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Review Article

Assessment of Genotoxicity, Hepatotoxicity and Reproductive Toxicity of Imidacloprid on Mammalian Models

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ABSTRACT:
Imidacloprid, a systemic neonicotinoid insecticide, isused widely to control various types of harmful insects and pests to prevent crop damage. Use of imidacloprid was increased tremendously in last few decades due to its lowtoxicityon mammals/vertebrates. Consistent use of Imidacloprid in enormous amount showed evidences of toxicity in exposed non-target invertebrates, vertebrates and also in mammals. This review focuses on the extent of toxicity induced at genetic, biomolecular, biochemical and histological level due to exposure of imidacloprid on mammalian models. Various parameters like antioxidant enzyme assays, cytotoxic assays, hematological parameters, histological parameters and reprotoxic assays are used to evaluate the toxicity of imidacloprid in mammalian models. Imidacloprid may damage DNA, alter histology and disturb antioxidant system of the body. The outcome will help in better understanding of imidacloprid toxicity on mammalian systems.

Keywords: Neonicotinoid, Imidacloprid, Mammalian models, Hepatotoxicity, Reproductive toxicity, Genotoxicity

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INTRODUCTION

Pesticides are the substances that were used to control, prevent or reduce the harmful organisms. In contemporary agriculture the use of pesticides is increasing to cater the food demands of continuously growing world population. According to a research, India is the largest producers of pesticides in world having worth of Rs 197 billion in 2018.In 2024 this market is estimated to grow approximately Rs 316 billion, with an Annual Growth Rate of 8.1 per cent. India lies at 10th position in the top ten pesticides consuming countries in the world. Total consumption of pesticides is maximum in Maharashtra after that comes Uttar Pradesh, Punjab and Haryana. Punjab (0.74 kg) has maximum consumption of pesticides per hectare

followed by Haryana (0.62 kg) and Maharashtra (0.57 kg) during 2016-17(Pesticide management bill, 2020). Globally about 5000 species of plant pathogens, more than 9000 species of insects and 8000 species of weeds that cause 13%, 14% and 13% loss of crops respectively (Pimental, 2009; Zhang et al, 2011). Approximately, 78% loss of fruits, 54% in vegetables and 32% in cereal production can be prevented by the use of pesticides (Cai, 2008). In spite of their beneficial effects in increasing food production and improving crop yield these also raised some adverse effects towards non-target organisms and humans as well (Mostafalou & Abdollahi, 2013). These pesticides can be classified as rodenticides, herbicides, nematicides, insecticides, miticides, fungicides, larvicides, bactericides etc. on the basis of target species. Insecticides are the most inevitable agrochemicals used in agriculture for the prevention of crops from insects inflicted damage (Sharma et al., 2020).

A new class of pesticides - neonicotinoids means "new nicotine like insecticides" were used as an ideal replacement of some widely used insecticides like carbamates and organophosphates (Shivanandappa et al, 2014). Neonicotinoids are synthetically derived from nicotine and most frequently used agrochemicals all over the world (Gibbons et al, 2015; Morrissey et al, 2015). The neonicotinoids were preferred over other insecticides due to their broad-spectrum toxicity, application flexibility (seed treatment, foliar treatment, spray), and low toxicity to non-target terrestrial and aquatic organisms (Anderson et al, 2015). Neonicotinoids can be categorised on the basis of their pharmacophore groups in three classes- as N-cyano-guanidines (Thiacloprid and Acetamiprid), N-nitro-guanidines (Imidacloprid, Thiamethoxam, Clothianidin, and Dinotefuran), and as nitromethylene species (Nitenpyram) (Jeschke et al., 2011). Although neonicotinoids are considered safe in mammals some studies reported their negative health disorders (Calderon-Segura et al., 2012; Cimino et al., 2017).

Imidacloprid is preferred widely for insect control due to its distinctive chemical and biological characteristics such as low application rates, wide range of insecticidal activity, favourable safety profile, fast imbibitions and transfer in plants as well as unique mode of action to have improved crop yield (Maienfisch et al, 2001). Imidacloprid being systemic, translocate rapidly across tissues after contact or ingestion (Fossen, 2006; Tomlin, 2006). Imidacloprid is one of the most frequently used neonicotinoid that is introduced by Bayer in 1992 and applied for more than 140 agricultural crops (Drobne et al., 2008; Shao et al., 2013). Imidacloprid is used in Indian states to control the insect infestation in crops and vegetables like Cotton, Paddy, Chilli, Mango, Grapes, Sunflower, Okra, Groundnut, Sugarcane, Tomato etc. Production of Imidacloprid in 2019-20 was 20 MT in India (Ministry of chemicals and fertilizers Annual Report 2020-21). Consumption of Imidacloprid was 309M.T. in 2019-20 and 372 M.T. in 2020-21 in India http://ppqs.gov.in/statistical-database?page=1. Demand of imidacloprid in 396.66 MT in 2016-17, 161.11 in 2017-18, 352.27 MT in 2018-19, 425.67 MT in 2019-20 and 365.53 in 2020-21 (DPPQS, 2021).

