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Neurobehavioral Assessment of Imidacloprid (Neonicotinoid insecticide) in Swiss albino mice

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ABSTRACT:
The behavioural effect of imidacloprid pesticide was investigated in Swiss albino mice when exposed for sub-acute period (7 days) utilizing open field (OF), Hole board test (HB) and social interaction test. For sub-acute toxicity study groups of adult mice (n=18) were given imidacloprid orally by gavage, one group was taken as control second group was taken as low dose (90mg/kg bw) and third group was taken as high dose (110 mg/kg bw).In open field test, the number of blocks crossed by mice in both low dose (90mg/kg bw) and high dose (110mg/kg/bw) groups of imidacloprid decreased significantly when compared with the control. In the HB test at high dose level of imidacloprid i.e., 110mg/kg bw significant increase in freezing time and decrease in exploration time was observed.A decrease in grooming, sniffing and crawling behaviour was also observed in social interaction test after imidacloprid exposure. However subacute exposure of imidacloprid has no effect on histopathology of brain areas.
KEYWORDS:Imidacloprid, sub-acute exposure, behavioural effects, histopathological effects, brain**.**

I. INTRODUCTION

In the last few decades, there is a growing concern that pesticides and other pollutants alone and in combination are found to cause harmful effects in organisms (Sharma et al 2019;2021). These chemicals might modify normal physiological functions of organisms (Kaur et al 2021 and Sharma 2014) and causes a variety of adverse effects including developmental, reproductive and behavioral effects.Imidacloprid is representative of the neonicotinoid insecticides, a fastest growing class of insecticides introduced to the market since the commercialization of pyrethroids (Nauen and Bretschneider, 2002). Neonicotinoids are broadly used to control sucking insects and flea on crops. Imidacloprid insecticide has been recorded with the world's fastest growing sales and is considered as a possible replacement for organophosphorus insecticides in agriculture. As per records, the global annual production of Imidacloprid was reported to be 20,000 tons in 2010 (Simon-Delso et al., 2015).

Neonicotinoids work like nicotine, they act as agonists at nicotinic acetylcholine receptors present in brain with similar action in insects and mammals (Tomizawa & Casida, 2003).Neonicotinoid's pesticides work as neurotoxicants. Imidacloprid has been reported to accumulate in soils, water and persist in crops, vegetables and fruits, thereby extensively exposing non-target animals and humans to neonicotinoids including imidacloprid (Bonmatinet al., 2015).Toxicological studies of imidacloprid are limited. Most of the studies were observed on insects. It acts at the distinct acetylcholine receptor (AChR) subtype in cockroach nervous system. It also induced facilitation of proboscis extension reflex habituation in the honeybee (S Buckingham et al., 1997). Few studies have reported that imidacloprid is less toxic to fish and but it is highly toxic to bees and house sparrow. (Agha et al, 2012, David et al., 2007,Mohammad et al., 2009).

Despite the lower toxicity of imidacloprid to mammals than to invertebrates, manyinvestigators have demonstrated it to be toxic to vertebrates, both aquatic and terrestrial (Mineau and Palmer, 2013). The toxicity assessment of imidacloprid onhuman peripheral blood lymphocytes, rat bone-marrow cells, and lymphocyte culture of rabbits has exhibited potential genotoxicity (Demsia et al., 2007; Stivaktakis et al., 2010). Also, imidacloprid was reported to induce an oxidative stress in male mice. It was also found to be hepatoxic and lead to impairment in male reproductive system of rats ((El-Gendy et al., 2010). In white Leghorn cockerels, it resulted in immunotoxic effects,endocrine disruption of the hypothalamic– pituitary– thyroid (HPT) axis in birds (Pandey and Mohanty, 2015); and neurobehavioral deficits in rat pups (Kara et al.,

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2015).A dose related reductions in body weight gain was observed in pregnant rats and rabbits (Becker et al., 1988). On human, it showed adverse effects i.e., chronic toxicity, reproductive effect and many more. The acute effects of Imidacloprid on behavior of vertebrates have notbeen very well characterized. A study on zebra fish showed neurobehavioral impairments caused bydevelopmental imidacloprid exposure, with significant decreased in novel tank exploration.

Similarly, the literature on histopathology of imidacloprid on rat is also scanty. Recently few histopathological studies different organs of rat liver, heart, kidneys etc (Mohany et al., 2011), In light of the above information weplannedneurobehaviouralassessmentofImidaclopridpesticide.

II. METHODOLOGY

Test animals

The adult Swiss albino mice were maintained on a 12 h light/dark cycle at 25±2°C, and relative humidity 50-70% and were given free access to food and water. Mice were habituated to the behavioural lab conditions prior to their use. For acute toxicity study three groups of adult mice (n=6) were given imidacloprid orally by gavage, one group was taken as control second group was taken as low dose (90mg/kg bw) and third group was taken as high dose (110mg/kg bw).

