CLINICAL AND HAEMATOLOGICAL STUDIES OF REPEATED DOSE ORAL IMIDACLOPRID TOXICOSIS IN WHITE LEGHORN BROILERS AND ITS AMELIORATION BY COW URINE DISTILLATE[#]

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ABSTRACT

Present study was planned to investigate the ameliorating effect of cow urine distillate on imidacloprid intoxicated white leghorn chicks. Chicks were exposed orally to different doses of imidacloprid either individually or in combination with cow urine distillate once daily for 45 days. No signs of toxicity were seen in birds of any groups up to fifth weeks of experiment. After fifth week, toxic symptoms *viz.* sudden onset of depression, reduced feed intake, dullness, ataxia, convulsion and circling movement were observed in birds of high dose group *i.e.* VI (5.20 mg/kg body weight) of imidacloprid. While birds received cow urine distillate along with imidacloprid did not show any observable signs of toxicity. Imidacloprid caused significant decrease ($P<0.05$)) in haemoglobin, packed cell volume, total leukocyte count and lymphocytes at high dose used in this study suggest that it has toxic influence on the haemopoetic system whereas non significant effect was observed in total erythrocyte count (TEC), monocytes, granulocytes, Mean corpuscular volume (MCV) ,Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in only imidacloprid treated groups. The ameliorative effects of cow urine distillate is supported by the non-significant change in haematological values in treatment groups received cow urine distillate along with imidacloprid in comparison to their respective groups that received only imidacloprid. Amelioration with cow urine distillate resulted in improvement in blood parameters.

***Key words*:** Cow urine distillate, imidacloprid, toxicity

Introduction

Insecticides are being extensively used in agriculture and veterinary field all over the world. However, extensive and indiscriminate use of insecticides has led to a global concern over the potential adverse effects of these chemicals on animal and human health (Al-Saleh, 1994) as these insecticides interfere the defence mechanisms of the host, which normally ensures its survival against invading pathogens (Casale *et al*., 1993; Rodgers and Xiong, 1996; Sodhi *et al*., 2006; Cabassi, 2007).There is number of evidences which suggest that the use of insecticides in crops, in poultry houses, in storehouses and livestock leaves behind its residue that may cause interference with some of the fundamental biological processes. Various cases of high lethality in poultry and other avian species are associated with acute exposure to insecticides (Kawano *et al*., 1967; Bahl and Pomeroy, 1978; Aulakh *et al*., 2005). Various classes of compounds that have been used as insecticides are organophosphates, organochlorines, pyrethroids, carbamates and various inorganic compounds.

Imidacloprid, relatively new and systemic neonicotinoid insecticide was introduced as the first chloronicotinyl insecticide to the market in 1991, and has become the most successful, highly effective and largest selling insecticide worldwide for agricultural use and as a veterinary medical remedy to control ectoparasitic insects in the last decade (Mencke and Jeschke, 2002). Imidacloprid has been developed for veterinary use due to its favourable mammalian safety characteristics and is used as flea control agents on cats and dogs.

There is paucity of information available concerning the effects of imidacloprid on animal health, as the insecticide that is likely to be used in future pest control programs. So one of the objective to study the effect over the haematological parameters of poultry to elucidate the toxicity of imidacloprid in dose dependent manner. In poultry, cow urine enhances the immune-competence status of birds and has positive effect on their body weight gain, haematological profiles (Mathivanan and Kalaiarasi, 2007) and immunomodulatory effect on both the humoral and cellular immune response (Kumar *et al.*, 2004). Cow urine is able to clear the toxins from the system and act as an anti-toxin that protects the body from various types of poisons (Jain *et al.,* 2010). Though, there are numerous claims of the efficiency of cow urine but its efficiency against pesticide-induced toxicity has been rarely explored. The present study is aimed to evaluate repeated dose or alimidacloprid toxicity and to determine ameliorative potential of cow urine distillate on imidacloprid-induced clinical and haematological alterations in poultry.

Material and Methods

Experimental animals

The study was conducted on day old healthy male White Leghorn chicks. The chicks were procured and housed in pens of Battery brooder house at Poultry farm, College of Veterinary and Animal Science, Bikaner. The chicks were provided with *ad libitum* standard feed and water. The experimental trials were approved by the Institutional Animal Ethics Committee (No.-CVAS/IAEC/CPCSEA-2044/GO/Re/SL/18/2019/10) and conducted under its guidelines.