It is applied in various ways like seed treatment, foliar spray, soil treatment etc. Its repeated and frequent use has negative impacts on some non-target organisms like Earthworm, Collembola pollinators like Honeybees, some beneficial insects which are helpful in natural pest management, aquatic organisms (fishes), sprayers, farmers and insecticides manufacturing industry workers. Imidacloprid cause chronic toxicity in bees and also change their behavior (Wu et al., 2017) disrupts bumblebees foraging rhythms and sleep (Tasman et al., 2020), it changes the T4 concentration in plasma by converting T4 to T3 in thyroid gland of lizards (Wang et al., 2020), in Drosophila it induces ROS that may lead to neurological and metabolic impairment (Martelli et al., 2020), it induces intestinal injury and oxidative stress in gut of Zebra fish (Luo et al., 2021) alters level of gene expression and biomarkers in common carp (Ozedemir et al., 2018).

GENOTOXIC EFFECTS OF IMIDACLOPRID IN MAMMALIAN MODELS

Imidacloprid acts as a toxicant. Various hematological tests are used to analyze toxic effect of imidacloprid on blood parameters such as hemoglobin (Hb) level, total erythrocytes count (TEC), total lymphocyte count (TLC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), differential leucocyte count (DLC), mean corpuscular volume hemoglobin (MCVH), mean corpuscular volume (MCV) of animals. Imidacloprid also cause harmful effect on the DNA of the cells. Genotoxicity of imidacloprid in various mammalian models was also tested by various cytogenetic test i.e. micronuclei assay, comet assay, chromosomal aberration assay. A brief explanation of these assays, their principle is given in the following paragraphs.

Comet Assay-This assay also known as single cell gel electrophoresis is based on the stretching of DNA supercoils after analysis and then relaxed by strand breaks forming a tail like configuration during electrophoresis. It is easy to perform, highly sensitive, quick and require small amount of biological substrate and used for both *invivo* and *in vitro* system for detecting genotoxicity of chemicals. Low concentration of Imidacloprid also induced DNA damage that remained persistent in subsequent cell cycles of human lymphocytes, erythrocytes. Imidacloprid acts as an alkylation agent that damages the DNA of cell. It reacts with the DNA sites that are rich in electrons due to its high electronegativity (alkylating property) and it covalently binds to nitrogenous bases of DNA and forms DNA adducts (Ostling and Johanson, 1984).

Micronuclei Assay-The cytokinesis block micronucleus assay is also a preferential method for testing genotoxicity as it highly sensitive for detection of aneugenic (change in chromosome number) and clastogenic (chromosome breaking) activity of toxicants (Fenech, 2007). Micronuclei contain whole chromosomes lacking centromere or smaller fragments of chromosomes that get separated during mitosis lag behind and assumes morphology of an inter phase nuclei and covered by a nuclear envelope. These are smaller, extranuclear bodies that are not included in either daughter nucleus at anaphase stage of cell division. These micronuclei are resulted from replication on a damaged DNA template, direct DNA breakage or inhibition of DNA synthesis. So more the number of micronuclei more will the genotoxicity of the toxicant.

Chromosomal Aberration assay- This assay is used to analyse the DNA damage at chromosomal level. Structural chromosomal abnormalities such as duplication, inversion, deletion, translocation, breaks and gaps and numerical chromosomal abnormities such as an euploidy or polyploidy occur during cell division due to chemical, physical or physiological factors. Thereportedimidaclopridinducedtoxicityandoxi dativestressaresummarized in tables 1-3 and with respect to genotoxic, hepatotoxic and reprotoxic parameters.

A dose dependent alteration in the level of haematological parameters were reported at 25%, 50% and 75% of LD_{50} when given for 28 days in mice (Kataria et al, 2016). In rats no significant changes were reported in these parameters upto 20mg/kg/day dose of Imidacloprid for 90 days (Bhardwaj et al, 2010). Imidacloprid showed various genotoxic effects in various animal models. Imidacloprid caused genotoxicity may vary with the amount of dose, period of treatment, age and sex of the animal, and the other chemical given with it. A significant dose and time dependent increase was reported in micronuclei and chromosomal aberrations, at 50mg/kg/day and above it for 90 days in rats (Karabay & Oguz, 2005), 120mg/kg in mouse twice in 24 hours (Kobir et al, 2020) and 22mg/kg in mice for 28 days daily (Bagri et al, 2016). Acute exposure of Imidacloprid in mice at a dose of 120mg/kg/b.w did not induce micronuclei in polychromatic erythrocytes however in combination with other pesticides like Imazalil and Tebuconazole micronuclei induction occur even at 30 and 60 mg/kg/b.w. when given twice at intervals of 24 hours (Ilyushina et al., 2020). Due to mutual influence of active substances or their metabolites or any other additives present in technical grade products Imidacloprid may cause synergistic genotoxic effects when given in a mixture with

tebuconazole and Imazalil (Ilyushina et al., 2020). Imidacloprid cause a significant alteration in inflammatory cells and other hematological parameters that affects body homeostasis and acts as a cofactor for other diseases and infections in rabbits (Kobir et al., 2020), mice (kataria et al., 2016) and rats (Soujanya et al., 2020).

