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Evaluation of ameliorative effect of cow urine distillate on serum biochemical parameters in imidacloprid intoxicated white leghorn broilers

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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 alone or in combination with cow urine distillate once daily for 45 days. The blood samples were collected just before termination of study for estimation of serum biochemical parameters. There was significant increase (P<0.05) in the Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Cholesterol, Triglycerides, Blood Urea Nitrogen (BUN), Creatinine and Uric acid whereas significant decrease in Glucose, Total protein and Albumin in high dose imidacloprid treated group (5.20 mg/kg body weight) in comparison to control group. Study is indicative of ameliorative effect of cow urine distillate as it protects the birds from being severely intoxicated by imidacloprid. Based on the results of the present study it may be concluded that imidacloprid caused toxicity in a dose dependent manner following repeated dose oral exposure for 45 days and cow urine distillate has produced ameliorative effect against the imidaclorid toxicity by restoring the values towards normally.

Keywords: Cow urine, distillate, imidacloprid, intoxication, serum biochemical

Introduction
Cow called gaumata in Indian culture, is regarded as the most valuable and sacred animal in India. Cow is the backbone of Indian rural economy which sustains human life and represents cattle wealth and bio-diversity. In traditional medicines, cow urine was used as an effective and simple medicine. Cow's urine was compared to the nectar in Veda, the sacred Hindu scriptures (Rig-Veda 10.15). It has a unique place in Ayurveda and has been described as the most effective secretion of animal origin with diverse therapeutic values in "Sushrut Sumhita" and Ashtanga Sangraha". Cow's urine having curative properties is referred in various ancient scriptures like Charaka- Samhita, Rajnighantu, Brahad-Wagbhatt, Sushruta Samhita and Amritsagar. Cow Urine was also considered as an antidote to various types of poisons (Sushruta Samhita). It has been patented as activity enhancer and availability facilitator for bioactive molecules including anti-infective and anti-cancer agents (US Patent No 6410059/2002) (Khanuja, 2002) [11]. Cow urine analysis has shown that it contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, citric, titric, succinic, calcium salts, Vitamin A, B, C, D, E, lactose and creatinine. Cow urine promotes power of wisdom in human beings and act like universal medicine (Chauhan, 2004 and Dhama *et al.*, 2005) [2, 3]. Due to its medicinal values, rural population in India use cow urine as a folklore remedy for various ailments. 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) [7]. Though, there are abundant claims of the efficiency of cow urine but its efficiency against pesticide-induced toxicity has not been much more explored so the present study was aimed to determine the ameliorative potential of cow urine distillate on imidacloprid-induced biochemical alterations in poultry.

Material and Methods
Day old healthy male white leghorn chicks used in this study were procured and kept in pens of Battery brooder house at Poultry farm, College of Veterinary and Animal Science, Bikaner. The chicks were offered standard feed and water ad libitum during entire experiment period. The experimental trial was approved by the Institutional Animal Ethics Committee

(No.-CVAS/IAEC/CPCSEA-2044/GO/Re/SL/18/2019/10) and performed as per its guidelines.

Commercial product of Imidacloprid (17.8%) was purchased from the local market of Bikaner, Rajasthan India. Apparent LD_{50} of imidacloprid 104.1 mg/kg (orally) was taken into consideration for calculation of different dose groups (Kammon *et al.*, 2010) [10].

Fresh cow urine was collected at morning time in sterile containers from healthy Rathi cows from a private dairy farm of Bikaner district of Rajasthan and filtered through Whatman filter paper no.1.Cow urine distillate was prepared from filtered cow urine using distillation apparatus and stored in air tight brown bottle for further use.

Experimental design

Birds were divided randomly into twelve groups containing 20 birds in each group. Birds of Group I served as normal control were administered only feed and water and birds of group II and group III were administered orally cow urine distillate@ 2 ml/kg and 4 ml/kg body weight respectively for 45 days. Birds of group IV, V and VI were administered imidacloprid orally @ 2.60 mg/kg ($LD_{50/40,}$ Low dose), 3.47 mg/kg ($LD_{50/30,}$ Medium dose) and 5.20 mg/kg ($LD_{50/20,}$ High dose) body weight respectively for 45 days and birds of group 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. 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. Birds were observed for any toxic symptoms throughout the experimental period and were also weighed weekly to monitor body weight gain. The blood samples were collected before termination of study and were analysed.

Biochemical estimations

The blood samples were collected in plain tubes (5ml) for serum biochemical parameters. Serum was harvested by centrifugation at 3000 rpm for 10 minutes. The separated serum was stored at -20 ^{0}C until analysis. The biochemical estimations were carried out using a fully automatic biochemistry analyzer 'Turbo Chem 100, Awareness Technologies, USA', with iChem 100 reagent kits. Biochemical parameters *viz.* serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), serum albumin, serum glucose, serum cholesterol, triglycerides (TGL), serum creatinine, urea and uric acid were estimated.

Statistical analysis

The values are expressed as the mean and standard error of the mean (SEM). Comparisons between treated groups 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 considered as a standard for statistically significant differences.