Chemical (Insecticide)

Commercial products of imidacloprid (17.8%) used in this

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study were purchased from the local market of Bikaner, Rajasthan India. Apparent LD_{50} of imidacloprid 104 mg/kg (orally) was taken into consideration for calculation of different dose groups (Kammon *et al*., 2010).

Cow urine distillate

The cow urine was collected from apparently healthy Rathi cows from a private dairy farm. Fresh cow urine at morning time was collected in sterile containers and then urine was filtered through Whatman filter paper No.1. Distillate was prepared from filtered cow urine using distillation apparatus. The distilled cow urine was stored in air tight brown bottle until use.

Experimental design

Birds were divided at random into twelve different groups containing 20 birds in each group. Birds of group I served as normal control and were administered only feed and water and birds of group II and group III were administered cow urine distillate @ 2 ml/kg and 4 ml/kg body weight respectively for 45 days .All the birds of groups IV, V and VI were administered imidacloprid orally @ 2.60 mg/kg ($LD_{50/40}$), 3.47 mg/kg ($LD_{50/30}$) and 5.20 mg/kg ($LD_{50/20}$) body weight respectively for 45 days and all the birds of groups VII, VIII and IX were administered imidacloprid orally @ 2.60 mg/kg, 3.47 mg/kg and 5.20 mg/kg body weight along with cow urine distillate @ 2 ml/kg body weight for 45 days, respectively. All the birds of groups X, XI and XII were administered imidacloprid orally @ 2.60 mg/kg, 3.47 mg/kg and 5.20 mg/kg body weight along with cow urine distillate @ 4 ml/kg body weight for 45 days respectively. All the birds were monitored for any observable toxic symptoms throughout the experimental period and they were also weighed weekly to monitor body weight gain. The blood samples were collected before termination of study and were analyzed for haematological parameters.

Haematological studies

Haematological studies were carried out on the day of collection of blood. The haematological estimations *viz.* haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) were carried out with fully automatic analyzer 'Mindray BC-2800 Vet'. Blood smears were prepared from fresh blood for differential leukocyte count (DLC) which was manually done. Differential leucocytes count (DLC) was done as per Jain (1986), using Leishman's staining. The percentages of different leucocytes were determined by examining the stained smear under oil immersion of light microscope. MCV, MCH and MCHC were calculated.

Statistical analysis

The results are expressed as the mean and standard error of the mean (SEM). Comparisons between treated and control groups were made on computer using ANOVA (Analysis of one Variance) by using SPSS software version 20. A probability (P) value of 0.05 was selected as a standard for statistically significant differences.

Results and Discussion

The birds were regularly and keenly observed throughout the experiment. No signs of toxicity were seen in birds of any groups up to fifth weeks of experiment. After fifth week, clinical symptoms were observed in birds of high dose group *i.e*. VI (5.20 mg/kg body weight) of imidacloprid. The most common signs were sudden onset of depression, reduced feed intake, dullness ataxia, convulsion and circling movement in high dose imidacloprid treated birds. While in medium and low toxic dose groups *i.e*. in V (3.47 mg/kg body weight) and IV (2.60 mg/kg body weight), clinical symptoms were of mild nature. Control group I did not show any visible clinical signs of toxicity. Birds of group (II) and III group expressed increase appetite, more activity than other birds. While birds of VII, VIII, IX, X, XI and XII did not show any observable signs of toxicity. Decrease in feed and water intake also observed in VIII and IX group.

Clinical manifestation of toxic symptoms in groups treated with high dose of imidacloprid alone revealed the toxic effects of the imidacloprid. Absence of toxic signs in groups treated with cow urine distillate along with imidacloprid showed the medicinal and detoxifying potential of cow urine distillate. Similarly, rats given an oral lethal dose of imidacloprid showed typical nervous system poisoning symptoms including emaciation, diarrhoea, laboured breathing, lethargy, staggering, trembling and spasms (Cox, 2001). Results are in accordance with Tiwari *et al.* (2017) who reported that clinical signs as ataxia, tremors episode were appeared after 45th day of exposure to the imidacloprid whereas mild reduction in feed and water intake was observed in birds treated with imidacloprid along with cow urine distillate. It was also reported by Chauhan *et al*. (2009) that cow urine serves as a potential booster of immunity if taken on daily basis.