Table 1: Genotoxicity and hematological toxicity caused by Imidacloprid

S. No.	Type of animal model /Treatment	Doses / Duration of treatment	Parameters	Results	References
1	Mouse and *S.typhimurium*/ Imdiacloprid, Imazalil and Tebuconazole	Imidacloprid 120mg/kg, Imazalil 300 mg/kg and Tebuconazole 1000mg/kg and their mixtures 15/1.82/1.07mg/kg, 30/3.64/2.14 mg/kg, 60/7.29/4.29 mg/kg b.w./d as low, middle and high dose combination, orally twice at interval of 24 hours in mouse and 48-72h in bacteria.	Ames test in *Salmonella typhimurium* and Micronuclei formation in mouse bone marrow erythrocytes	None of the three induced gene mutation in *S. typhimurium* strain and micronuclei formation in erythrocytes of mouse bone marrow when applied single. But MN-PCE incidence increased significantly in mice and decrease in Ames test at middle and high dose combination mixtures of three pesticides were observed	(Ilyushina et al., 2020)
2	Swiss Albino male mice / Imidacloprid	5.5mg/kg, 11mg/kg and 22 mg/kg b.w./d for 7 (Group I), 14 (Group II) and 28 days (Group III)	Micronuclei and chromosomal Aberration assay	Chromosomal aberration and micronuclei frequency was increased in a dose and time dependent manner in bone marrow. A significant increase was observed in Group III (28 days) at highest dose tested (22 mg/kg).	(Bagri et al., 2016)
3	Female mice/ Imidacloprid	37.5mg/kg (low), 75mg/kg (medium) and 112.5mg/kg (high) b.w. dose for 24 h.	CA, MN and hematological parameters	Medium and high doses showed significant mitotic inhibition and chromosomal abbreations (CA) ($p<0.001$) and at high dose micronuclei	(Kataria et al., 2016)

				formation ($p<0.005$) was reported. A significant dose dependent decrease in hematological parameters red blood cells, erythrocytes sedimentation rate and Hemoglobin) was also observed.	
4	Female albino wistar rats/ Imidacloprid	30g/kg/b.w./d for 30 days	Hematological parameters and body weight	A significant reduction ($P<0.05$) in hemoglobin, packed cell volume, total erythrocytes count, total lymphocyte count, MCV, MCHC and MCH however a significant increase ($P<0.05$) in neutrophil count was reported. A significant reduction in weekly body weight was also observed.	(Soujanya et al., 2020)
5	Wistar albino rats and *Salmonella typhimurium*/ Imidacloprid and Methamidiphos	50mg/kg and 100 mg/kg b.w. of imidacloprid and 2.5mg/kg and 5 mg/kg b.w. of methamidiphos and their mixtures for 90 days	Chromosomal aberrations, micronuclei test and Bacterial mutation assay	Chromosomal aberration frequency was increased with increase in concentration of both pesticides and MN frequency increased significantly in all treated groups as compared to control ($P<0.05$)	(Karabay & Oguz, 2005)
6	Imidacloprid residues in dog blood and gloves of care takers	364mg/kg Imidacloprid applied topically to dogs to control fleas	Imidacloprid residues were detected by RP-HPLC in both blood samples of dogs and gloves of care takers after 5 minutes of rubbing	Imidacloprid residues detected in blood of dogs is 54.06±3.00 ppb and in gloves 254.16±25.49 ppm after 24 hours and not detected after 5th week.	(Craig et al., 2005)
7	Rabbits/ Imidacloprid and 6-CINA	0.05, 0.1, 0.25, 1 and 10g/ml	MN assay and CBMN assay, LC-APCI- MS	Statistically significant increase ($P<0.001$) in	(Stivaktakis et al., 2016)

				frequency of BNMN and MN was reported in exposed groups as compared to control. But no genotoxic effects were observed with time and dose.	
8	Rabbits/ Imidacloprid exposed diet	100mg/l Imidacloprid exposed diet for 15 days	Changes in hematological parameters	Significant alterations in inflammatory cells mainly lymphocytes, neutrophils and eosinophils affect body homeostasis, a non-significant decrease in hemoglobin, total erythrocytes count and packed cell volume was also observed.	(Kobir et al., 2020)
9	Male Wistar rats/Imidacoprid	0.06mg/kg, 0.8mg/kg, 2.25mg/kg/ b.w. for 28 days	Oxidative stress, acetylcholinestrase activity, comet assay,	Significant increase in ROS and DNA damage in peripheral blood leukocytes at lowest dose and GSH and SOD at highest dose was observed. However non-significant changes in the activity of butyrylcholinestrase, acetylcholinesterase and cholinesterase activities were observed in brain and plasma.	(Katic et al., 2021)

