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Toxicological Investigation of Single Oral Dose Administration of Imidacloprid in Male Wistar Rats

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Abstract

The aim of this study was to evaluate the acute toxic effect of Imidacloprid (IM) on adult wistar rats. Rats were divided into four different groups. Animals from Group I and II were received water and corn oil, respectively, while, Group III and IV were received oral dose of IM @ 150mg/kg and 300mg/kg body weight, respectively. Thereafter, animals were subjected to series of neuro-toxicity related parameters (general behavioral changes, motor activity, pentobarbitone induced sleeping time and measurement of pain response through mechanical stimulation, radiant heat and tail clip method). IM administration in rats produced significant behavioral depression which resulted into decrease in alertness, awareness and grip strength, and there was reduction in the response to sound, touch and pinna reflexes as compared control. Further, IM resulted in significant decrease in spontaneous locomotor activity and forced locomotor activity (rota-rod performance). IM administration significantly increased the pentobarbitone-induced sleeping time without altering the onset of sleeping time. Orally administered IM did not produce any significant analgesic or hyperalgesic effect. The present study suggests that IM has pronounced CNS effect without effecting analgesic activity during acute toxicosis in animals.

Keywords: Analgesic Activity, Imidacloprid, Motor Activity, Sleeping Time

1. Introduction

Pesticides with different chemical structures are being used worldwide in agriculture. It has significant public health benefit by increasing food production and productivity to fulfill demand of food forever increasing human and animal population. At present, India is the largest producer of pesticides in Asia. Among the class of pesticides, neonicotinoid insecticides, one of the newer class and fastest growing pesticides, are widely used to treat crops as well as livestock against a broad range of commercially important sucking and chewing pests[1,2]. Neonicotinoid insecticides act as agonists at the insect nicotinic acetylcholine receptors (nAChRs), which plays an important role, in synaptic transmission, in the central nervous system[3,4]. However, indiscriminate use of neonicotinoid insecticides in agricultural systemic has resulted in the unintended contamination of aquatic environments and other form of life[5].

Imidacloprid (IM), a chlorinated analog of nicotine belongs to neonicotinoid insecticides, which is basically neuro-active insecticide, irreversibly binds to the nicotinergic receptors in postsynaptic nerves and preventing binding of acetylcholine[6]. It acts as a nicotinic acetylcholine (nACh) partial agonist and not degraded by acetylcholinesterase[7]. It has a higher binding affinity for insect nerve receptors as compared to mammalian receptors[8] due to its specificity for this type of receptors, which are found more often in nervous system of insects and zooplankton than that of other animal species (except

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earthworms and a few species of fish). IM has low mammalian toxicity, but chronic exposure may cause heart, kidney and other organ damages and even death besides gastrointestinal irritation and neurological symptoms[9,10]. There are some reports on IM having genotoxic effect on different animal species[11,12], teratogenic[13], carcinogenic[14], acute neurotoxic[15–17] and toxic effects on male and female reproduction[18–20] in animals and humans.

Long and short term consequences resulting from IM exposure has been studied in detail. However, no system study is available on acute toxic effect of IM on motor activity and analgesic activity with exposure to high doses IM. Thus, study was planned to investigate the toxic manifestation of IM exposure in male wistar rats.

2. Materials and Methods

2.1 Chemicals

Technical grade imidacloprid (purity 95.45%) was kind of gift from M/S Indofil Chemical Company, Division of Indofil Organic Industries Ltd., India. All the other chemicals were used of analytical grade and purchased from standard manufacturers from India.

2.2 Experimental Animals

Adult male Wistar rats of 8 weeks and weighing 110-120g were procured from Laboratory Animal Resource Section, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly for the present study. The rats were housed and maintained at 23±1°C with 57-60% relative humidity under standard managemental condition with 12 h dark and 12 h light cycle. During this period provided free access to standard ration (Feed Unit, IVRI) and water *ad libitum*. The rats were housed 7 days' prior experimentation for acclimatization before commencing the experiment. Prior start of study approval was obtained from the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA), Ministry of Environment and Forests, Government of India.

