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EVALUATION OF IMMUNOTOXIC POTENTIAL OF FIPRONIL IN WISTAR RATS WITH SPECIAL REFERENCE TO HUMORAL IMMUNE RESPONSE

Amarnath G. Somwanshi[1], Vikas V Karande[2], Nitin D. Jadhav[3] and Madhumanjiri M. Gatne[4]*

[1]*Department of Pharmacology, Bombay Veterinary College, Parel, Mumbai*
[2*]*Department of Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist-Satara*
[3]*Department of Pharmacology and Toxicology, COVAS, Parbhani, Maharashtra*
[4]*Department of Pharmacology, Bombay Veterinary College, Parel, Mumbai*

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Abstract: *A number of biological or chemical agents have the capacity to alter the functionality of the immune system in humans and animals. There are several studies on immunotoxicity of most groups of pesticides viz. organochlorines, organophosphates, carbamates and pyrethroids. However, no reports seem to be available on immunotoxicity of phenylpyrazole class of pesticides in animals. In view of this, it was imperative to explore the immunotoxic effects of fipronil. These investigations will help in understanding the specific immunological alterations caused by fipronil. The present study was therefore undertaken with the objectives of generating data on immunotoxic potential (if any) of fipronil by assessing humoral immune responses. The finding of the tests performed for assessment of humoral immunity (antibodies titers against SRBCs) revealed that fipronil has adverse effect on humoral immunity. Present study warns immunotoxicity as one of the risks associated with chronic exposure to fipronil.*

Keywords: *Humoral immunity, HA titer, Fipronil, Immunotoxicity.*

Introduction

In today's world of chemicals human beings and animals are continuously getting exposed to different chemicals knowingly or unknowingly. After World War II, introduction of different chemicals for increasing crop production started on large scale. This undoubtedly was like a boon for increasing food production. However, it also carried the risk of getting exposed chronically to these chemicals either in the form of environmental contaminants or residues in food and food products. Potential of the chemicals to cause the adverse effects on immune system was also realized subsequently and the number of studies to assess effects of chemicals on immune system also gradually increased. Some of the pesticides were shown to have adverse effects on immune system.[1-7]

Arsenic was one of the earliest insecticides used. In the mid-twentieth century, a major revolution took place in the field of insecticides with the introduction of organochlorine compounds in 1939, organophosphates in the 1950's and pyrethroids in 1970's.[8] Insecticides of these groups enjoyed the privilege of their widespread use till the hazards and toxicities (both to the host and the ecosystem) associated with them were realised. The development of resistant insects was another problem faced by the users of these insecticides. The continuous efforts towards finding out pesticides with high selective toxicity resulted in development of phenylpyrazoles.[9] Fipronil, is an insecticide that was discovered and developed by Rhone-Poulenc in year 1985-1987 and was approved for marketing in year 1993. Although it is effective in veterinary field, there are concerns about its environmental and human health effects. Fipronil is a member of the phenyl pyrazole class of pesticides. Fipronil became the leading imported product in the area of veterinary and agriculture field.Fipronil is used in a wide variety of pesticide products, including granularproducts for grass, gel baits, spot-on pet care products, liquid termitecontrol products, and

products for agriculture. There are more than 50registered products that contain fipronil all over the world.

A number of biological or chemical agents have the capacity to alter the functionality of the immune system in humans and animals. There are several studies on immunotoxicity of most groups of pesticides viz. organochlorines, organophosphates, carbamates and pyrethroids.However, no reports seem to be available on immunotoxicity of phenylpyrazole class of pesticides in animals. Although a few pharmacological and toxicological studies have been conducted, substantial reports are lacking on immunotoxicity studies of the phenyl pyrazole.

In view of this, it was imperative to explore the immunotoxic effects of fipronil. These investigations will help in understanding the specific immunological alterations caused by fipronil. The present study was therefore undertaken with the objectives of generating data on immunotoxic potential (if any) of fipronil by assessing humoral immune responses.

Material and Methods:

Experimental animals:

The study was conducted in healthy Wistar rats weighing 200-250g. Rats (30) were procured from institutional animal house and maintained in the Department of Pharmacology and Toxicology, Bombay Veterinary College, Parel, Mumbai under standard laboratory conditions.