HEPATOTOXICEFFECTS OF IMIDACLOPRID IN MAMMALIAN MODELS

Liver being the principal organ responsible for detoxification and metabolism of toxins, drugs etc. The various serum and/or biochemical toxicity assessment parameters due to imidacloprid or its metabolites exposure on liver are given in table 2. Liver act as a vital organ for the metabolism and detoxification of drugs and chemicals like Imidacloprid. To evaluate hepatic damage various serum enzymes like SGPT (Serum Glutamate Pyruvate Transaminase)/ALT (Alanine Transaminase), GGT (Gamma Glutamyl Transpeptidase), SGOT (Serum Glutamate Oxaloacetate Transaminase)/ AST (Aspartate Transaminase), ALP (Alkaline Phosphatase) are the best parameters. Any toxic chemical when enters the body it alters the level of these enzymes. Imidacloprid did not show any change in the level of liver enzymes of rats

when given at a dose of 10 mg/kg/b.w/ day orally for 60 or 90 days (Bhardwaj et al., 2010; Toor et al., 2013; Vohra et al., 2014; Vohra & Khera, 2016). Imidacloprid at a dose up to 20 mg/kg/b.w./day showed a non-significant increase in the level of these enzymes in rats (Kapoor et al., 2010; Vohra et al., 2014; Vohra & Khera 2016; Chakroun et al., 2017). However, a significant increase in the level of these enzymes were reported at a dose of more than 20mg/kg of Imidacloprid in rats (Chakroun et al., 2017; Mehmood et al., 2017; El-Ela & Abdel-Aziz, 2019).

Imidacloprid and Antioxidant enzymes- Imidacloprid decreases the level of antioxidant enzymes that protect the cell from reactive oxygen species likesuperoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and increase the level of pro-oxidant enzymes that leads to formation of ROS (reactive oxygen species), LPO (lipid Peroxidation) and DNA damage. SOD (superoxide dismutase), GPx (glutathione Peroxidase) CAT (catalase) GSH (glutathionea non-protein antioxidant) may affect oxidant molecules of tissues and are active in defence against oxidative cell injury due to their free radical scavenging activity. Imidacloprid may cause excessive production of ROS (reactive oxygen species) that alter the cellular antioxidant defence system and affect succeptibilty to oxidative stress. Imidacloprid at a dose of 38mg/kg/day (Lohiya et al., 2016) and 45 and 90mg/kg (Lonare et al., 2016) significantly induced the oxidative stress to rats as evidenced by increase in level of LPO and decrease in activities of SOD, GPx and GSH. A significant increase in concentration of triglycerides and cholesterol in serum was observed maximum in rats at 550mg/kg dose given. It may be due to direct utilization of cholesterol and triglycerides as an antioxidant which will eventually lead to termination of free radicals that may induce oxidative stress. Similar results were reported for the level of ALT, AST and ALP in the blood plasma of rats due to degeneration and necrosis of hepatocytes which cause an increase in permeability of cell membrane resulting in an increase in transaminase into blood stream. Imidacloprid at higher doses significantly decreased the body weight. As it may be due to decrease in average feed intake (Mehmood et al., 2017).Based on haematological parameters, enzymatic changes, urine analysis, intoxication, mortality and neurobehavioral examination 10mg/kg/b.w./d dose of Imidacloprid is considered as NOEL (No observed effect level) and 20 mg/kg/b.w./d as LOEL (Low observed effect level) in female rats (Bhardwaj et al., 2010). 15mg/kg/day dose of Imidacloprid induced significant toxicological effect to mice in terms of biochemical and liver histopathological terms (Arfat et al., 2014). Below 15mg/kg dose non-significant changes were observed in serum parameters (Reda, 2018).

A foul smell and repellent activity of imidacloprid cause reduction in consumption of food that results a decrease in body weight (Arfat et al., 2014). Chronic exposure of imidacloprid causes inflammation and oxidative stress in liver of rats (Duzguner and Erdogan, 2012). Oxidative stress upregulates stress related HO_1 genes. This will increase the MDA and free radicals that in turn increase DNA and protein degradation and LPO in liver (Timoumi et al., 2019).Imidacloprid may cause downregulation of Keap1 genes that in turn downregulate Nrf2 genes that leads to increase in mitochondrial ROS production and hence cell death by apoptosis or inflammation (Shibata et al, 2008). Imidacloprid also cause oxidative and nitrosative stress induced response by activating a series of caspase cascades and by JNK pathway it cause apoptosis. Imidacloprid cause hepatic apoptosis by Casp3 dependent and independently by activating JNK pathway. Nrf2 down regulation also alter permeability of mitochondrial membrane due to disruption of Na/K/ATPase and ionic balance (Khalaf et al., 2020, Morgan et al., 2021). Mitochondrion regulates NADH and ATP production by oxidative phosphorylations that are essential cellular bioenergetics. Imidacloprid inhibit NADH and ATP productions hence cause cellular dysfunction (Mehta et al., 2005). Imidacloprid disturbs mitochondrial function by opening mitochondrial pores and increasing cytosolic calcium level that may activate various other enzymes and degrade proteins and cause

DNA damage (Hassanen et al., 2022). Imidacloprid cause oxidative stress by increasing free radicals and increased reactive oxygen and nitrogen species production that ultimately succumb the antioxidant system of the organism (Dhawan, 2014). Oral intake of Imidacloprid directly cause gradual exhaustion of antioxidant enzymes like SOD, CAT, GST and GPx that cause metabolic disturbance, increased LPO and cellular damage (Hayes et al., 2005).