Results and Discussion

Effect of cow urine distillate on serum biochemical parameters in imidacloprid intoxicated white leghorn broilers is depicted in table 1and table 2. Significant increase in SGOT and SGPT after 45 days oral administration of imidacloprid was observed in medium dose (V) and high dose group (VI) and significant increase in ALP, cholesterol, triglycerides, BUN, creatinine and uric acid level was observed only in high dose of imidacloprid treated group (VI) in comparison to control group (I). Non-significant change was observed in SGOT, SGPT, ALP, cholesterol, triglycerides, BUN, creatinine and uric acid level in birds that received cow urine distillate @ 2 ml/kg (II) and @ 4 ml/kg (III) as comparison to control group (I). Non significant change was observed in SGOT, SGPT, ALP, triglycerides, BUN and creatinine level in birds that received cow urine distillate along with imidacloprid (VII to XII) in comparison to their respective groups that received only imidacloprid (IV, V and VI) whereas significant change in cholesterol and uric acid level was found in the group XII when compared with their respective only imidacloprid treated group (VI).

A dose related but non-significant decrease was observed in total protein and albumin after 45 days oral administration of imidacloprid at low lose (IV) and medium dose (V) in comparison to control group (I) whereas significant decrease was observed in high dose group (VI) in comparison to control group (I). Non-significant change was observed in total protein in birds that received cow urine distillate @ 2 ml/kg (II) and @ 4 ml/kg (III) as comparison to control group (I) whereas significant increase was found in albumin value in birds that received @ 4 ml/kg (III) as comparison to control group (I). Non-significant increase was found in the group VII, VIII, X and XI group when compared with their respective only imidacloprid treated group (IV, and V) whereas significant increase was found in the group IX and XII in total protein level when compared with their respective only imidacloprid treated group (VI). Non-significant effect was found in the group VII, VIII, IX, X and XI group when compared with their respective only imidacloprid treated group (IV, V and VI). Whereas significant increase was found in the group XII in albumin when compared with their respective only imidacloprid treated group (VI)

Significant decrease inglucose level was observed in medium and high dose group in comparison to control group (I). Non-significant change was observed in glucose level in birds that received cow urine distillate (II and III) as comparison to control group (I) whereas significant change was found in birds received cow urine distillate along with imidacloprid (VII to XII) in comparison to animals that received only imidacloprid respectively (IV, V and VI).

Present findings are in accordance with Gururaja *et al.* (2009) [6] who evaluated attenuation of carbon tetrachloride induced haepato-toxicity by cow urine distillate in rats and reported that cow urine distillate decreased the levels of SGOT, SGPT and ALP in a dose dependent manner and investigated protective effect of cow urine distillate on liver and kidney function which might be due to antioxidants present in cow urine. Wate *et al.* (2011) [13] observed the cow urine, distillate, redistillate and residue as a good source of compounds with antioxidant properties and they exhibited significant free radical scavenging activity and reducing power activity.

Similarly, Joshi and Chauhan (2012) [9] evaluated the anticancer effect of Taxus Bacatta alone and in combination with cow-urine distillate on biochemical profile of mice treated with Diethyl Nitrosamine and reported that the extracts alone and its formulation with CUD treatments of test groups showed the positive response by significantly decreasing the AST, ALT and alkaline phosphatase values. They also reported significant improvement in Cholesterol, triglycerides Creatinine, BUN and Uric acid levels**.**

Present finding is also in agreement with Tiwari *et al.* (2017) [12] who investigated the imidacloprid induced hemato biochemical changes and its amelioration by cow urine distillate in white leghorn broilers and found that there is marked increase in serum total protein and albumin in birds received cow urine distillate along with imidacloprid in comparison to their respective only imidacloprid treated birds. Tiwari *et al.* (2017) [12] also reported that there was significant increase in cholesterol and creatinine in intoxicated groups in comparison to control group and there was decrease in the cholesterol and creatinine level in birds received cow urine distillate along with imidacloprid in comparison to their respective high dose imidacloprid treated birds cementing the beneficial and ameliorative role of cow urine distillate against the toxicant.

Garg (2004) [4] also reported that supplementation of cow urine to white leghorn layer showed significant amelioration in serum total cholesterol. Beneficial effects of cow urine on serum biochemical profile (serum protein and glucose) of laying birds were also observed by Garg*et al.* (2004) [4]. Ayoub *et al.* (2011) [1] reported hepatoprotective, antioxidant and pancreatic beta cell neogenesis in STZ-induced diabetic rats on *Momordica charantia* in cow urine treatment. Jarald *et al.* (2008) [8] found that cow urine has antioxidant and antimicrobial activities.

