



Immunomodulatory impact of dicamba in Black Bengal goats following repeated oral administration*

M KHAN¹, A K CHAKRABORTY² and T KMANDAL³

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 037 India

Received: 4 January 2010; Accepted: 11 May 2011

Key words: Black Bengal goat, Haemolytic plaque assay, Immunomodulation, Sandwich ELISA

Most of the pesticides exert immunosuppression effect on both wings of immunosystem i.e. humoral and cell mediated immunity. Organochlorines, organophosphates, carbamates and synthetic pyrethroids were found immunotoxic at no adverse effect dose levels in poultry, sheep, and bovine calves (Pruett *et al.* 1992). Immunotoxicity of pesticides would not only be responsible for vaccination failure, reduced immunocompetence, occurrence of disease epidemics but also lead to increased incidence of neoplasm in both animals and man. Dicamba-dimethyl-amine salt, a lipid soluble herbicide having probability and acceptability for field application to control weeds. Now a-days considerable interest has arisen to study the immunomodulatory effect of agrochemicals. Study of immunomodulatory effect of Dicamba is scarcely available. Considering the above, an attempt has been made to screen the immunomodulatory effect of Dicamba.

Clinically healthy 9 Black Bengal male goats (1–1.5 year age) weighing between 9–12 kg were caged individually in custom made metabolic cage and kept in controlled condition. Before starting the experiment animals were dewormed twice with a mixture of albendazole (7.5 mg/kg) and rafoxanide (7.5 mg/kg) at 21 days interval. After 1 month of last dosing of anthelmintic, animals were acclimatized for 7 days.

Animals were divided into 3 equal groups. The experimental group (group 3) of goats were fed with Dicamba suspending in 25 ml of 1% carboxy methyl cellulose (CMC) at a maximum non toxic oral dose of 466.67 mg/kg once daily for a consecutive period of 35 days, while levamisole (standard immunostimulant dose) was administered at immunostimulating dose (Robertson 1993) to experimental control (group 2) group of animals.

The control group (group 1) of goats received 25 ml of 1% CMC once daily for consecutive 35 days. On 36 day, all goats were immunized with 5% SRBC intraperitoneally and

on day 5 post dosing SRBC, animals were sacrificed for conducting haemolytic plaque assay.

Blood samples were collected from all 9 goats before administration of herbicide (0 day) and thereafter at every 7 days interval till 35 days post dosing. Serum was separated and preserved at –20°C for estimation of IgG level. A small portion of spleen from each goat was collected in ice-cold RPMI -1640 (pH 7.4) media for haemolytic plaque assay.

Estimation of serum immunoglobulin G (IgG) level: Before performing sandwich ELISA, goat IgG was purified by ion-exchange chromatography according to Himmelhoch (1971). Anti goat hyperimmune sera was raised by repeated intramuscular injection of purified goat IgG in 2 rabbits. Anti-species conjugate and substrate were procured from commercial farm.

Purification of goat IgG: Serum (15 ml) was collected from blood of a healthy goat and centrifuged at 3000 rpm for 15 min. Before loading to column, protein concentration of the solution was measured as per Lowry *et al.* (1951). The concentration of protein in the sample was 54.5 mg/ml.

Absorbance of eluted material of each fraction was measured by spectrophotometer at 280 nm wave length. Tubes showing peak were pooled and the volume was concentrate by reverse osmosis using sucrose.

Anti goat HIS was raised by injecting goat IgG into the gluteal muscle of 2 rabbits. The day of injection and amount of dose injected were:

Day	Amount of injected IgG (µg)
0, 5, 10, 15, 20, 28, 38	400, 450, 500, 500, 600, 650, 650

Samples were pooled and protein concentration was estimated by Lowry's method. Sandwich ELISA was performed as per Nemzek *et al.* (1999). Optimum anti-goat HIS dilution and conjugate dilution were calculated to be 1: 8000 and 1: 2000 respectively. IgG levels in serum sample were deduced from standard curve prepared before using fortified goat-IgG as capturing antibody. Haemolytic plaque assay was done following Jerne and Nordin (1963). For immunization, 5 ml suspension of 20% SRBC in PBS was

*From MVSc thesis of the first author.

Present address: ³Professor (e mail: drtkm48 @yahoo.co.in),

^{1,2}Department of Veterinary Pharmacology and Toxicology.

Table 1. Effect of dicamba on serum immunoglobulin G (IgG) level (mg/ml) in Black Bengal male goats following consecutive oral administration for 35 days

Animal/day	0	7	14	21	28	35	41
Control ^I	13.25±0.64	12.5±0.55	10.75±0.88	12.25±0.52	13.0±0.52	13.25±0.72	11±0.70
Experimental Control ^{II}	9.25 ^e ±0.44	10.5 ^e ±0.64	8.5 ^e ±0.23	19.5 ^{cd} ±1.04	24.5 ^{bcd} ±0.75	20.5 ^{bcd} ±0.75	18.00 ^d ±0.81
Group Experimental ^{III}	10.17±0.33	11.3±0.73	12.5±0.50	12.1±0.97	13.33±1.99	9.58±1.99	9.67±0.44

Mean values of three replicates with S E; ^I, administered CMC (25 ml) once daily by oral route for consecutive 35 days; ^{II}, administered levamisole (2.25 mg/kg) for consecutive 3 days followed by 3 days rest and again for subsequent 3 days by subcutaneous route; ^{III}, administered dicamba (466.67mg/kg) once daily by oral route for consecutive 35 days; mean values with different superscript in a row are significantly different (P<0.01)

Table 2. Haemolytic plaque assay (%) by caprine spleen cell sacrificed on 5 day post-immunization with SRBC