Table 2: Hepatotoxicity caused by Imidacloprid

S. No.	Type of animal model or cell line /Treatment	Doses / Duration of Treatment	Parameters	Results	References
1	Female albino rats / Imidacloprid	10mg/kg and 20 mg/kg b.w./d for 60 days	Histological tests for tissues and biochemical tests for liver enzymes (AST, ALT and ALP)	Higher dose cause congestion and dilation of central vein, and degeneration of hepatocytes but no significant effect was observed in the level of ALP, ALT and AST enzymes	(Vohra et al., 2014)
2	Female Wistar rats / Imidacloprid	1mg/kg b.w. /d for 30 days	RT PCR for mRNA of nitric oxide synthase quantification, biochemical assays like ALT, AST, MDA, LDH, SOD, GSH	Imidacloprid induced mRNA transcription of 2 isoforms of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide syntahase (eNOS) in liver, increased lipid peroxidation and myeloperoxidase activity in liver	(Duzguner and Erdogan, 2012)
3	Female rats/ Imidacloprid	20 mg/kg after 6, 12, 24 and 48h after exposure	Disposition of imidacloprid metabolites in brain, liver, kidney and body fluids were monitored by HPLC and LC/MS	Imidacloprid metabolites such as 6 CINA ranged from 27.12 µg/ml/h to 1006.42µg/ml/h 6 HNA 14.98 µg/ml/h to 302.74 µg/ml/h, and Imidacloprid is 35 µg/ml/h to 358 µg/ml/h, in fluids and various organs of body. Maximum clearance was found in ovary followed by kidney, liver and minimum in blood.	(Kapoor et al., 2014)
4	Female rats/	5 mg/kg, 10	Hematological	Hematological	(Bhardwaj et

	Imidacloprid	mg/kg, 20 mg/kg b.w./d for 90 days	parametersfor RBC, MCV etc. and biochemical parameters for GOT and GPT level	parameters not changed significantly while an increase in level of GPT, GOT and glucose levels was observed along with significant histopathological changes in liver and kidney of female rats and decrease in body weight was reported.	al., 2010)
5	Female rats/ Imidacloprid	9 and 45 mg/kg b.w./d for 28 days	Histology of liver and biochemical assays for AST, AMP and AKP	Significant changes in liver histology at 45mg/kg dose and also reduction in weight of liver and body while an increase in the level of AST, ALP and AKT was observed	(Toor et al., 2013)
6	Male albino mice / Imidacloprid	5 mg/kg, 10 mg/kg and 15 mg/kg b.w./d for 15 days through oral gavage	Serum parameters SGOT, SGPT, TBIL and ALP	Significant effect on liver function, body weight and kidney and elevation of serum parmeters	(Arfat et al., 2014)
7	Wistar albino rats / Imidacloprid	19 mg/kg and 38 mg/kg b.w./d for 10, 20 and 30 days	Liver weight activities of various enzymes and histology of liver	High dose of imidacloprid significantly increased organ weight and levels of GSH and SOD and GPx	(Lohiya et al., 2017)
8	Rat / Imidacloprid	10 µM / 2 h	Detection of mRNA expression level and biochemical parameters for enzyme level detection	Concentration of nitric oxidase increased in brain and liver. Inflammatory cytokines such as IL-6, IL-1β and TNF α were upregulated by 2 to 5 fold while IL-10 mRNA down regulated	(Duzguner and Erdogan, 2010)
9	Male adult albino rats / Imidacloprid	Control, Imidacloprid treated-	Biochemical (ALT, AST, ALP and MDA), histopathological (liver,	Imidacloprid induced significant increase in liver	(Mohany et al., 2012)