Treatment with pesticide

Commercial formulation of imidacloprid was used for treatment of mice. Adequate dilutions were made with distilled water to achieve the test concentrations. The experiment was conducted by dividing animals into three main groups (Groups I–III), The dose levels administered were as follows: Group I - control with standard diet and distilled water only; Group II - with IMD 90mg/kg bw (low), and Group III - 110(high) mg/kg bw respectively.

Experiment

To evaluate behavioural alterations by imidacloprid toxicity mice were transferred to the testing room in their home cages, and were kept for 45 minutes to get habituated. Behavioural analyses using open field, hole-board, social interaction test was done. All behavioural sessions were recorded between 10:00 and 12:00 a.m.

The open-field test

The open-field test was conducted in a square wooden area measuring 60 ×60 ×15 cm (Michael et.,al 2015) the mice were placed individually at the center of the open field arena and behavioural parameters were monitored for 5 minutes. The open field apparatus was then thoroughly cleaned with 95% ethanol before placing the next mice to preclude the possible cueing effects of odours left by previous mice. The behavioural parameters observed were locomotor activity (the number of floor sections entered by both feet), rearing (the number of times the animal stood on its hind legs), grooming (the number of grooming movements), and immobility (freezing) duration (time period for which animals shows no movements). The open field was divided into two squares, and the number of entries and time spent in the central area were recorded.

Hole Board Test

Hole board maze is an arena with holes in the floor. It is recommended as a test that can provide measure of exploration and curiosity behaviour of mice. The test was conducted in a square wooden area measuring 60 ×60 ×15 cm. In this mouse were allowed to move around and dip it's head into the holes. Poking the nose into a hole is a normal behaviour of mice. The hole board was cleaned with alcohol behavioural session. Then mice were placed at the corner of hole board maze and general exploratory behaviour, number of head dipping, duration of head dipping, grooming frequency and freezing frequency were observed for 5 min.

Social Interaction Test

Social interaction tests were conducted in their home cages for 10 minutes. The design of the social interaction test is suitable for use with naïve animals. Pairs of mice were allowed to freely interact in an arena while time spent interacting was recorded. The experiment involves one mice receiving treatment while the other serves as a control, then interaction time initiated by the treated mice were measured. Social interaction behaviour was observed between pairs of mice that were group housed for 3–6 week and are unfamiliar. The social interaction test consists of monitoring the duration of time for which the test animal was allowed to interact (genital investigation, sniffing, following, grooming, kicking, crawling under or over the partner, and touching) with a naïve mouse.

Histopathology of brain tissue

The animals were housed in a group of four in a wire topped polypropylene cage with saw dust. The control and tested animals were given food in pellet form and water ad libitum. The body weights of control as well as treated mice were taken before

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the start of the treatment and then on the day of sacrifice. On the completion of experiment, the mice were sacrificed by cervical dislocation and were perfused transcardially with fixative formalin and with phosphate buffer solution then whole brain was transferred to a formalin (10mL) vial tube for 48hr and then transferred to 30% of sucrose solution and after brains were processed for sectioning.

For light microscopic examination, paraffin sections of brain tissues were cut into 20 µm thickness and stained with hematotoxin and eosin protocol. Thestained slides were studied under an optical light microscope and the architecture in different regions of mice brain i.e., cortex, cerebellum and hippocampus were screened for any histopathological changes in their neurons

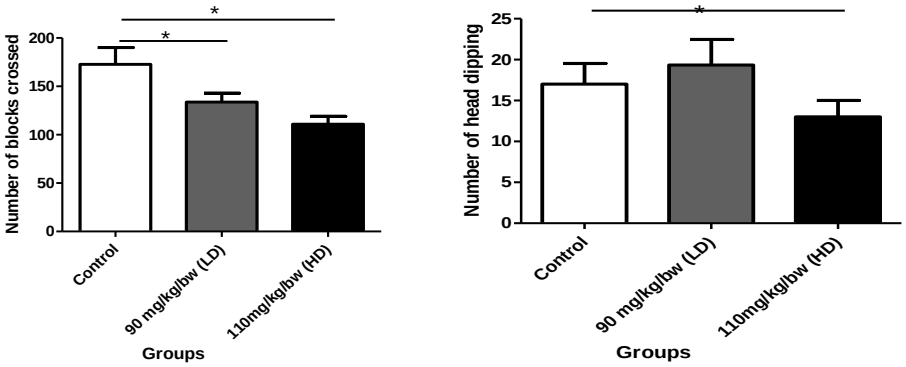

Figure-1a. Effect of acute exposure of imidacloprid on behaviour of Swiss albino mice. The data are represented as mean ± SEM of (a).Number of blocks crossed in open field test b. Number of head dipping in hole board test. Data were analysed by one-way ANOVA followed by"Dunnett's Multiple Comparison Test". N = 6 in each group. * $p < 0.05$, ** $p < 0.01$.