Animal group	% of plaque formed
Control ^I	8.16 ^b ±0.60
Experimental control ^{II}	20.00 ^a ±1.15
Experimental ^{III}	9.54 ^b ±0.44

^I, Administered CMC (25 ml) once daily by oral route for consecutive 35 days; ^{II}, administered levamisole (2.25 mg/kg) for consecutive 3 days followed by 3 days rest and again for subsequent 3 days by subcutaneous route; ^{III}, administered dicamba (466.67mg/kg) once daily by oral route for consecutive 35 days; mean values with different superscript in a column are significantly different (P<0.01)

injected intraperitoneally into all 9 goats on day 36. Immunized goats were sacrificed on fifth day post immunization. For preparation of single cell suspension of immunized goat's spleen, it was taken from immunized goats in 5–7 ml of ice-chilled RPMI-1640 media. It was minced and suspension was filtered. Viable cells were counted by mixing equal volume of filtrate with trypan blue (0.1%) by trypan blue dye exclusion test (Hudson and Hay 1980). The results were analysed using one-way analysis of variance (Snedecor and Cochran 1959). Results were expressed as mean±standard error (SE).

Serum IgG level: The serum IgG level at different days against 0 day of all 3 groups of animals (Table 1) revealed that the vehicle CMC had no effect on serum IgG level in control group of animal. The values of serum IgG level in group 2 animals, which received levamisole at immunostimulating dose, showed significantly (P<0.01) increased serum IgG level. But serum IgG level did not change throughout the period of observation in animals of group 3 (treated with dicamba (466.67 mg/kg) orally once daily for consecutive 35 days).

Stogaus and King (1995) reported that oral levamisole (18 mg/kg) decreased cortisone level in rats under antigenic challenge by ovalbumin. This review exerted strongest support on the immunostimulant effect of levamisole in rats.

Chronic exposure to DDT and another organochlorine

insecticide which caused depressed immune response in rodents did not change vaccinal responses in exposed children (Descotes 1999). From the above study it might be concluded that dicamba, a benzoic acid derivative had no effect on immune status of goats during the 35 days observation period.

Haemolytic plaque assay: The % of haemolytic plaque by spleen cells in group 1, and experimental groups 2 and 3 are given in Table 2. No significant variation in the % of plaque formation was observed between control and experimental group. But the value was significantly higher (P<0.01) in experimental control group.

Liubin and Kus'mitski (1997) observed the intragastric administration of trimesone, at new synthetic immunostimulant for increased humoral and cell mediated immune response to thymus dependent antigen, the SRBC. Levamisole, being an immunostimulant also increased the humoral immune response to SRBC in this experiment.

Estimation of plaques against sheep red blood cells in a heterologous host gives an indication of humoral immune status of the host. Direct IgM or indirect IgG plaques can be measured depending on the day of slaughter after immunization. In this experiment, haemolytic plaque assay may indicate the presence of IgM as the animals were sacrificed on day 5 post immunization. The unchanged level of IgM by dicamba indicated that dicamba had no effect on humoral immune status of host during this observation period.

From the above study, it may be concluded that dicamba had no effect on humoral and cell mediated immunity.

SUMMARY

The study was conducted to evaluate immunomodulatory effect of the herbicide, dicamba on goats. Black Bengal (9) male adult goats were divided into 3 groups, each containing 3 animals. Control (group 1) group received carboxy-methyl cellulose, while experimental control (group 2) group received levamisole at immunostimulating dose and experimental group (group 3) was given Dicamba daily for 35 days. On day 36, all goats were immunized with 5% SRBC intraperitoneally and on day 5 post dosing SRBC animals were sacrificed for conducting haemolytic plaque assay. Total serum IgG level was estimated by Sandwich ELISA. No

significant variation in serum IgG level was observed at different days against 0 day in control and experimental group. But serum IgG level was increased significantly in experimental control group. No significant variation was observed in % of plaque formation in control and experimental group but the value was significantly higher in experimental control group. Results indicated that dicamba had no effect on immunomodulation of goats during this observation period.

REFERENCES

- Descotes J. 1999. *An Introduction to Immunotoxicology*. 1st edn. Taylor and Francis Ltd., London.
- Himmelhoch S R. 1971. Chromatography of proteins on ion-exchange adsorbents. *Methods in Enzymology* **22**: 273–86.
- Hudson L and Hay F C. 1980. *Practical Immunology*. 2nd edn. Blackwell Scientific Publication.
- Jerne N K and Nordin A A. 1963. Plaque formation in other by single antibody producing cells. *Science* **140**: 405.
- Liubin G S and Kuzmitskii B B. 1997. The immunological properties of trimesone and levamisole on models of humoral and cell-mediated immunogenesis. *Eksp.Klin.Farmakol.* **60**(1): 50–52.
- Lowey O H, Rosenbrough N J, Farr A L and Randel R J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**: 265–75.
- Nemzek J A, Newcomb D E, Call D R and Remick D K. 1999. Plasma interference in an enzyme diluted immunosorbant assay using at commercial matched antibody pair. *Immunological Investigation* **28**(4): 209–21.
- Pruett S B, Han Y, Munson A E and Fuchs B A. 1992. Assesment of cholinergic influences on a primary humoral immune response. *Immunology* **81**: 157–62.
- Robertson L E. 1993. Antinematodal drugs. *John's Veterinary Pharmacology and Therapeutics*. 5th edn, Pp.820. (Eds) Book N H and McDonald L E. Kalyani Publishers, New Delhi.
- Stogaus R and King M G. 1995. Is oral levamisole immunostimulant in rats mediated by reduced levels of free plasma corticosterone? *International Journal of Immunopharmacology* **17**(8): 635–40.
- Snedecor G W and Cochran W G. 1968. *Statistical Methods*. 6th edn. Iowa State University Press, Iowa, USA.