		0.21mg/kg b.w., imidacloprid and thymoquinone treated- 1mg/kg b.w./d for 28 days imidacloprid	spleen, thymus) and immunological (total leucocyte count, total immunoglobulin level) assays	enzymes as well as total leucocyte count and total IgGs compared to control group.	
10	Balb C mice / Imidacloprid	2.5 mg/kg, 5 mg/kg and 10 mg/kg b.w./d for 28 days	Histology of liver and spleen, cellular and humoralresponse like hemaglutinating antibody titer to sheep RBCs, DTH response and adecrease in T-lymphocytesstimulation	Dose dependent suppression of DTH activity and decreased stimulation of T-lymphocytes to PHA 2.5 mg/kg has no observable adverse effects on immunotoxicity.	(Badgujar et al., 2013)
11	Male Albino mice/ Imidacloprid	2.6 mg/kg/b.w. for 28 days/ 5 doses per week orally	Measurement of level of ALT, AST, Glucose, urea, Cholesterol, Triglycerides and HDL	Significant reduction in the AST and α-amylase level while a significant increase in the level of ALT, Glucose, Urea, Triglycerides, Cholesterol and HDL were reported.	(Reda, 2018)
12	Male Albino Rats/ Imidacloprid	Mixture of Imidacloprid and Fipronil at a dose of 0.547, 0.409 and 0.820 mg/b.w./day for 28 days orally	Measurement of liver enzymes, urea, uric acid, Albumin, Globulin, total protein levels and hematological parameters	A significant increase in the level of ALT, AST, ALP, GGT, Urea, Uric acid, Albumin, Globulin, total protein level however a decrease in the level of RBC, WBC, Hb, Hematocrit levels in a dose dependent manner.	(Badawy et al., 2018)
13	Adult Male Rats/ Imidacloprid	45mg/kg and 90mg/kg Imidacloprid daily for 21 days	AST, ALT, total cholesterol, glucose, total proteins	Singnificant increase ($p<0.05$) in dose dependent manner in AST, ALT, at ($p<0.001$) in total cholesterol and glucose levels however a significant reduction total serum proteins and albumin	(El-Ela and Abdel-Aziz, 2019)

				($p<0.001$) was observed.	
14	Male mice/ Imidacloprid	30mg/L of Imidacloprid for 10 weeks	Liver weight and morphology, serum and bile acid level	A significant reduction in the relative liver weight and impairment in hepatic tissue morphology and a decrease in serum and hepatic total bile acids were observed	(Yang et al., 2020)

REPROTOXIC EFFECTS OF IMIDACLOPRID IN MAMMALIAN MODELS

Most of the people come in contact with a wide range of pesticides in their life. This exposure may be due to intake of insecticides contaminated fruit and vegetables, high fat content food like fish, meat, dairy products, eggs (Cimino et al., 2017). Imidacloprid is reported to reduce reproductive potential of organisms especially mammals. In males it cause reduction in sperm motility, spermviability, sperm count and increase in sperm abnormalities/deformities that leads to male infertility (Najafi et al., 2010; Lonare et al., 2016; Mehmood et al., 2017). Imidacloprid increases inflammatory cytokines like TNFα and IL-1β at higher doses. At lower doses no reduction in body weight hence no change in physical health of rats. Leydig's cells of the testes synthesizes testosterone and estradiol for proper spermiogenesis and reproductive fitness of the organism. Imidacloprid cause imbalance in gonadotropin hormones may be due to directly affecting Leydig's cell or indirectly by binding on nAChRs as agonist of nACh (Mehmood et al., 2017). Chronic exposure of low dose of Imidacloprid decreases testosterone level by directly injuring testes without affecting hypothalamus pituitary glands but at high doses it inhibit hypothalamic pitutary axis. This will lead to decrease in sperm quality and spermiogenesis (Tetsatsi et al., 2019). Oxidative stress and free radical generation also take part in decreasing sperm quality (Lonare et al., 2016). Due to the presence of high content of polyunsaturated fatty acid in plasma membrane of sperm and testicular tissue these are highly susceptible to LPO (Adedara et al., 2018). 14mg/kg/b.w./d dose of Imidacloprid is considered as a NOAEL (Non- observed adverse effect level) in male rats. Treatment with Imidacloprid at doses lower than NOAEL (8mg/kg) for 90 days declined the epididymal sperm concentration and sperm motility (Bal et al., 2012). Treatment with increased dose of Imidacloprid than NOAEL is correlated with deterioration of sperm properties, increased apoptotic index of germ cells and an increase in sperm morphology as well as decreased weight of reproductive organs (Najafi et al., 2010; Lonare et al., 2016). Imidacloprid also cause down regulation of steroidogenic acute regulatory protein genes (CYP19A, CYP11A1, CYP17A1, HSD17B3, StAR (steroidogenic genes and inhibit testosterone biosynthesis in testes (Mehmood et al., 2017; Yuan et al., 2020). Imidacloprid exerts reprotoxic effects in male rats by inducing testicular oxidative stress and apoptosis, inhibition of steroidogenesis, disruption of sex hormones and down regulation of PCNA (proliferating cell nuclear antigen).10mg/kg is (Kapoor et al., 2010; Bhardwaj et al., 2010) through antioxidant enzymes and LPO.

In female rats Imidacloprid cause abnormalities in the shape and size of ovarian follicles, imbalance in hormones, alteration of histology and reduction in body weight. 10mg/kg dose of Imidacloprid considered as NOAEL in female rats changes the shape and size of follicles, reduced the number of days of estrous cycles, reduction in body weight, reduction in food

consumption, changes in hormonal level, alteration in biochemical as well as haematological parameters, reduction in developmental immunotoxicity (Kapoor et al., 2010; Bhardwaj et al., 2010; Gawde et al., 2013; Naubini et al., 2015; Vohra and Khera 2016). Imidacloprid given above NOAEL level caused significant changes in the concentration of various hormones like Lutenizing hormone (LH), Follicular stimulating hormone (FSH), Testosterone, Estradiol, Prolactin (Hafez et al., 2016; Najafi et al., 2010). In female mammalsestrogen and estradiol were released from ovary and FSH from pituitary gland in response to gonadotropin releasing hormone from hypothalamus. Imidacloprid cause oxidative damage to ovarian follicles and decreases the level of estrogen and estradiol that give a negative feedback to increase synthesis of FSH that in turn increases the growth of ovarian follicles. Imidacloprid directly attack on mature ovarian follicles that cause a persistent elevation in FSH and reduction in estrogen.