Imidacloprid caused significant decrease ($P<0.05$)) in haemoglobin, packed cell volume, total leukocyte count and lymphocytes at high dose used in this study suggest that it has toxic influence on the haemopoetic system whereas non significant effect was observed TEC, monocytes, granulocytes, MCV, MCH and MCHC in only imidacloprid treated groups as depicted in Table1 and Table 2. The ameliorative effects of cow urine distillate is supported by the non-significant change in in haematological values in treatment groups received cow urine distillate along with imidacloprid (VII to XII) in comparison to their respective groups that received only imidacloprid (IV, V and VI). Amelioration with cow urine distillate resulted in improvement in blood parameters.

Tiwari *et al.* (2017) investigated the imidacloprid induced haemato-biochemical changes and its ameilioration by cow urine distillate in white leghorn broilers. The haematological parameters including haemoglobin, PCV, TEC and TLC have shown significant decline in the groups provided with 1/10th and 1/20th doses of LD50 of imidacloprid for 60 days. The study is the indicative of the beneficial role of cow urine supplement on daily basis as it protects the birds from being severely intoxicated. Cow urine has haematinic and immune boosting potential that may help the birds to counteract the undergoing stress following imidacloprid mediated toxicity.

Mishra (2014) found that the TEC, TLC, Hb and PCV were significantly ($P<0.01$) decreased in Acephate intoxication and Amelioration with cow and goat urine distillate resulted in improvement in blood parameters.

Table 1: Effect of daily oral dosing of imidacloprid alone and cow urine distillate along with imidacloprid on haemoglobin (g/dl), packed cell volume (%), total erythrocyte count (10^6/µl) and total leucocytes count (number/µl)in WLH cockerel chicks.

Group	Haemoglobin (g/dl) Mean ± SE (n=20)	Packed cell volume (%) Mean ± SE (n=20)	Total erythrocyte count (10^6/µl) Mean ± SE (n=20)	Total leucocytes count (number/µl) Mean ± SE (n=20)
I	9.51± .12^{cdef}	29.43±.12^{cde}	2.14±.02^{abc}	15718.35±17.04^{bc}
II	9.60±.10^{def}	29.54±.10^{e}	2.19±.02^{c}	15725.90±16.66^{bc}
III	9.82±.09^{f}	29.64±.12^{e}	2.20±.01^{c}	15835.30±23.22^{d}
IV	9.36±.13^{bcde}	29.17±.11^{bcd}	2.12±.02^{ab}	15707.55±17.37^{b}
V	9.26±.12^{abcd}	29.11±.12^{bc}	2.11±.01^{a}	15660.45±18.12^{b}
VI	8.95±.11^{a}	28.73±.13^{a}	2.10±.03^{a}	15155.45±58.23^{a}
VII	9.49±.13^{cdef}	29.49±.10de	2.15±.01^{abc}	15719.45±17.14^{bc}
VIII	9.36±.12^{bcde}	29.39±.10^{cde}	2.15±.00^{abc}	15670.65±18.96^{b}
IX	9.06±.11^{ab}	28.68±.15^{a}	2.14±.01^{abc}	15168.95±58.84^{a}
X	9.70±.11^{ef}	29.65±.07^{e}	2.18±.01^{bc}	15815.55±17.39^{cd}
XI	9.50±.11^{cdef}	29.41±.08^{cde}	2.15±.00^{abc}	15710.95±14.76^{b}
XII	9.17±.11^{ab}	28.84±.13^{ab}	2.15±.01^{abc}	15242.65±51.62^{a}

Values Indicate Mean ± S.E. (figures in the bracket indicate 'n') Means having different superscripts at a particular period of treatment differs significantly (P≤0.05)

Table 2: Effect of daily oral dosing of imidacloprid alone and cow urine distillate along with imidacloprid on Lymphocytes (%), Monocytes (%), Granulocytes (%), MCV(fl), MCH (pg/dl)and MCHC (g/dl) in WLH cockerel chicks.