III. RESULTS AND DISCUSSION

The effects of Imidacloprid in the open field behaviour are summarized in the figure 1a.In open field test, the number of blocks crossed with both feet were observed and here we observed that the number of blocks crossed at low dose(90mg/kg bw)and at high dose (110mg/kg/bw) decreased significantly when compared with thecontrol.The open-field test is considered as a marker of emotional condition of the living organisms and therefore utilized for the assessment of agents that act on the CNS (Eidman., et al1990).It helps in the measuring behavioral responses like exploratory behaviour.The decrease in number of blocks crossed is an indicator of decreasesexploration in imidacloprid treated mice suggesting that IMD could increase the emotionality, thereby decreasing the locomotor activity. This could be related to the exploration acting as a good index of stress response in rodents(Eidman., et al 1990).

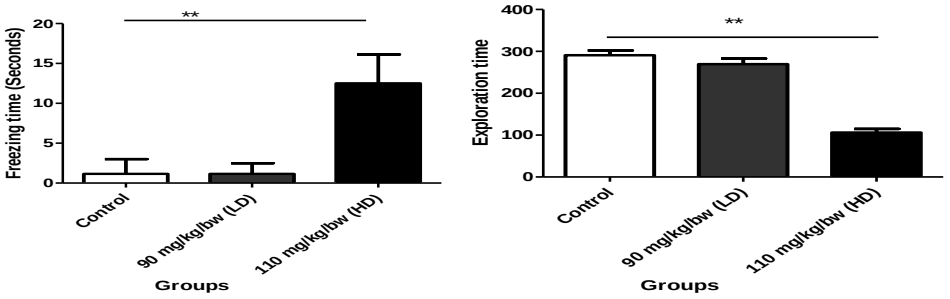

Figure-2a. Effect of acute exposure of imidaclopridon behaviour of Swiss albino mice. The data are represented asmean ± SEM of (a). Freezing time in Hole board test (b). Exploration time in Hole board test. Data were analysed by one-way ANOVA followed by"Dunnett's Multiple Comparison Test" N = 6 in each group. * $p < 0.05$, ** $p < 0.01$.

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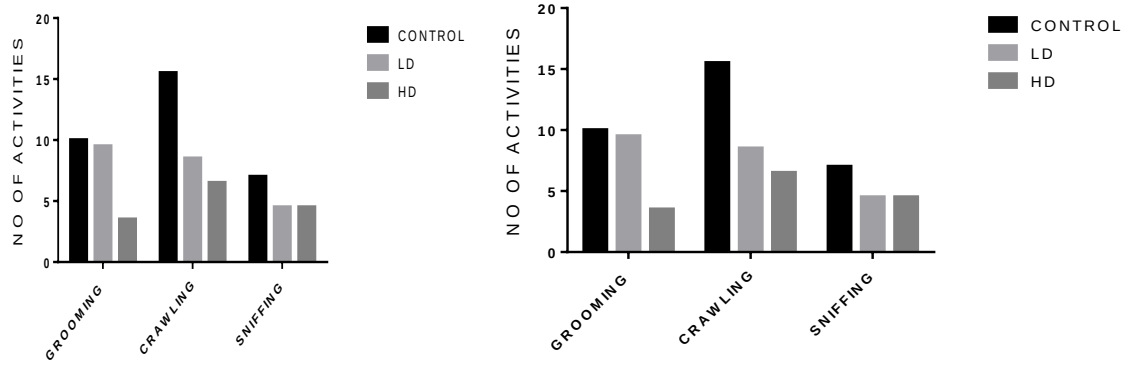

Figure-3. Effect of acute exposure of imidaclopridon social behaviour of Swiss albino mice. Number of grooming, Crawling and Sniffing attemptsN = 6 in each group.

Hole Board Test

The effects of imidacloprid in hole board behaviour are summarized in the figure 1b and figure 2a, b. After exposurecuriosity and exploratory behaviour was quantified by observing number of head dipping, duration of head dipping,grooming frequency, exploration time and freezing frequency of animal in hole board. We observed low dose of imidacloprid i.e., 90mg/kg bw did not affected the curiosity behaviouri.e., number of head dipping, duration of head dipping, grooming frequency as compared to control but at high dose level of imidacloprid i.e., 110mg/kg bw significant increase in freezing time and decrease in exploration time was observed, which is an indicator of increase in anxiety behaviour due to imidacloprid exposure.

Hole board test provide an independent measure of exploration and motor activity. In our experiment animals exposed to imidacloprid at 110mg/kg bw increased freezing and decreased exploration in hole suggesting a stimulatory effect of imidacloprid in the central nervous system of mice.

Social interaction test - The effects of imidacloprid in social interaction test are summarized in the figure 3. We investigated number of behaviour parameter like genital investigation, following, kicking and touching were not affected when treated with imidacloprid but a decrease in grooming, sniffing and crawling (under or over the partner) were observed as compared to control. This again reflects the anxiety in animals.

Histopathologicalexamination

In the present study we have observed different areas of brain like cerebral cortex, cerebellum and hippocampus.But at these dose levels we have not observed any pathological change in nerve cell bodiesand other cells of these areas.

IV. CONCLUSION

In conclusion, sub-acute oral exposure to imidacloprid causes effects related to emotionality, and exploratory activity but does not cause histopathological changes in brain area.

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