Histological changes in testis- Imidacloprid above NOAEL causes various histological changes in testis like increased thickness of tunica albuginea, atrophied seminiferous tubules, decrease in leydig's cells, vasodilation and thrombosis, vascular degeneration of germinal epithelium, loss of spermatids. Various changes in sperms like reduction in sperm viability, sperm velocity and abnormality which potentially can cause infertility (Najafi et al., 2010; Bal et al., 2012; Hafez et al., 2016). The extent of reproductive toxicity induced due to exposure of imidacloprid in laboratory animals (*in vivo*) are given in Table 3.

Table 3: Reproductive Toxicity caused by Imidacloprid

S. No.	Type of animal model/cell lines / Treatment	Doses/ Duration and route of administration	Parameters	Results	References
1	Developing wistar male albino rats / Imidacloprid	0.5 mg/kg, 2 mg/kg, 8mg/kg b.w./d for 90 days oral (gavage)	TUNEL assay, sperm morphology, GSH and Malondialdehyde (MDA) level, serum testosterone and lipid level in testis	Decreased weight of seminal vesicle, epididymis and epididymal sperm concentration, GSH in testis at 8mg/kg, increased abnormalities in sperm structure and DNA fragmentation, total cholesterol, testicular fatty acids and MDA at 2 and 8mg/kg	(Bal et al., 2012)
2	Testicular cells of male adult rats / Imidacloprid	112 mg/kg and 225 mg/kg/d for 60 days oral (gavage)	Testicular weight determination, histopathological analysis, sperm count, quantitativesperm motility and morphology,serum sampling and hormonal analysis, tubular differentiation	Adverse effects on testicular tissue, decreased sperm viability and sperm motility, testicular serum level	(Najafi et al., 2010)

3	Male adult rats/ Imidacloprid	45 mg/kg, 90 mg/kg and 450mg/kg. b.w./d for 15 days through oral gavage daily and in 1group 450mg/kg dose once	Histopathological analysis of seminiferous tubules and biochemical assays for LH, FSH, testosterone and estradiol hormones	Decreased LH, FSH, Estradiol 2, testosterone and Prolactin in Testis, decrease in sperm count, viability, motility and inhibition of spermatogenesis at 45 and 90 mg/kg, chronic exposure leads toinfertility	(Hafez et al., 2016)
4	Rats / Imidacloprid and CMN	45 mg/kg and 90 mg/kg b.w./d for 28 days oral (gavage)	Biochemical tests of enzymes levels and sperm mobility and viability	Significant decrease in sperm count, sperm mobility, and increased alteration in testis and epididymis. Decreased activity of CAT, SOD, GPx and GST. Toxic effects are restored by administration of CMN along with Imidacloprid	(Lonare et al., 2016)
5	Female adult rats / Imidacloprid	5 mg/kg, 10 mg/kg and 20 mg/kg/b.w./d orally for 90 days (gavage)	Biochemical measurements of LPO, SOD, GSH, CAT in liver, kidney and brain	Imidacloprid did not showed oxidative stress at 5mg/kg and 10 mg/kg but induced reduction of antioxidants at 20mg/kg. Hence 10mg/kg dose of Imidacloprid was taken as (NOEL)	(Kapoor et al., 2010)
6	Pregnant female wistar rats/ Imidacloprid	10mg/kg bw / from day 7 to 21 in gestation period through tail vein	Dax 1 gene expression by RT PCR and serological tests and histology	Imidacloprid caused down regulation of DAX1 expression and significant reduction in follicle diameter, corpus luteum no. and levels of estradiol and progesterone.	(Nabiuni et al., 2015)
7	Female adult rats/ Imidacloprid	100 ppm, 250 ppm and 700 ppm dose per day for 84 days in postnatal1 and for 105 days for postnatal 2 before mating, gestation and lactation oral	Body weight gain and reproductive parameters	No negative effects on reproductive system, decreased body weight gain and food consumption in Postnatal1 at 700 ppm	(Suter et al., 1990)
8	Female rats/ Imidacloprid	10mg/kg/bw and 20mg/kg/bw / For 28 days for both F_0 and F_1 generation	Biochemical, histological and physiological assays	Imidacloprid at 20mg/kg showed loss in body weight, ovarian weight and food consumption but	(Vohra and Khera, 2016)