Group	Lymphocytes (%) Mean ± SE (n=20)	Monocytes (%) Mean ± SE (n=20)	Granulocytes (%) Mean ± SE (n=20)	MCV (fl) Mean ± SE (n=20)	MCH (pg/dl) Mean ± SE (n=20)	MCHC (g/dl) Mean ± SE (n=20)
I	52.61±.01^{bcd}	8.32±.01^{abc}	39.06±.02^{ab}	137.24±1.4^{ab}	44.39±.84^{ab}	32.32±.44^{abc}
II	52.59±.02^{bcd}	8.34±.01^{bc}	39.30±.08^{abc}	135.05±1.6^{ab}	43.89±.67^{ab}	32.51±.37^{abc}
III	52.78±.13^{d}	8.35±.01^{c}	39.21±.06^{abc}	134.89±1.1^{ab}	44.71±.54^{b}	33.15±.34^{c}
IV	52.48±.08^{bcd}	8.30±.01^{abc}	39.21±.07^{abc}	137.37±1.4^{ab}	44.15±.90^{ab}	32.11±.46^{abc}
V	52.23±.18^{ab}	8.29±.01^{ab}	39.26±.13^{abc}	138.09±1.3^{b}	44.00±.87^{ab}	31.83±.47^{abc}
VI	51.93±.21^{a}	8.28±.01^{a}	39.38±.15^{bc}	137.16±2.0^{ab}	42.72±.72^{ab}	31.18±.43^{a}
VII	52.58±.11^{bcd}	8.32±.01^{abc}	39.09±.11^{abc}	136.87±.76^{ab}	44.06±.66^{ab}	32.19±.46^{abc}
VIII	52.47±.20^{bcd}	8.31±.01^{abc}	39.20±.20^{abc}	136.52±.72^{ab}	43.52±.64^{ab}	31.88±.45^{abc}
IX	52.24±.10^{ab}	8.30±.015^{ab}	39.45±.11^{c}	133.59±1.1^{a}	42.17±.50^{a}	31.60±.44^{ab}
X	52.68±.07^{cd}	8.32±.01^{abc}	38.99±.07^{a}	135.91±.88^{ab}	44.47±.58^{b}	32.71±.35^{bc}
XI	52.63±.07^{bcd}	8.32±.01^{abc}	39.04±.07^{ab}	136.26±.76^{ab}	44.00±.56^{ab}	32.30±.42^{abc}
XII	52.28±.05^{abc}	8.31±.01^{abc}	39.40±.05^{bc}	133.66±.95^{a}	42.5±.51^{ab}	31.83±.40^{abc}

Values Indicate Mean ± S.E. (figures in the bracket indicate 'n') Means having different superscripts at a particular period of treatment differs significantly (P≤0.05)

Chauhan *et al.* (2009) reported that cow urine taken on daily basis, serves as a potential booster of immunity. Thus, the amelioration with cow urine distillate had a beneficial effect on the health of the birds. The decrease in Hb, PCV, TEC and TLC might be due to moving of fluid from extra vascular compartment to the intravascular compartment in order to manage the normal cardiac output and stroke volume and also pooling of the blood cells in the blood reservoirs such as spleen etc. The ameliorative effects of cow urine distillate is justified by the increase in haematological values in treatment groups VII to XII as compared to their respective only imidacloprid treated groups. This is an indicator of ameliorating potential of cow urine distillate that stimulate the bone marrow and reticuloendothelial system to generate the more number of erythrocytes and other blood cells to fight against the imidacloprid induced toxicity. Bapu (2001) reported that cow urine posses the haematinic properties due to the presence of traces of iron in it. Chauhan *et al.* (2001) reported that there was increase in B and T lymphocyte in mice when treated with 1 ml Kamdhenu ark in water for a period of 90 days. Garg *et al.* (2005) reported that bird treated with cow urine shows increase in T-cell blastogenesis. Ambwani *et al.* (2006) reported significant increase in avian B and T cell blastogenesis when treated with cow urine.

The study concluded that imidacloprid produced the dose dependent toxicity on repeated dose oral administration in white leghorn cockerels and the cow urine distillate was proved to be having ameliorative effect against the imidacloprid induced toxicity by restoring the values towards normal value by continuous administration for 45 days.

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