

V. DISCUSSION

The present study was conducted to assess the toxic and pharmacological efficacy of cypermethrin and amitraz combination pesticide in rats, rabbits and guinea pigs. The results obtained in the study under acute oral toxicity, repeated dose 28 day toxicity, dermal toxicity, dermal irritation, skin sensitization, in vivo pharmacological efficacy against different stages of ticks in dogs, cattle and sheep studies are discussed here.

5.1 Acute oral toxicity study

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short term exposure by the oral rate. It is traditionally a step in establishing a dosage regimen in other studies by providing initial information on the mode of toxic action of a substance.

The oral LD\textsubscript{50} determined under acute toxicity study for cypermethrin and amitraz combination pesticide rats indicated that the cypermethrin and amitraz pesticide combination in male and female rats were 224.13 and 218.73 mg/kg body weight, respectively. The LD\textsubscript{50} values in female rats was slightly lower than that of the male rats. In the present study the LD\textsubscript{50} values recorded for male and female rats indicated that the technical grade cypermethrin and amitraz pesticide combination was very toxic as per toxicity rating (Clarke and Clarke, 1975).

The animals showed toxicity signs of hyperexcitibility, profuse salivation, tremors, burrowing behavior, frequent maturation, diarrhea, hunched back, muscular incoordination, weakness, altered gait, startle response, writhing movements of neck, violent twisting movements sometimes lifted from the body from floor, labored breathing, gasping, convulsions and death. The toxic signs were indicative of CNS involvement and the toxic signs produced by pesticide combination may be classified as Type II toxicity syndromes as has been reported for α-cypermethrin (Manna et al., 2004).

The clinical signs were in accordance with Rose (1982) who reported clonic convulsions, piloerection and salivation when α-cypermethrin administered in corn oil. The oral LD\textsubscript{50} value was reported to be 798 mg/kg in mice when administered orally.

In another study Coombs et al. (1976) reported the acute oral toxicity of cypermethrin to be ranging from 400 mg/kg in aqueous solution in rats. Coombs et al. (1976) and Dewar and Owen (1979) reported that the factors which increased the oral LD\textsubscript{50} value of cypermethrin included concentration, vehicle, temperature, age and the strain of animals used.

Rose and Dewar (1978) reported that α-cypermethrin was moderately to highly toxic and three to four times more toxic than cypermethrin. The clinical signs of toxicity were typical for a cyano
containing pyrethroid intoxication. The majority of mortalities occurred within the first three hours and surviving mice recovered within seven days.

Seawright (1982) reported that toxicity in dogs was expressed as CNS depression, atoxia, hypothermia, bradycardia and cardiovascular collapse. Fatalities have been reported and are more in common in small breeds. Amitraz produces a characteristic syndrome of hyperactivity, irritability and decreased motor activity as well as numerous other behavioural and physiological effects in dogs as observed by Moser et al., (1987).

Moser and MacPhail (1985) reported in mice that an i.p. dose of 600 mg/kg of amitraz caused the cumulative mortality with 80 per cent in one week; no death was observed in the first 48 hrs following amitraz administration.

Iyaniwura and Okonkwo (2004) determined the effects of acute cypermethrin toxicity in rats and its dose response characteristics. The intraperitoneal LD$_{50}$ of cypermethrin from the study was 44 mg/kg body weight and the symptoms of toxicity were muscular weakness, swaying gait and respiratory distress and prostration occurred at higher doses and convulsions preceded death apparently due to respiratory failure.

### 5.2 Repeated dose 28-day oral toxicity study

In the assessment and evaluation of the toxic characteristics of Pesticide combination, the determination of oral toxicity using repeated doses was carried out after initial information on toxicity was obtained by acute oral toxicity testing. This study provides information on the possible health effects and hazards likely to arise from repeated exposure over a relatively limited period of time.

Cypermethrin and amitraz combination pesticide was administered orally by gavage daily for 28 days to five groups of male and female rats separately at 0, vehicle 30, 60 and 120 mg/kg body weight.

