose level compared to the control (4.518 ± 1.82). Curcumin co-treatment resulted in a non-significant increase in the number of blocks crossed (207.33 ± 63.63) and exploration percent time (89.88 ± 0.80) while freezing percent time (4.7733 ± 0.98) was found to be reduced in the group exposed to 2 mg.kg^{-1}.bw^{-1} IMI + 100 mg.kg^{-1}.bw^{-1} Cur in comparison to the group exposed with 2 mg.kg^{-1}.bw^{-1} IMI.

Morris Water Maze test: The effects of imidacloprid in the Morris water maze test are summarized in Table 3. It is one of the most widely used behavioral tests for studying spatial learning and memory. In the target quadrant, latency to locate the platform in percent time was increased in the animals exposed to 1mg.kg^{-1}.bw^{-1} (27.66 ± 7.21) and 2 mg.kw^{-1}.bw^{-1} (12.33 ± 5.56) IMI compared to the control (11.998 ± 1.334). However, it is not significant. Further, curcumin co-treatment resulted in an even more reduction in the latency period to locate the platform (6.32 ± 1.22) compared to the control.

Acetylcholine Esterase Activity (Table 4): Developmental exposure to IMI has resulted in a non-significant decrease in AchE activity in both 1 mg.kg^{-1}.bw^{-1} (0.003146 ± 0.0005) and 2 mg.kg^{-1}.bw^{-1} (0.003659 ± 0.0008) IMI-exposed animals compared to control (0.004857 ± 0.0012). However, no change in the acetylcholine esterase activity was observed in the curcumin co-treated group (0.003360 ± 0.0007) compared to the 2 mg.kg^{-1}.bw^{-1} group.

Lipid Peroxidation (Table 4): LPO estimated in the brain tissue at the end of the exposure period was significantly ($p<0.05$) higher in 2 mg.kg^{-1}.bw^{-1} (0.11735 ± 0.0134*) IMI exposed animals. No change was seen in the 1 mg.kg^{-1}.bw^{-1} (0.042075 ± 0.0048) compared to the control (0.046325 ± 0.0072). However, it was found to be lowered significantly ($p<0.05$) by curcumin in the co-treated (0.047575 ± 0.0206) group compared to the 2 mg.kg^{-1}.bw^{-1} group.

Catalase (Table 4): IMI treatment resulted in a decrease in brain catalase levels at 1 mg.kg^{-1}.bw^{-1} (17.69 ± 4.38**) dose level when compared to the control (41.325 ± 3.65)

Table 3: Effect of IMI and Cur exposure in Swiss albino mice on Latency to Reach Platform. IMI: imidacloprid; Cur: curcumin.

Parameters	Group I Control	Group II 1mg/kg bw IMI	Group III 2mg/kg bw IMI	Group IV 2mg/kg bw IMI + 100mg/kg bw Cur
Latency in finding a platform	11.998 ± 1.334	27.66 ± 7.21	12.33 ± 5.56	6.32 ± 1.22

Values represent Mean ± SEM bearing different superscripts in the same rows differ significantly ($p<0.05$). One-way analysis of variance followed by Tukey's multiple comparison test.

Table 4: Effect of IMI and Cur exposure in Swiss albino mice on Acetylcholinesterase (AchE) Activity, Lipid Peroxidation, Catalase Activity, Superoxide Dismutase Activity IMI: imidacloprid; Cur: curcumin.

Test	Group I Control	Group II 1mg/kg bw IMI	Group III 2mg/kg bw IMI	Group IV 2mg/kg bw IMI + 100mg/kg bw Cur
Catalase (µmole H202 decomposed/ min//mg protein)	41.325 ± 3.65	17.69 ± 4.38**	42.47 ± 4.96	19.72 ± 3.49*
Superoxide Dismutase (Units/mg protein)	558.33 ± 121.66	509.41 ± 56.52	439.58 ± 76.63	521.58 ± 105.18
Lipid Peroxidation (n moles /mg protein)	0.046325 ± 0.0072	0.042075 ± 0.0048	0.11735 ± 0.0134*	0.047575 ± 0.0206
Acetylcholine (µM acetylthiocholine hydrolysed/min/mL)	0.004857 ± 0.0012	0.003146 ± 0.0005	0.003659 ± 0.0008	0.003360 ± 0.0007

