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Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats

Upasana Kapoor, M.K. Srivastava, L.P. Srivastava *

Pesticide Toxicology Division, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), P.O. Box No. 80, Mahatma Gandhi Marg, Lucknow 226 001, India

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ABSTRACT

Technical imidacloprid was evaluated for its effect on ovarian morphology, hormones and antioxidant enzymes in female rats after 90 days oral exposure. Luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone levels were estimated in serum of rats and activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and level of reduced glutathione (GSH) and lipid peroxidation (LPO) were estimated in ovary after oral administration of imidacloprid (5, 10, and 20 mg/kg/day) for 90 days. Decreased ovarian weight together with significant patho-morphological changes in follicles, antral follicles and atretic follicles were observed at 20 mg/kg/day. Imidacloprid at 5 and 10 mg/kg/day has not produced any significant changes in ovarian morphology, hormones and antioxidant status of ovary. However 20 mg/kg/day dose has produced significant alterations in the levels of LH, FSH and progesterone. Similarly significant changes in SOD, CAT, GPx, GSH, and LPO were observed at 20 mg/kg/day dose level. Therefore, it is concluded that imidacloprid at 20 mg/kg/day dose level has produced significant toxicological impact on ovary of female rats as evident by pathomorphological changes, hormonal imbalance and generating oxidative stress and can be considered primarily as Lowest Observed Effect Level (LOEL) for chronic study.

1. Introduction

Imidacloprid, a neonicotinoid the newest class of major insecticide has outstanding potency and systemic action for crop protection against piercing and sucking insects pests and also highly effective for control of flea on cats and dogs (Tomizawa and Casida, 2005). It shows selective toxicity to insects over vertebrates attributable to high affinity for insects' nicotine acetyl choline receptors (nAChR). Relative toxicity of several nicotinoids co-relates closely with their relative affinity for insects nAChRs and suggested that nAChR are principle site of action of these compounds (Buckingham et al., 1997; Liu et al., 2005). Imidacloprid is extensively used for crop protection and animal health care. Few cases of acute human poisoning have been reported following ingestion of imidacloprid formulations (Wu et al., 2001; Proenca et al., 2005). Recently imidacloprid has raise concern because of egg shell thinning; reduce egg production and hatching time which are considered as signs of possible endocrine disruptors (Berny et al., 1999; Matsuda et al., 2001). Many pesticides having endocrine disruptors properties are known to adversely impair reproductive competence of male and females (Yousef, 2010). Endocrine disruptor compound mimic the structure of natural hormones found in the animal/human body and their adverse effect are related with their ability to interfere with sex steroid action which finally causes adverse effects on adult reproductive system (Kumar et al., 2008). Since imidacloprid is now being considered for replacement of many existing organochlorine pesticides; therefore an exhaustive toxicological studies are needed to know its relative risk and hazards. Increasing use of imidacloprid and its potential toxicity among human warrants its heightened awareness of this compound. Therefore, this study has been undertaken in continuation of our earlier studies (Bhardwaj et al., 2010; Kapoor et al., 2010) to know its in-depth toxicological information related to morphology of ovary, ovarian hormones, and antioxidant profiles in female rats after 90 days oral exposure.

2. Material and methods

2.1. Chemicals and animals

Imidacloprid technical 96% pure, 1[(6-chloro-3-pyridinyl) methyl]-*N*-nitro-2-imidazolidinimine, [CAS No. 138261-41-3] was obtained from Bharat Rasayan, India Limited, New Delhi, India. Forty adult female rats (*Rattus norvigicus* Wistar strain) weighing 150–155 g of Indian Institute of Toxicology Research, Lucknow breeding colony were maintained under condition of controlled temperature (22 ± 3 °C) and humidity (30–70%) with 12 h light and dark cycle. The animals were given synthetic pellet diet (Ashirwad India Ltd, India) and water *ad libitum*. Rats were acclimatized for 1 week prior to the start of experiment. An approval from animal ethics committee of the Institute was obtained for the use of animals (ITRC/IAEC/04/2010.).

* Corresponding author. Tel.: +91 522 2627586/2613786; fax: +91 522 2628227/2611547.
E-mail address: lpsrivastava@iitr.res.in (L.P. Srivastava).

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2.2. Treatment

After acclimatization period of 7 days, female rats were divided into four groups having 10 rats in each. Group I served as control and was given corn oil (0.4 ml/rat) as vehicle through gavage. Groups II, III, IV were orally administered imidacloprid (5, 10, and 20 mg/kg/day) suspended in corn oil to female rats daily for period of 90 days. Clinical signs of toxicity were observed and recorded throughout the period of exposure.

2.3. Histopathology of ovary

Ovary of control and treated groups were fixed in 10% formal saline solution. After routine processing, paraffin sections were cut at 5 μm thicknesses and stained with heamatoxylin and eosin for microscopic examination.

2.4. Hormones assay

Level of serum follicles stimulating hormone (FSH), luteinizing hormones (LH) and progesterone were assayed using ELISA kit (Herchinson diagnostics Pvt. Ltd., USA). The sensitivity of progesterone, FSH and LH was 0.3 ng/ml, 1.0 ml U/ml and 1.5 ml U/ml, respectively. The coefficient of inter and intra run precision was 2.4% and 2.6% for progesterone, 4.64% and 5.37% for FSH and 6.80% and 5.70% for LH, respectively.