Experimental Design:

Study was carried out in 30 rats. Considering the LD_{50} (97 mg/kg) [10] and finally three dose levels of exposure were selected as 5 mg/kg, 7.5 mg/kg and 10 mg/kg. Rats were divided into five groups, out of which group III, IV and V were used for HA test. Group III, IV and V comprised of six rats in each that received 5 mg/kg, 7.5 mg/kg and 10 mg/kg fipronil respectively via oral gavage for consecutive 28 days. Group I and II served as control for group III, IV and V. Groups III, IV and V were exposed to the sheep RBCs antigen on day 14th and at sacrifice (day 29) of study. HA test was performed to find the antibody titer against sheep RBC antigen. Details of treatment and grouping are presented in table 1.

Table 1: Design of experiment:

Group	Treatment	Dose (for 28 days)	Immunization with SRBCs (on 14th and 29th day)
I	Distilled Water	1 ml/ 100g BW	+
II	Tween80 0.01% (v/v)	1 ml/ 100g BW	+
III	Fipronil	5 mg/ kg BW	+
IV	Fipronil	7.5 mg/ kg BW	+
V	Fipronil	10 mg/ kg BW	+

Chemicals: standard Fipronil was obtained from Cipla ltd Mumbai, whereas Nutrient broth, Hank Balanced Salt Solution (HBSS) and Culture of *Staphylococcus aureus* procured from Himedia laboratories Pvt. Ltd. Mumbai.

Evaluation of humoral immune response:

The procedure of HA test was followed as per Cunningham andThomas associates. [11-12]

Preparation of Sheep RBCs:

Blood (5 ml) was collected from jugular vein of sheep and mixed with equal volume of Alsever's solution. It was then centrifuged at 3000 rpm for 10 minutes to separate out plasma and cells. The plasma and buffy coat were discarded and to the remaining RBC pack equal volume of NSS was added. After proper mixing, the sheep RBC's (SRBC's) suspension was centrifuged again and the procedure was repeated thrice so that at the end there was only a packed cell volume of SRBC's in the test tube after removal of the supernatant layer of NSS. NSS (10 ml) added in the packed cell volume of SRBC. This suspension contained 5 $\times 10^9$ SRBC/ml counted by Haematoanalyzer (Diatron Abacus). Further it was diluted 10 times to use for rat immunization and for the antibody titer against SRBC determination.

Immunization of rat:

For evaluation of HA titer, rats were pretreated with the drugs for 14 days and each rat was immunized with0.5×10^9 sheep red blood cells (SRBCs) intraperitoneally, including control rats. Fipronil gavage was continued for further consecutive 14 days and blood sampleswere collected from each rat on day 29th. The highest dilution ofserum showing haemagglutination wasexpressed as HA titer.[12]

Haemagglutination (HA) test:

The HA test was performed as mentioned by Cunningham (1961). In microplate 50 µl of chilled NSS was added in first 11 out of 12 wells. Then 50 µl serum was added in well no. 1 and 12.From well no. 1 to 10 serial two fold dilutions of the contents of well was carried out by transferring 50 µl volume into subsequent well after through mixing. 50µl volume from well no. 10 was discarded. After that, 50 µl SRBCs suspension was added in wells 1-12.Thus 11th well was control for SRBC (50 µl NSS + 50 µl of SRBCs) and 12th well was serum control (50 µl serum + 50 µl of SRBCs).The microtiter plate was rotated gently.The plate was covered with the foil and kept at room temperature for 2 hours and at the time of interpreting the results care was taken to find button formation in the serum control wells.The reciprocal of the highest dilution of the serum that produced complete agglutination (matt formation) of the SRBCs was taken as the HA titer or HA unit/50 µl.

Statistical analysis:

HA titers obtained during the study were analyzed statistically with completely randomized design**.**

Results and Discussion:

Antibody titer against Sheep red cells (SRBCs) by Haemagglutination test (HA):

Antibody titers against SRBCs were measured at the end of the experiment in treated groups and control groups. Antibody titers as mean HA units against sheep red blood cells (SRBCs) antigen in experimental rats are presented in Table 1 and Figure 1. The present investigation revealed reduction in antibody titer against SRBCs in dose dependent manner in rats exposed to fipronil. Antibody titers of groups III, IV and V were compared with Groups I and II. Antibody titer of group of highest dose (10 mg/kg) was found lowest among all groups indicating its adverse effect on immune system. In case of treatment groups antibody titers reduced highly significantly ($P \leq 0.01$) in group V (26.67 ± 3.37) as compared to the group IV (85.33 ± 13.5) and group III (234.67 ± 21.33) receiving fipronil @ 10 mg/kg, 7.5 mg/kg and 5 mg/kg respectively.