There were clinical signs of toxicity viz., salivation, tremors, hyperexitability depression, isolation from group, burrowing behaviour, decreased feed intake, hunched back, altered gait, muscular incoordination, writhing movements at neck, frequent maturation, anaemia, diarrhea and mortality was noticed in mid and high dose group administered 60 and 120 mg/kg body weight in both male and female treated groups. There was no mortality in the group administered 30 mg/kg body weight. The group administered with vehicle viz., odorless kerosene did not show any clinical signs.

#### 5.2.1 Feed consumption

There was no change in feed consumption of all the treated groups compared to control group.
On the contrary, Buckwell and Butterworth (1977) reported that cypermethrin mixed diet at 1500 mg per kg for 13 weeks showed diminished feed intake in beagle hound dogs with weight loss, diarrhoea, anorexia, licking and chewing of paws.

5.2.2 Body weight gain

There was significant decrease in body weight gain in the mid dose and high dose groups of both male and female animals when compared to respective control groups from I to IV week. The decrease in body weight gain was in accordance with Clark (1982) who reported a decreased growth which correlated with decreased feed intake in both male and female Wistar rats fed with 540 mg/kg α-cypermethrin.

Hend and Butterworth (1976) had reported reduction in body weight gain in rats fed 1600 mg cypermethrin per kg for three months. The growth in rats was reduced at 750 mg of cypermethrin per kg for five weeks.

There was weight loss in beagle dogs fed α-cypermethrin at 200 mg per kg for seven days as reported by Green Vough and Goburdun, (1984).

5.2.1 Haematolgocial parameters

Haematological parameters were estimated using blood samples collected from all the animals on Day 7, 14, 21 and 28. The haematological parameters viz. Total erythrocyte count (TEC), Haemoglobin concentration (Hb), Haematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCV), Mean corpuscular haemoglobin concentration (MCHC), Total leucocyte count (TLC) and Differential leucocyte count (DLC) were determined following standard methods.

In the present study there was significant (P<0.05) decrease in TEC count (10⁶/mm³), haemoglobin concentration (g%) and haematocrit (%) values in Group V male rats and Group X female rats administered with 120 mg/kg body weight high dose cypermethrin and amitraz pesticide combination on Days 7, 14, 21 and 28 in male rats compared to their respective control group values.

The decrease in TEC, haemoglobin concentration and haematocrit values were in accordance with Rose (1982) who reported a decreased haemoglobin concentration, haematocrit value and RBC counts with anaemia when mice were fed with cypermethrin upto 1600 mg/kg for 101 weeks.

Hend and Butterworth, (1975) had reported that female rats receiving upto 1600 mg cypermethrin / kg feed for three months showed decreased haemoglobin concentration and RBC count.
Mansee (1998) reported a reduction in RBC count and PCV in rats when cypermethrin and permethrin were administered at a single dose of 10 mg/kg body weight of each pyrethroid.

Yousef et al. (1998) reported oral administration of cypermethrin at 6 or 12 mg/kg body weight decreased haemoglobin, RBC and PCV in barki sheep.

Shakoori et al. (1988) reported that at the end of the stipulated period haemoglobin content and white blood cell (WBC) count remained unaltered, while red blood cell (RBC) count and packed cell volume (PCV) decreased significantly in assessing the effects of six months feeding of cypermethrin mixed diet uninterruptedly to male albino on the blood and liver was studied by rats consumed cypermethrin at a dose of 420 mg active ingredient per kg body weight per day.

On the contrary, Clark (1982) found no effects on haematological and clinical parameters in rats fed α-cypermethrin upto 27 mg/kg body weight for 13 weeks. In another study there was no changes in haematological and clinical chemistry value change were reported in rats fed cypermethrin upto 1000 mg/kg diet for two years as reported by Mcausland et al. (1978).

5.2.2 Serum biochemical parameters

Serum obtained from blood samples collected on Days 7, 14, 21 and 28 of experiment period were used to estimate AST, ALT, ALP, Creatinine, BUN, Glu, TSP and Alb for both male and female rats separately.