Table 1: Effect of cow urine distillate on SGOT, SGPT, ALP, Serum Total Protein, Serum Albumin in imidacloprid intoxicated white leghorn broilers

Group	SGOT (U/L)	SGPT (U/L)	ALP(U/L)	Serum Total Protein (g/dl)	Serum Albumin (g/dl)
I	183.50±1.53^{a}	23.05±0.15^{a}	931.10±3.71^{a}	3.9435±0.002^{c}	1.5530±0.001^{cde}
II	183.75±1.68^{a}	23.10±0.14^{ab}	937.10±3.84^{a}	3.9430±0.006^{c}	1.5610±0.001^{ef}
III	184.55±1.60^{a}	23.20±0.09^{ab}	942.10±3.77^{a}	3.9450±0.005^{c}	1.5675±0.003^{f}
IV	187.25±2.29^{a}	23.30±0.16^{ab}	962.10±7.05^{a}	3.9195±0.005^{c}	1.5460±0.001^{cd}
V	241.90±2.66^{cd}	23.55±0.13^{b}	976.65±13.86^{a}	3.9145±0.011^{c}	1.5400±0.002^{c}
VI	252.60±2.65^{e}	24.55±0.19^{c}	1150.75±31.18^{b}	3.5890±0.034^{a}	1.4685±0.008^{a}
VII	189.15±2.43^{a}	23.15±0.13^{ab}	957.45±4.90^{a}	3.9355±0.002^{c}	1.5520±0.001^{cde}
VIII	235.40±3.20^{bc}	23.40±0.13^{ab}	973.70±13.24^{a}	3.9325±0.002^{c}	1.5425±0.001^{cd}
IX	247.10±2.97d^{e}	24.45±0.18^{c}	1141.25±28.48^{b}	3.7450±0.018^{b}	1.4760±0.008^{ab}
X	184.30±1.87^{a}	23.10±0.06^{ab}	949.00±4.60^{a}	3.9375±0.002^{c}	1.5550±0.001^{def}
XI	228.90±3.26^{b}	23.25±0.12^{ab}	966.20±8.42^{a}	3.9350±0.002^{c}	1.5470±0.001^{cd}
XII	241.60±2.94^{cd}	24.25±0.19^{c}	1139.75±28.08^{b}	3.7800±0.021^{b}	1.4860±0.007^{b}

Values Indicate Mean ± S.E. (n=20). Means having different superscripts differs significantly (P≤0.05) when compared vertically within the same column

Table 2: Effect of cow urine distillate on Glucose, Cholesterol, Serum Triglyceride, BUN, Serum Creatinine, Serum Uric Acid in imidacloprid intoxicated white leghorn broilers

Group	Glucose (mg/dl)	Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Blood Urea Nitrogen (mg/dl))	Serum Creatinine (mg/dl)	Serum Uric Acid (mg/dl)
I	162.20±0.21^{ef}	131.95±0.221^{a}	33.77±0.094^{a}	0.6505±0.007^{a}	0.3480±0.001^{a}	5.2950±0.015^{ab}
II	162.90±0.26^{ef}	132.00±0.229^{a}	33.81±0.085^{a}	0.6485±0.006^{a}	0.3435±0.001^{a}	5.2900±0.012^{a}
III	163.85±0.38^{f}	132.10±0.260^{a}	33.82±0.084^{a}	0.6445±0.006^{a}	0.3455±0.001^{a}	5.2800±0.009^{a}
IV	158.20±1.18^{de}	132.35±0.274^{a}	33.92±0.141^{ab}	0.6605±0.009^{ab}	0.3565±0.004^{a}	5.3200±0.015^{ab}
V	147.20±1.31^{c}	133.10±0.216^{a}	34.12±0.186^{ab}	0.6685±0.008^{abc}	0.3660±0.008^{a}	5.3450±0.016^{bc}
VI	117.70±1.17^{a}	137.60±0.650^{c}	34.32±0.203^{b}	0.6935±0.012^{c}	0.4775±0.013^{b}	5.4500±0.027^{e}
VII	163.50±0.66^{f}	132.20±0.224^{a}	33.87±0.101^{a}	0.6515±0.006^{a}	0.3515±0.003^{a}	5.3050±0.013^{ab}
VIII	155.70±1.67^{d}	133.00±0.177^{a}	33.88±0.099^{a}	0.6585±0.007^{a}	0.3605±0.009^{a}	5.3150±0.015^{ab}
IX	132.70±3.49^{b}	137.15±0.572^{bc}	34.12±0.173^{ab}	0.6875±0.010^{c}	0.4600±0.011^{b}	5.4100±0.021^{de}
X	165.80±1.01^{f}	132.05±0.256^{a}	33.72±0.095^{a}	0.6465±0.006^{a}	0.3435±0.002^{a}	5.2900±0.010^{a}
XI	161.85±2.53^{ef}	132.45±0.211^{a}	33.77±0.158^{a}	0.6555±0.006^{a}	0.3545±0.007^{a}	5.3000±0.012^{ab}
XII	144.70±1.28^{c}	136.35±0.493^{b}	33.95±0.153^{ab}	0.6850±0.009^{bc}	0.4570±0.010^{b}	5.3800±0.011^{cd}

Values Indicate Mean ± S.E. (n=20). Means having different superscripts differs significantly (P≤0.05) when compared vertically within the same column

Conclusion

Based on the results of the present study it may be concluded that imidacloprid caused toxicity in a dose dependent manner following repeated dose oral exposure for 45 days and cow urine distillate has produced ameliorative effect against the imidacloprid toxicity by restoring the values towards normally.

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