2.3 Maximum Tolerable Dose (MTD) Determination

The OECD method was used to determine MTD in rats[21]. Test material was orally administered in graded doses and animals were monitored for signs of toxicity, behaviour and lethality/ mortality at 1, 2, 6, 12 extended upto 24h intervals after test material administration. Test material was found to be well tolerated up to 300mg/kg.

2.4 Experimental Design

Adult male Wistar rats were divided into four different groups comprising of 6 animals each. Group I and II were received deionized water (control) and corn oil (Vehicle control: 1ml/kg body weight; *po*), respectively. Group III and IV was treated with single oral dose of IM @ 150mg/kg and 300mg/kg body weight; *po*, respectively. One hour after administration of drug, animals were screened for evaluation of neuro-toxicity related parameters.

2.5 General Behavioral Profiles

The animals were under observation for the behavioral changes and sign of toxic symptoms, if any, at 30 min intervals in the first two hours and at the hourly intervals for the next 4 hours. The awareness, alertness, righting reflex and touch response was recorded.

2.6 Motor Activity

2.6.1 Measurement of Spontaneous Locomotor Activity (SLA)

The animals were exposed to photoactometer activity cage before administration of drug and observed for 5 min for their locomotor activity. Spontaneous Locomotor Activity (SLA) was evaluated 1 h after dosing. Animals were individually placed in photosensitive cages by using automated photoactometer animal activity monitors (UGO Basile Comerio-VA, Italy). The activity was monitored for horizontal as well as vertical movements for the duration of 5 min.

2.6.2 Measurement of Forced Locomotors Activity (FLA)

FLA was measured 1 h after drug administration using an automated rotating rod using rota rod instrument. Fresh rats were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 30 rpm. The rats capable of remaining on the top for one min or more, in five successive trails were selected for this study. Number of fall within five minute on horizontal plane from rotating rod

was recorded and time spend on the rotating rod was calculated[22].

2.7 Measurement of Pain Threshold

Pain threshold was measured by mechanical method (Randall-Selitto assay and tail clip assay) and radiant heat latency method.

2.7.1 Pain Measurement by Randall-Selitto Assay

Mechanical stimulation was done by applying progressive pressure in grams on hind paw of rats by Randall-Selitto assay method[23] using Randall-Selitto analgesiometer (UGO Basile Varese, Italy). The test consisted on the progressive application of an increasing point-pressure over the hind paw, which evokes a pain reaction characterized by a fighting reaction (struggle) and a vocalization as manifestations of the pain sensation. Recording of both hind paws was done.

2.7.2 Pain Measurement by Tail Clip Assay

All the rats were screened by applying a metal artery clip to the base of the tail with its jaw sheathed with thin rubber tubing. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. The animals that did not attempt to dislodge the clip within 10 s were not used for the experiment[24]. Response was evaluated 1h after administration drugs and reaction time recorded in second.

2.7.3 Pain Measurement by Radiant Heat Method

Paw-flick latency in seconds was recorded as paw withdrawal by the animal against infrared radiant heat (Ugo, Basil, Varese, Italy). Rats were screened out for sensitivity before the administration of test drug and animal withdrawing the paw within 10 second were selected and used for this parameter.

2.8 Pentobarbitone Induced Sleeping Time

The induction of sleep (time) and sleeping time measured as the time interval between the loss and regaining of the righting reflex[25] were recorded in different treatment groups. Animals received 40mg/kg (*i.p.*) pentobarbitone sodium 1hr after the administration of IM to all the groups and time of induction of sleep and duration was recorded.

3. Results

3.1 Effect on General Behavior Profile

Oral dose administration of IM produced significant behavioral changes exposed animals (Table 1). IM administration produced depression amongst animal which resulted into decrease in alertness, awareness and grip strength as compared control. Change in response to sound, touch and pinna reflexes were slightly less in IM treated groups as compared to control group. Moderately less righting reflexes were observed 1 h after IM administrations.