In the present study there was significant (P<0.05) increase in AST, ALT, GLU, BUN, TSP and Alb values in Group IV and Group V male rats administered with 60 and 120 mg/kg body weight mid dose and high dose of cypermethrin and amitraz combination pesticide on Days 7, 14, 21 and 28 in male rats compared with their respective control group values.

In the present study there was significant (P<0.05) increase in AST, ALT, GLU, BUN, TSP and Alb values in Group IX and Group X female rats administered with 60 and 120 mg/kg body weight mid dose and high dose cypermethrin and amitraz pesticide combination on Days 7, 14, 21 and 28 in female rats compared to their respective control groups values.

In Group IX and Group X female rats administered with 60mg/kg body weight mid dose cypermethrin and amitraz pesticide combination, there was significant (P<0.05) increase in creatinine concentration on Day 21 and Day 28 compared to their respective control group values on Days 21 and 28.

The decrease in AST was in accordance with Shakoori et al. (1988) who had reported that at the end of the stipulated period there was decrease in Glutamate oxaloacetate transminase (GOT), creatine phosphokinase (CPK) and cholesterol content in a study conducted to assess effects of six months feeding of cypermethrin mixed diet fed uninterruptedly at a dose of 420 mg active ingredient per kg body weight per day.
The increase in serum BUN was in accordance with Filazi et al. (2003), who reported no effect on serum glucose, serum calcium and bilirubin concentrations but increased urea, phosphorus, aspartate transaminase and alanine amino transferase concentrations were observed in the group given 45 mg/kg and a decrease in creatinine and alkaline phosphatase concentration was observed in all groups in a study conducted to assess the effect of amitraz on the biochemical parameters in mice given 15 or 45 mg/kg body weight of amitraz orally, diluted in dimethylsulphoxide (DMSO). Hend and Butterworth, (1975) had reported that female rats receiving upto 1600 mg cypermethrin / kg feed for three months reported an increased plasma urea concentration and plasma alkaline phosphatase activity. Quetroz et al (2002) reported an increase in BUN value in horses given intravenous injection of amitraz. The mean value for urea was significantly more from the mean value obtained for the control group.

On contrary to the findings in the present study, Yousef et al. (1998) reported 6 or 12 mg/kg body weight oral administration of cypermethrin decreased total protein, albumin, globulin and increase total leucocyte count with decreased AST, ALT and ALP concentration in barki sheep.

The increase in glucose concentration was in accordance with Hsu and Schaffer (1988), who reported that amitraz induced hyperglycemia partly by inhibiting insulin release when 3.78 L (containing 2.1g) of amitraz (twice the recommended concentration) was applied to five dogs four hours before glucose (0.6 g/kg of body wt.) administration i.v. The plasma glucose concentration was increased, but the increase in plasma insulin concentration, which usually follows i.v. administered glucose, was suppressed. Hugnet et al. (1996) reported rapid hyperglycemia and no change in insulin concentration in dogs given 100 mg/kg amitraz. Administration of atipamezole at doses of 50, 100, 200 µg/kg b.wt. i.m enhanced insulin secretion, resulting in a decreased blood glucose concentration.

5.2.3 Organ weights and organ to body weight ratio

These were no statistically significant changes in organ weights and organ to body weight ratio.

On the contrary, Green Vough and Goburdhun (1984) reported that when α-cypermethrin fed in diet at the rate of 270 mg/kg for 13 weeks to beagle dogs did not show any pathological changes in organs and tissues.

Pathology

At autopsy none of the treated and control rats showed any gross pathological lesions. Histopathology of liver, kidney, spleen, heart, lungs and brain did not reveal any abnormal changes. The histopathology of the tissues showed normal appearance.
Satellite high dose group administered with 120 mg/kg body weight of cypermethrin and amitraz combination pesticide showed normal recovery in haematological and biochemical parameters, and histopathological studies did not show any significant changes compared to satellite control group values.

5.3 Dermal toxicity

A 21/28-day Dermal toxicity study will provide information on possible health hazards likely to arise from repeated dermal exposure to the test substance for a period of 28-days. This study provided useful information on the degree of percutaneous absorption, target organs, the possibilities of accumulation and can be of use in selecting dose levels for longer term studies. Repeated dose dermal study is used to evaluate the toxic effects associated with repeated doses of a chemical or test substance over a part of life span of the test animal.