Values represent Mean ± SEM bearing different superscripts in the same rows differ significantly * indicates ($p<0.05$) and ** indicates ($p<0.01$). One-way analysis of variance followed by Tukey's multiple comparison test

($p<0.05$). However, catalase activity was significantly ($p<0.05$) increased in 2 $mg.kg^{-1}.bw^{-1}$ (42.47 ± 4.96*) exposed animals compared to the low dose (1mg/kg bw) group, reaching almost control values. Treatment with curcumin has shown a significant ($p<0.05$) decrease (19.72 ± 3.49*) in comparison to the control.

Superoxide Dismutase (Table 4): Superoxide dismutase activity was not affected by IMI exposure across all the treatment and co-treatment groups compared to the control.

DISCUSSION

The growing evidence of the toxic effects of various pesticides, including imidacloprid in animals and humans, as non-target organisms, calls for assessing the adverse health effects it could exert. The most available literature on the toxicity of such chemicals is based on high doses and the adult population. While the effects of acute and sub-chronic low-level exposure, especially in young and developing population, has not been well investigated. The effects of such xenobiotics ought to be well studied among the most vulnerable population, i.e., neonates and young offspring. The developing brain goes through various changes and is far more susceptible to these harmful toxins than the adult brain. Thus, to better understand the toxicity of imidacloprid, the present study was designed to evaluate whether a consecutive 40 days of exposure to imidacloprid at doses (1 $mg.kg^{-1}.bw^{-1}$ and 2 $mg.kg^{-1}.bw^{-1}$) lower than what most past studies have been based on (El-Gendy et al. 2009, Badgujar et al. 2013, Lonare et al. 2014). These are environmentally relevant with respect to mammals and humans and produce neurobehavioral effects and oxidative stress in young male mice weanlings (Kara et al. 2015, Khalil et al. 2017, Burke et al. 2018). The present doses were selected based on several toxicological reference values (acceptable daily intake, no-observed effect level dose, and acute oral LD50). They were applied orally to 21 days old male Swiss albino weanlings.

Results have indicated that imidacloprid treatment of male mice weanlings has increased body weight gain in treated animals compared to the control group at the end of 40 days of exposure. This increase in body weight in the treated animals can be attributed to the fact that male Swiss albino weanlings were in their growing age during the exposure period. Further, imidacloprid has been reported to impair glucose and lipid metabolism, leading to insulin resistance and weight gain, consistent with the present finding (Kim et al. 2013, Sun et al. 2016).

In the open field test, locomotory behavior indicates locomotor and exploratory activities, whereas freezing can positively correlate with fear and emotionality (Khalil et al.2017). In the current study, IMI exposure led to a non-significant decrease in exploratory behavior. This suggests that exposure to low doses of IMI during the development phase does not affect the locomotor and exploratory activity of young Swiss albino mice. Similar results have been reported by Terçariol & Godinho (2011), where open-field behavior was not affected significantly at the low dose level of fipronil with respect to the other dose levels used in the study.

In the Morris water maze (MWM), performance was used to assess spatial learning and memory in young mice. In the present study, a non-significant increase in latency to reach the platform in the target quadrant was observed at both the dose levels compared to the control. Indicating that cognitive skills are not affected due to sub-chronic low-dose exposure to IMI in developing Swiss albino male mice. The present finding is consistent with Koslowski et al. 2020 where long-term low-dose exposure to another neonicotinoid fipronil did not affect memory and cognitive skills.

Acetylcholinesterase (AchE) is a cholinergic enzyme at postsynaptic nerve endings. It breaks down the naturally occurring neurotransmitter acetylcholine. Results from the

present study have indicated a non-significant decrease in the levels of acetylcholinesterase activities at both the dose levels compared to the control. Similar results have been reported by Tariba Lovaković et al. (2020) at 0.06 and 0.8 $mg.kg^{-1}.bw^{-1}$ IMI dose levels.

Antioxidant enzymes such as catalase, superoxide dismutase, and lipid peroxidation protect the cellular components from reactive oxygen species by deactivating the free radicals (Kurutas 2016).