3.2 Effect on Motor Activity

3.2.1 Spontaneous Locomotor Activity

Single oral administration of IM *(150 and 300mg/kg)* resulted in significant decrease ($p>0.05$) in horizontal and vertical activity as compared to control group

Table 1. Effect of single oral dose administration of IM on general behaviour profile in male rats

Behaviour type	Control	Corn coil	IM-I	IM-II
Alertness	+ + + +	+ + + +	+ +	+
Awareness	+ + + +	+ + + +	+ +	+
Sound response	+ + + +	+ + + +	+ +	+ +
Touch response	+ + + +	+ + + +	+ + +	+ +
Pinna reflex	+ + + +	+ + + +	+ + +	+ + +
Righting reflex	+ + + +	+ + + +	+ + +	+ +
Grip strength	+ + + +	+ + + +	+	-

IM: Imidacloprid;
+ + + +: very high activity; + + +: high activity; + +: moderate activity; +: very low slight activity; - : completely Nil,

(Table 2). Non-significant (p>0.05) decreased in SLA was observed between the treatment groups (IM-I and IM-II).

3.2.2 Force Locomotor Activity

IM treatment groups showed significant decrease in time spent on the rotating rod when compared with control group (Table 2). A non-significant (p>0.05) decrease in time spent on rod was noticed between the IM treatment groups.

3.3 Effect on Pain Threshold

3.3.1 Pain Response in Randall-Selitto Method

Orally administered IM (150 and 300mg/kg) did not produce any significant (p>0.05) analgesic or hyperalgesic effect in rats after post administration as compared to control (Table 3). No significant change in paw withdrawal threshold was recorded in different treatment groups.

3.3.2 Pain Response in Radiant Heat Stimulation

In radiant heat stimulation, IM (150 and 300mg/kg; *po*) did not produced significant (p>0.05) effect on reaction time after 1 h post administrations as compared to control animals (Table 3).

3.3.3 Pain Response in Tail Clip Method

Oral administration of IM (150 and 300mg/kg) did not produced significant effect on reaction time against mechanically induced pain by tail clip as compared to control animals (Table 4).

Table 2. Effect of single oral dose administration of IM on spontaneous locomotor activity (SLA) and force locomotor activity (FLA) in male rats

Treatment	Dose	Spontaneous locomotor activity		Force locomotor activity (Time spend in Sec.)
		Horizontal activity	Vertical activity	
Control	-	916.167±32.99[a]	148.00±5.46[a]	200.00±12.62[a]
Corn oil	1 ml/kg	929.67±39.93[a]	157.50±6.14[a]	216.67±15.01[a]
IM-I	150mg/kg	279.50±16.69[b]	49.50±3.05[b]	40.99±3.17[b]
IM-II	300mg/kg	269.00±15.75[b]	41.33±2.23[b]	23.64±2.38[b]

IM: Imidacloprid; Values (Mean ± SEM, n=6) bearing different superscript in the same column differ significantly (p<0.05) in Tukey's multiple comparison post hoc test.

Table 3. Effect of single oral dose administration of IM on mechanical and radiant heat stimulation in male rats

Treatment	Dose	Mechanical stimulation (pressure in gm)	Radiant heat stimulation (reaction time in Sec)
Control	-	49.17±2.71	3.31±0.15
Corn oil	1 ml/kg	48.33±3.47	3.37±0.16
IM-I	150mg/kg	42.67±2.79	3.33±0.07
IM-II	300mg/kg	44.83±3.91	3.25±0.17

IM: Imidacloprid; Values (Mean ± SEM, n=6) bearing different superscript in the same column differ significantly (p<0.05) in Tukey's multiple comparison post hoc test.