Cypermethrin and amitraz combination pesticide containing amitraz (1%) and cypermethrin (1%) emulsified concentration in odourless kerosene was applied to the skin of five groups of male and female Wistar Albino rats separately at zero, vehicle, one, two and four ml/litre of water. The animals treated with cypermethrin and amitraz combination pesticide did not show any clinical signs of toxicity during the experimentation period.

5.3.1 Haematological parameters

In the present study, none of the animals in the treated groups of male and female rats showed any statistically significant alterations in the haematological parameters such as TEC, Hb, Hct, MCV, MCH, MCHC, TLC and DLC to cypermethrin and amitraz combination pesticide on dermal application indicating that the combination pesticide did not had any effect on haematological parameters estimated.

5.3.2 Serum biochemical parameters

Serum biochemical parameters viz., AST, ALT, ALP, BUN, creatinine, glucose, TSP and albumin did not show any statistically significant alterations in the Cypermethrin and amitraz combination pesticide applied on male and female rats indicating that the combination pesticide did not had any effect on serum biochemical parameters estimated.

5.3.3 Organ weights and organ to body weight ratio

There were no statistically significant changes in organ weights and organ to body weight ratios indicating that the combination pesticide did not had any effect on organ weights.

5.3.4 Pathology
At autopsy none of the treated and control rats showed any gross pathological lesions. Histopathology of liver, kidney, spleen, heart, lungs, brain and lymphnodes did not reveal any abnormal changes. The histopathology of the tissues showed normal appearance.

Histopathology of skin revealed parakarotosis at mid dose and high dose treated in male and female rats and other treated groups did not show any significant changes.

Satellite high dose group applied dermally with cypermethrin and amitraz combination pesticide 4 ml/litre of water did not show any significant change in haematological and biochemical parameters when compared to satellite control group. Histopathological studies of skin and other organs did not show any significant changes when compared to satellite control group.

In the present dermal toxicity study, there was no change in the haematological and biochemical parameters in treated and control groups. This is in accordance Al-Qarawi et al (1999) who reported that no effects were observed in three month old male Wistar rats sprayed with 250, 500, 1000 and 2000 ppm of amitraz in a study to evaluate the effects of amitraz given by different routes on rats.

On the contrary, Seawright (1982) reported that since the treatment with amitraz involves whole body application, the possibility of percutaneous absorption of amitraz may lead to intoxication in calves under hot humid conditions, the main effect being tranquillization which may lead to recumbency.

Luty et al. (1998) studied the immunotoxic effects of dermally applied alpha-cypermethrin in female wistar rats at 50 and 250 mg/kg applied to the fall skin four hours daily for 28 days. Dermal application resulted in slight histological changes in liver, kidney, lung and brain with ultrastructural pathological changes in heart and there was stimulated bactericidal and phagocytic activity of neutrophils after administration of 50 mg/kg alpha-cypermethrin.

Cypermethrin at 200 mg per kg when applied topically for six hours a day for 13 weeks reduced feed intake and body weight gain in New Zealand white rabbits (Henderson and Parkinson, 1978).

5.4 Immunotoxicity

Immunotoxicity study was conducted to assess the effect of cypermethrin and amitraz combination pesticide on immune response in five groups of male and five groups of female Wistar albino rats consisting of 10 animals each with saline (control), vehicle, 30, 60 and 120 mg/kg body weight administered orally for a period of 28 Days.

Immunological parameters
At mid dose and high dose groups of both male and female rats there were statistically significant decrease in body weight gain, TSP, albumin and HAT.

The decrease in proteins such as TSP, albumin and HAT values were in accordance with Khurana et al. (1998), who reported that day-old broiler chicks when fed 100 mg/kg cypermethrin in feed for 8 weeks showed reduction in humoral immune responses with a decrease in serum globulins, \( \gamma \)-globulin concentration and antibody titers indicating immunosuppressive effect of cypermethrin.