2.5. Necropsy and tissue homogenate preparation for oxidative status

Rats were euthanized and necropsied after 90 days treatment. Ovaries were quickly removed, trimmed of extraneous tissue, washed with ice-cold physiological saline solution and weighed. Pooled (group wise) ovaries were divided into two parts. One half was homogenized with ice-cold 0.15 M KCl solution (10% w/v) for lipid peroxidation (LPO), reduced glutathione content (GSH) and catalase activity (CAT) while another half was homogenized with 0.1 M phosphate buffer solution for superoxide dismutase (SOD) and glutathione peroxidase (GPx).

2.6. Biochemical measurements

2.6.1. Lipid peroxidation (LPO)

Levels of malionaldehyde (MDA), an end product of polyunsaturated fatty acid peroxidation (lipid peroxidation) were measured in ovary homogenate on the basis of the reaction with thiobarbituric acid (TBA) to form a pink colored complex. MDA produced was determined with the absorbance coefficient of the MDA–TBA complex at 532 nm on GBC Cintra 20 Spectrophotometer using 1,1,3,3-tetraethoxypropane as standard (Ohkhawa et al., 1979).

2.6.2. Determination of glutathione (GSH) content

GSH level was determined using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) for color development and reading was taken at 412 nm on GBC Cintra 20 Spectrophotometer after 15 min. A standard curve using reduced glutathione was used for calibration (Ellaman, 1959).

2.6.3. Superoxide dismutase (SOD)

Activity of SOD was determined in the tissue homogenates by modified method of NADH-phenazinemethosulphate-nitriblue tetrazolium formazan inhibition reaction spectrophometrically measured at 560 nm on Spectrophotometer Genesys 10 UV (Kakkar et al., 1984).

2.6.4. Glutathione peroxidase (GPx)

Activity of GPx was determined and expressed in terms of μmole GSH consumed/min/mg protein measured at 420 nm on Spectrophotometer Genesys 10 UV (Flohe and Gunzler, 1984).

2.6.5. Catalase (CAT)

The activity of CAT was determined by the method of Sinha, 1972, in which catalase preparation is allowed to decompose H_2O_2 for a fixed period of time. The reaction was then stopped by addition of dichromate–acetic acid reagent followed by heating for 15 min in boiling water bath. The remaining H_2O_2 was determined by measuring chromic acetate generated at 570 nm on GBC Cintra 20 Spectrophotometer.

2.6.6. Determination of protein

Protein was assayed using Bovine serum albumin (BSA) as standard and optical density read at 690 nm on GBC Cintra 20 Spectrophotometer (Lowry et al., 1951).

2.7. Statistical analysis

Statistical significance between control and experimental values were calculated by Student'*t*' test (Fisher, 1950). All parameters were compared at 5% level of significance.

3. Results

3.1. In-life parameters and relative ovary weight

Repeated oral administration of imidacloprid at 5 and 10 mg/kg/day did not produce any signs of toxicity and mortality during 90 days exposure. However mild to significant toxic symptoms, together with significant decrease in weight gain appear in rats exposed to high dose (20 mg/kg/day) of imidacloprid for 90 days. The relative weight of ovary of rats did not show any change at 5 and 10 mg/kg/day dose levels however significant decrease was observed at 20 mg/kg/day dose (Table 1).

3.2. Histopathology of ovary

Histopathological observation of ovary of control rats showed presence of many developed follicles, antral follicles and atretic follicles (Fig. 1). However repeated exposure of high dose of imidacloprid (20 mg/kg/day) for 90 days showed presence of cytoplasmic clumping and abundant lipofuscin elements in granulosa cells of follicles (Fig. 2). Morphology of ovary of rats exposed to imidacloprid (5 and 10 mg/kg/day) for 90 days have shown about normal architecture.

3.3. Hormone analysis

Serum FSH level was significantly increased while LH and progesterone levels were decreased in rats exposed to imidacloprid at 20 mg/kg/day for 90 days as compared to their respective control. No significant alterations were found in any of these hormones at 5 and 10 mg/kg/day dose levels (Table 3).

3.4. Biochemical studies

There were no significant changes in level of LPO (measured in terms of MDA), GSH content, SOD, CAT and GPx activity in ovary of rats at 5 and 10 mg/kg/day dose levels. However, a significant

Table 1
Clinical signs of toxicity and relative weight of ovary of rats exposed to imidacloprid for 90 days.

Dose (mg/kg/day)	Initial body weight (g)	Final body weight (g)	Weight gain (%)	Total deaths	Signs of toxicity	Absolute weight (g)	Relative weight (%)
0	155.00 ± 2.16	254.00 ± 6.18	64	0	Nil	0.15 ± 0.02	0.05 ± 0.009
5	150.05 ± 2.30	244.00 ± 3.37	63	0	Nil	0.12 ± 0.02	0.05 ± 0.002
10	151.00 ± 2.62	247.50 ± 1.93	63	0	Nil	0.13 ± 0.002	0.04 ± 0.01
20	154.00 ± 2.28	236.66 ± 2.58*	53	0	Salivation diarrhea, piloerection, dyspnea, weight loss (symptoms occurred and persisted during 10–13 weeks of experiment)	0.07 ± 0.002*	0.02 ± 0.006*

Values represent the mean ± SE of 10 animals in each group.
* Significant at the level of $p < 0.05$.