Table 4. Effect of IM on tail clip response and petobarbitone sodium induced sleeping time in male rats

Treatment	Dose	Tail clip response (Reaction time in Sec)	Pentobarbitone induced sleeping time	
			On set of sleep in min)	Sleeping time in min)
Control	-	3.52±0.21	4.33±0.03	57.50±2.11[a]
Corn oil	1 ml/kg	3.51±0.22	4.17±0.03	68.83±2.57[a]
IM-I	150mg/kg	3.50±0.16	4.50±0.07	180.83±4.56[b]
IM-II	300mg/kg	3.33±0.19	4.50±0.05	216.17±4.61[c]

IM: Imidacloprid; Values (Mean ± SEM, n=6) bearing different superscript in the same column differ significantly (p<0.05) in Tukey's multiple comparison post hoc test.

3.4 Effect on Sleeping Time

IM administrations (150 and 300mg/ kg, orally) did not alter induction of sleeping time/onset of sleep (min) but significantly increased the duration of sleeping time induced by pentobarbitone sodium in dose dependant pattern as compared to control group (Table 4).

4. Discussion

In present study, acute oral dose of IM (single large dose - 150 and 300mg/kg) administration produced general behavioural change in animals, significant depression amongst animal, and decrease in alertness, awareness and grip strength. Animals showed Low response to sound and touch 2 h post administrations. Similarly, acute dose of IM has been shown to produce twitching, cramps, spasms, apathy, respiratory disturbances, decreased mobility, staggering gait, narrowed palpebral fissures, transient trembling and muscle weakness leading to asphyxia and fatigue in different species of animals[7,26]. It results in severe hypertension, tachycardia, mydriasis with sluggish reaction to light and loss of consciousness, further, manifested bradycardia, eupnea, cardiopulmonary arrest and death[27]. IM significantly decreased horizontal and vertical activity in SLA and time sent on the rotating rod in FLA was observed in this study. Likewise, tremors, uncoordinated gait and decrease activity were observed in male rats fed high dose of IM and dose related decrease in motor and locomotor activity[28]. Further, reduced locomotion, and seizures, behaviors with predominantly central components has been reported, suggesting a central site of action on nicotinic AChRs[29]. It has been reported that IM produce direct toxic effect through action on nAChR which were reflected from earlier studies while, delayed toxic effect may be due the guanidine (C=NH) metabolite which is highly active against mammalian nAChR and more toxic than that of parent compound[30].

Acute dose of IM administration did not alter onset of sleeping time but significantly, increased pentobarbitone induced sleeping timing in present study. Similar reports have been reported on pesticide exposure. Alpha-cypermethrin caused significant motor incoordination, decrease in the time of onset of sleep and increase in the duration of sleeping time induced by petobarbitone[31]. IM potentiated the effect of pentobarbitone induced sleeping time. IM itself produced sluggish reaction and loss of consciousness, cardiopulmonary arrest and some time death[27] after systemic absorption from GIT. This might be responsible for increase in duration of sleeping time. It also indicates that IM does not have any inducing effect on liver metabolizing enzymes which is responsible for pentobarbitone metabolism. Otherwise, that might be reflected in terms of decrease in sleeping time and induction of sleeping time.

Acute dose of IM did not produce significant effect on central analgesia which was seen in mechanical stimulation (Randal Selitto) and time latency or reaction time in response to radiant heat and tail clip application in the present study. However, it has been reported that, antinociception property is not associated with IM insecticides even though IM is derivative of nicotine[32] and nicotine have modest analgesic (antinociceptive) activity of short duration in rodent tail-flick tests[33]. From previous study it has been cleared that IM does not have analgesic effect and does not have all the activities similar to that of nicotine though it is derived from parent compound nicotine.

Results of the present study indicates that accidental exposure to high doses of IM may results in non-specific decrease in motor and other kinds of activities through central depressant mechanism without affecting metabolizing enzymatic activity. Further IM may or not have central and peripheral analgesic activity. Finally, this study suggests that high dose administration of IM responsible for altered CNS activity without producing analgesia in animals.

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