The decrease in TSP, albumin and HAT values indicated that the pesticide combination has adverse reaction of decreasing humoral immune response or humoral immunosuppression at mid and high dose groups of both male and female rats.

Luty et al. (2000) reported that \( \alpha \)-cypermethrin had a suppressive effect on IL-12p70 production in Swiss mice at 25 and 10 mg/kg body weight administered per o.s daily for a period of 29 days. Subacute poisoning of mice resulted in decreased bacterial activity of neutrophils in both dose groups with stronger stimulatory effect on phagocytic activity in low dose group. Significantly higher numbers of monocytes and lymphocytes were noticed in the blood of male mice poisoned with low dose. There was significant decrease in IL-12p70 serum secretion. Histopathologic and ultrastructural changes were observed in the liver and kidneys.

The delayed type of hypersensitivity reaction was depressed in cypermethrin fed birds by dinitrofluorobenzene (DNFB) skin sensitivity test as evidenced by reduction in skin thickness and mild inflammatory reaction (Khurana et al., 1999).

A suppression in total leucocyte count, absolute lymphocyte count, and a delayed type hypersensitivity reaction were noted in chicken fed 100 ppm a low dose of cypermethrin in feed (Khurana et al., 2000).

Cell mediated immune response and humoral immune response were suppressed in cross bred calves administered 60 mg/kg cypermethrin (Barathrin 25% EC) daily for 30 days (Patel et al., 1996).

On the contrary, Mikula et al. (1992) found reduction in the phagocytic index and reduction in the number of rosette forming lymphocytes in 7 month old sheep fed 300ppm cypermethrin for 4 weeks.

Tamang et al. (1988) reported that cypermethrin suppressed both cell mediated and humoral immune response in mice administered cypermethrin 50 mg/kg i/p for 26 days.

Oral administration of 30, 60 and 120 mg/kg permethrin per day for 14 days to mice did not alter the primary and secondary humoral immune response and protein concentrations. A dose of 120 mg/kg reduced the CMI response and reduced TLC and ALC (Shah and Gupta, 1998).

5.5 Acute eye irritation
In the assessment and evaluation of the toxic characteristics of a substance, the determination of irritant and corrosive effects on eyes of mammals is an important initial step. The information derived from acute eye irritation test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes and associated mucous membranes to the test substance.

Acute eye irritation study was conducted to assess the effects of cypermethrin and amitraz combination pesticide on mammalian eyes of New Zealand white rabbits.

Clinical examination of eyes of all rabbits in treated groups and control group did not reveal any corrosive changes or irritation in eyes. Lacrimation was noticed in 2 rabbits of Group III and 3 rabbits of Group IV applied with 2 ml/ ltr and 4ml/ltr cypermethrin and amitraz combination pesticide respectively at 1 hour and 24 hours after instillation.

The scoring done at 0, 1, 24, 48 and 72 hours did not show much changes except the scoring for lacrimation in Group III and Group IV.

Many works on different insecticides and pesticides were reported with respect to their effect on mammalian eyes, but there are no reports on the effects of either amitraz or cypermethrin on mammalian eyes.

From the present study, it is evident that the cypermethrin and amitraz combination pesticide did not produce irritant effects on rabbit eyes at the recommended concentration.

Acute eye irritation study in rabbits revealed that cypermethrin and amitraz combination pesticide at applied concentrations did not produce any corrosive changes or irritation in eyes.

5.6 Skin sensitization study

Determination of the potential to produce or elicit skin sensitization reactions is an important element in evaluating a chemicals toxicity. The information generated from skin sensitization test serves to identify the possible health hazards to a population exposed repeatedly to a test substance.

Buehler test was conducted in Guinea pigs to evaluate the effects of cypermethrin and amitraz combination pesticide on skin sensitization.

Approximately 24 and 48 hours after remaining the challenge patch in Group IV animals applied with cypermethrin and amitraz combination pesticide at 4 ml/ltr of water concentration, there was discrete and patchy erythema on skin surface. This was not observed in other treated group animals.