				showed no significant effect at 10mg/kg dose	
9	Pregnant wistar rats / Imidacloprid	10, 30 and 90 mg/kg/bw / Oral administration from gestation day 6 to 21 oral ingestion to pups from post natal day 21 to 42	Histological and biochemical parameters, necropsy and macroscopic examination of utereo dams and pups	90 mg/kg dose of imidacloprid reported to affect implantation leading to increased chances of abortion and alterations in soft tissues and skeleton also.	(Gawde et al., 2013)
10	Male and female mice/ Acetamiprid Imidacloprid	500µM / 30 min for both Acetamiprid and Imidacloprid	Sperm motility assay, sperm chromatin dispersion assay	No significant change in DNA integrity and sperm motility after 30 minutes treatment. But *in vitro* fertilization process as well as zygotes were significantly affected by imidacloprid exposure ($P<0.05$).	(Gu et al., 2013)
11	Mice/Imidacloprid and Mancozeb	0.5% of LD_{50} of each pesticide, 131mg/kg for imidacloprid and 800mg/kg/b.w. for Mancozeb / From postnatal day 1 to 28^{th} day	Body mass index, waist hip ratio, hormonal assay lipid profiles and in silico analysis using bioinformatics	Imidacloprid alone did not show any significant changes but its exposure with Mancozeb decreased the plasma T_3 level and increased prolactin and TSH level. Total cholesterol, triglycerides and LDL were also increased significantly.	(Bhaskar & Mohanty, 2014)
12	Male mice/ Imidacloprid	3mg/l, 10mg/l and 30mg/l Imidacloprid for 10 weeks	Testicular morphology, level of androgen receptors and testosterone	A significant decrease in the level of testosterone and androgen receptors expression was observed and a severe impairment in the testicular morphology at all the three doses.	(Yuan et al., 2020)
13	Female Wistar rats/Imidacloprid and chloropyrifos	44 mg/kg/b.w./d throughout gestation period	Determination of blood glucose, insulin, lipid profile and GLUT4 and NF-KB levels in mothers and offsprings	Induction of hyperglycemia, insulin resistance and dyslipidemia in female rats and their offsprings. Reduction in HDL and increase in LDL levels. Decrease in expression of GLUT-4	(Ndonwi et al., 2020)

				level. However an increase in the level of NF-KB was observed.	
14	Male wistar rats/ Imidacloprid	0.06mg/kg and 0.6mg/kg b.w./d for 90 days	Determination of LH, FSH, testosterone and estradiol level as well as level of Imidacloprid in serum, histopathology	Decrease in sperm concentration, testosterone level and activity of CYP450 and increase in sperm abnormalities at both doses	(Zhao et al., 2021)
15	Sprague-Dawley rats/Imidacloprid	22.5mg/kg/b.w./d for 56 days	Analysis of sperm motility, sperm count, hormonal levels and antioxidant enzymes level, analysis of apoptosis and steroidogenesis gene levels	Increase in LH, FSH and MDA levels decrease in antioxidants, GSH, testosterone, body weight, sperm parameters was reported. A significant downregulation in mRNA of steroidogenic genes of testis.	(Saber et al., 2021)
16	Male Wistar rats/Imidacloprid	0.06 mg/kg, 0.80 mg/kg and 0.25 mg/kg/b.w./d for 28 days	Antioxidant status, weight of testis and epididymis, comet assay	A significant increase in the level of SOD and GPX at all tested concentration but a decrease in GSH at lowest dose. A significant DNA damage was also observed in testicular cells.	(Lovakovic et al., 2021)
17	Male albino rats/Imidacloprid	9mg/kg/b.w. 5 times a week for a month	Body weight, various sperm parameters, hormonal assay, antioxidant assay and protein carbonyl content analysis	A significant decrease in sperm count, sperm motility, sperm viability, SOD, GSH, testosterone, LH and FSH levels. However a significant increase in LPO, PCC and catalase was reported.	(Abdel-Razik et al., 2021)
18	Female albino wistar rats	30mg/kg/b/w./d for 30 days	Estimation of hormones FSH, LH and estrogen by ELISA	A significant increase in length of estrous cycle and FSH level whereas a decrease in estrogen was observed.	(Soujanya et al., 2022)

CONCLUSION

Imidacloprid - a neonicotinoid insecticide used for safeguarding crops, horticulture, ornamental plants, tree nurseries, forestry, agriculture lands and soon. After exposure of the target and non-target species including mammals, Imidacloprid acts as a neurotoxicant that affect mainly

nAChRs and oxidativestress, which is one of the most common cause of toxicity induced. The differential extent of imidacloprid toxicity is due to difference in dose, tissue, sex, age of animal, extent and mode of exposure as per reviewed and referred reports in *vivo* in mammalian models. The residues of imidacloprid and its metabolites were detected in body fluids (blood, serum, milk, plasma and urine), hairs and tissues and acts as an exposure biomarker. Alteration in molecular, biochemical, behavioural, histopathological, genetic and enzymaticend points were used as biomarkers of Imidacloprid toxic effects. More studies are required to search new drugs, magic molecules and novel biomarkers having higher sensitivity, reproducibility and reliability in order to mitigate toxic effects of pesticides as well as conduct of more awareness programs so that users prefer to follow the prescribed precautions to have safe and healthy future generations.

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Conflict of Interest
All authors declare that there is no conflict of interest.

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