Peroxidation of the membrane lipids affects the structure, functions, and activity of various membrane-bound enzymes and transport mechanisms. In the present study, imidacloprid exposure to mice at 2 $mg.kg^{-1}.bw^{-1}$ has significantly increased brain LPO levels. The present finding is consistent with results obtained by (El-Gendy et al. 2009, Duzguner & Erdogan 2009, Lonare et al. 2014). These studies were carried out on doses much higher than those selected in the present study. Present findings further strengthen the hypothesis suggesting that oxidative stress is one of the central pathways by which such pesticides exert their cytotoxic effects (Mahaboob Khan & Kour 2007, El-Gendy et al. 2009).

After an initial decrease at the 1mg/kg bw dose level, a significant increase in the activity of the antioxidant enzyme catalase was observed at the 2 $mg.kg^{-1}.bw^{-1}$ dose level compared to the 1 $mg.kg^{-1}.bw^{-1}$. It acts as the first line of defense against the oxy-free radicals generated due to the toxicity of imidacloprid. This has been attributed to the fact that these defense mechanisms against oxidative stress are in the process of attempted cellular repair. The present finding is consistent with the results obtained in studies where catalase activity was found to increase when male Swiss albino mice were exposed to $1/10^{th}$ of LD50 of IMI and in another study where animals exposed to IMI intravenously (El-Gendy et al. 2009) but in much higher dose levels compared to those selected in the present study.

Plant-based drugs help alleviate oxidative stress induced by various environmental neurotoxicants. Evidence has shown that consuming naturally occurring antioxidants can decrease oxidative stress markers (Vouldoukis et al. 2004, Lonare et al. 2014). Curcumin has been known Scavenge free radicals and induce detoxification of enzymes protecting against degenerative disease and cancer (Kim et al. 2014, Duan et al. 2014). The antioxidant mechanism of Cur has been attributed to its unique conjugated structure, uses an intramolecular Diels-Alder reaction, and uses linoleate as oxidizable poly-unsaturated lipid (Masuda et al. 2001, Guo et al. 2011, Lonare et al. 2014).

Co-treatment with curcumin resulted in a decrease in body weight gain and an increase in the neurosomatic index, indicating that curcumin could partially reverse the toxic effects of IMI. However, no effect was seen in the open field and Morris water behavior after curcumin co-treatment.

While results from the present study have indicated that the animal group co-treated with curcumin and imidacloprid has shown significant improvement in the antioxidant parameters such as catalase activity and lipid peroxidation, at the same time, no effect was seen in the activity of superoxide dismutase and acetylcholine esterase.

Adolescent brains undergo several anatomical, physiological, and biochemical changes during puberty. Such maturational modifications can influence the pharmacokinetics of these xenobiotics (Blakemore et al. 2010; Kaur et al. 2023). Further, it may also be due to the lower absorption of active insecticide metabolites from the digestive system of young male mice weanlings following oral administration, which results in lesser bioavailability of IMI to tissues (Kim et al. 2007). It has been reported that the major enzymes responsible for the metabolization of IMI P450 (CYP450), especially CYP2C19 and flavin monooxygenase (FMO), have age-dependent functional changes and have been reported to display comparatively greater catalytic effectivity in adolescent liver samples compared to adult samples (Schulz-Jander & Casida 2002, Basaran & Can-Eke 2017). Thus, it can be said that the consequences of IMI metabolism may differ in young and adults. Thus, adolescent mice could detoxify IMI more effectively (Zane et al. 2018; Zhang et al. 2015). It has also been reported that adolescent renal and hepatic clearance capability surpasses the adult capability to do the same. Hence, they metabolize pesticides more easily than adults (Bruckner 2000; Kirti 2023).

CONCLUSION

Our data show that low doses of pesticides like imidacloprid can disrupt the biochemical profile, leading to the generation of oxidative stress in the brains of the developing population; however, behavior and cognitive skills appear unaffected. The young developing population can metabolize and detoxify the pesticides more effectively than adults.

Further, staying on top of oxidative stress is essential in the increasingly toxic world. Results from the present study have indicated that curcumin, a naturally occurring antioxidant, can restore the antioxidant enzyme profile effectively. Thus, dietary intake of such naturally occurring substances by individuals who come in direct and regular contact with pesticides is beneficial in combating the deleterious effects of imidacloprid.

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