Grading of skin reaction after challenge period i.e. at 24 and 48 hours for all the Groups was nil where as 2 animals in Group IV were graded as 1.
Many works on different insecticides and pesticides were reported with respect to their effect on skin, but there are no known reports on the effects of either amitraz or cypermethrin on skin sensitization.

Skin sensitization study of cypermethrin and amitraz combination pesticide in Guinea pigs revealed that at the applied concentrations did not cause any adverse skin sensitization and systemic reaction.

5.7 *In vitro* pharmacological efficacy of cypermethrin and amitraz combination pesticide against different stages of *Rhipicephalus sanguineus* ticks

*Rhipicephalus sanguineus*, a brown-dog tick, parasite of dogs and a wide variety of mammals and birds, known for its vector potential to transmit infectious organisms like *Babesia canis*, *Ehrlichia canis*, *Coxiella burnetti*, *Rickettsia spp.*, *Anaplasma marginale*, *Borrelia spp.*, *Babesia bigemina*, *Theileria annulata*, etc. They are sturdy insects, a female laying around 4000 eggs, egg hatching 17 to 30 Days or longer, larvae engorging up to 6 Days and moult in 5 to 23 days nymphs, engorge up to 9 days and moulting in 11 to 73 days. The female engorges for 6 to 21 days, unfed larvae can survive up to 8 and a half months, unfed nymph can survive up to six months and unfed adults can survive up to 19 months (Soulsby, 1982). Finding an effective pesticide with a minimal environmental contamination to control the parasite on animal and its vicinity and to prevent the spread of the disease among animals was the objective of conducting the present study. The results obtained for the efficacy of cypermethrin and amitraz combination pesticide against various stages of the *Rhipicephalus sanguineus* is discussed.

The *in vitro* pharmacological efficacy of cypermethrin and amitraz combination pesticide against different stage of *Rhipicephalus sanguineus* ticks viz., eggs, larvae, adult male, unengorged female and engorged female ticks was determined. The cypermethrin and amitraz combination pesticide concentrations used in the present study were control (water) 0.25, 0.50, 1.0 and 2.0 ml/ ltr of water.

Of the several methods generally used for acaricidal sensitivity on ticks, the immersion test as described by Mira-Shah-Fisher and Ralph say (1981) was employed in the present study.

5.7.1 Immersion Test

The sensitivity of a tick species *Rhipicephalus sanguineus* was assessed to determine the optimum chemical concentration in an emulsion or suspension. The immersion test shows whether the liquid has normal
acaricidal activity by comparing with the results of immersion in known concentrations.

Cypermethrin and amitraz combination pesticide at 2.0 ml/ltr of water concentration was found to be highly effective in inhibiting the hatchability of tick eggs and causing 100 per cent mortality of ticks larvae, adult male, unengorged and engorged female ticks. 1.0 ml/ltr of water of cypermethrin and amitraz combination pesticide was also found to be effective against different stages of ticks.

In Immersion test, the cypermethrin and amitraz combination pesticide at 2.0 ml/ltr of water concentration was found to be highly effective in inhibiting the hatchability of tick eggs and causing 100 per cent mortality of tick larvae, adult male, unengorged and engorged female ticks. 1.0 ml/ltr of water of Cypermethrin and amitraz combination pesticide was also found to be effective against different stages of ticks.

Maske *et al* (1994) reported that *in vitro* studies on efficacy of amitraz against *B. microplus* ticks of cattle showed 100% mortality of adult males in 30 h after exposure to amitraz at dilution of 0.05% and 75% and 55% mortality at 0.03% and 0.01% concentrations respectively. Engorged females treated with 0.05%, 0.03% and 0.01% dilutions of amitraz were immobile at 36 h, 48h and 60 h respectively. Treated females did not lay eggs.

5.8 *In vivo* pharmacological efficacy of cypermethrin and amitraz combination pesticide against *Ixodid* ticks in different species of animals

The *in vivo* pharmacological efficacy of cypermethrin and amitraz combination pesticide was studied in naturally infested dogs, cattle and sheep against *Ixodid* ticks.