The level of the serum antibodies against SRBCs is the conventional index of the assessment of humoral immunity, which could be measured accurately by HA titer. The decline in HA titer to SRBCs in the present study was similar as reported by some workers.[13-16]Decline in HA titer to SRBCs due to the role of acetamiprid in the inhibition of the degradation of antigen by the reticuloendothelial system[16], inhibition of immunoglobulin synthesis,[13-14] or increased lysosomal digestion of immunoglobulin.[15] The decline in HA titer to SRBCs may also be due to the depletion of T- lymphocyte because SRBCs are thymic dependent antigen and require T and B cell co-operation for antibody synthesis.[17]

Table 15: Mean (± S.E.) haemagglutinationtiter (HA) from different groups of rats immunized with SRBCs.

Groups	Group I	Group II	Group III	Group IV	Group V
Treatment	Control	Vehicle control	5 mg/kg	7.5mg/kg	10mg/kg
Mean±S.E.	768 ± 114.5^a	768 ± 114.5^a	234.67 ± 21.3^b	85.33 ± 135^c	26.67 ± 3.37^d

Values (n= 12) in the same row bearing at least one common superscript do not differ significantly.
Treatments found Significant at 1% and 5% level of significance. C.D. (0.01) = 288.894 and C.D. (0.05) = 213.533.

Table 3: Analysis of Variance:

ANOVA	Source of Variation	SS	DF	MS	F	C.D. value at 5 %
HA titer	Treatment Error Total	3202935 805888 4008823	4 25 29	800733.9 32235.52	24.84011**	213.533

** Significant at P ≤ 0.01

From the present study it is logical to expect possible increase in susceptibility of fipronil exposed animals to various infections because of the declined humoral immune response. The antigen-antibody reaction results in agglutination. The relative strength of an antibody titer is defined as the reciprocal of the highest dilution which is still capable of causing visible agglutination. The antibody titer is useful to measure the changes in immune response.

On scanning the literature, hemagglutination test was found to have been used as a method for assessing humoral response. Antigens used for HA test were sheep red blood cells, ovalbumin and virus. The procedure of use of SRBCs as described in material method was followed by several workers for assessing immunological response in laboratory animals.[18-23]

There was progressive and proportional decrease in HA titer in treated rats with imidacloprid as compared with vehicle control rats.[3]Another study by Mondal and associates with acetamiprid toxicity revealing effect on humoral immunity showed significant reduction in HA titer in rats.[6]Pal*and associates also* reported the dose dependent reduction in HA titer after administration of endosulfan on humoral response in rats.[7]

Conclusions:

The finding of the tests performed for assessment of humoral immunity (antibodies titers against SRBCs) revealed that fipronil has adverse effect on humoral immunity.

Present study warns immunotoxicity as one of the risks associated with chronic exposure to fipronil. This factor should be borne in mind while the issue of chronic exposure to fipronil is a question.

Humans continuously associated with during process of production, transport or uses of fipronil are the ones likely to get exposed to it chronically.

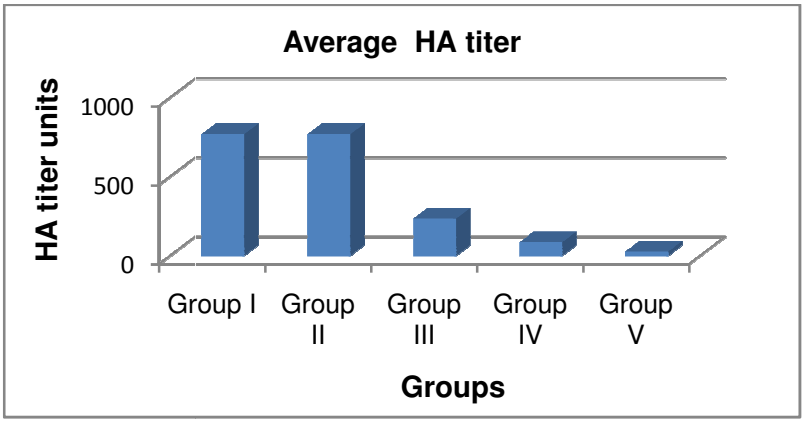

Figure 1: Average HA titers from different groups of rats.

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