5.8.1 Acaricidal efficacy of cypermethrin and amitraz combination pesticide against *Rhipicephalus sanguineus* ticks of dogs

Amitraz acts by inhibiting monoamine oxidase enzyme. It has been considered as potent tickicide which is effective against all economically important species of ticks affecting cattle and sheep and has been widely used (Auer *et al*., 1984). In the present study it was also observed that some of the dogs which were regularly bathed with organophosphates, ticks and fleas were cleared in 24 hours after a single application of amitraz. This indicated that amitraz is effective in situations where ticks and fleas have developed resistance to organophastes. No side effects were observed either on the animals or on the bathing personnel involved.

The acaricidal efficacy of cypermethrin and amitraz combination pesticide in dogs was found to be in accordance with Estrade-Pena and Ascher (1999), who did comparison of an amitraz
impregnated collar with topical administration of fipronil for prevention of experimental and natural infestations by brown dog tick (*Rhipicephalus sanguineus*) in dogs. Fipronil and amiraz were acaricidal agents and inhibited attachment and feeding. Amitraz had a significantly greater effect than fipronil on number of live, feeding ticks, egg hatchability and larval viability, indicating partial ability to interrupt the tick life cycle. In field conditions amitraz remained effective over the entire observation period and had stronger and more sustained effects against tick infestation than fipronil.

Brown dog tick (*Rhipicephalus sanguineus*) and American dog tick (*Dermacentor variabilis*) populations were eliminated and repelled for up to four weeks post treatment with a topical formulation of amitraz applied at 250 ppm active drug as a single treatment to dogs (Folz *et al.*, 1986).

Many clinical studies conducted by different researchers had shown the efficacy against demodicosis. In a prospective study conducted by Estrada-Pena and Reme (2005) in 72 dogs fitted with test collars impregnated with amitraz (9%), amitraz (9%) and priproxyfen (PPF) or only excipients against experimental tick infestations by *Rhipicephalus sanguineus*, *Ixodes ricinus* and *Ixodes scapularis* revealed collars impregnated with amitraz were efficient in preventing tick infestations in dogs but did not inhibit ovi position in few surviving female ticks. Incorporation of PPF into amitraz impregnated collar resulted in impairment of reproductive ability of ticks.

Amitraz topically applied as 0.025 or 0.05 percent aqueous suspension once or twice weekly for up to 8 weeks cured three cases of generalized squamopapular to pastular demodecic mange and effectively controlled the condition in dogs (Farmer and Seawright 1980). At these doses the drug was non-toxic and non-irritant to skin and mucous membrane.

Folz *et al.* (1983) reported that multiple treatments with liquid concentration of 250 ppm amitraz (Mitaban; Upjohn) were highly efficacious and safe for the treatment of generalized demodicosis without any ocular dermatological or other clinical side effects. When 42 dogs (26 treated and 16 controls) were given three to six treatments topically at 14 day intervals. All (100%) the dogs responded clinically and the mean rate of improvement at four weeks of treatment was 99.1 per cent.

Davis (1985) reported that in 27 clinical cases of canine demodecosis, three of four applications of a wash containing 0.025 per cent amitraz together with antimicrobial and antipruritic therapy were necessary and sufficient to produce clinical care in 25 out of 26 cases which were mildly to severally affected. One case which was very severally affected, nine weekly applications together with microbial and antipruritic therapy effected clinical and parasitological care.

Folz *et al.* (1985) evaluated the bioactivity and safety of a liquid concentrate formulation of amitraz at a concentration of 250 ppm active drug with or without the addition of a nonionic detergent in 20 dogs naturally acquired generalized demodicosis at 14 days intervals. The liquid concentrate with or without detergent was equally effective and safe as a dermotherapy for demodicosis and the addition of nonionic detergent grossly improved the wetting characteristics of the treatment mixture however it did not alter the biological activity or the safety of the therapy.
Hugnet et al. (2001) conducted a clinical study to assess the efficacy of 1.25% amitraz solution applied weekly in the treatment of eight dogs with generalized demodicosis and five dogs with sarcoptic mange associated with antidote treatment atipamazole 0.1 mg kg\(^{-1}\) i.m. once and Yohimbine 0.1 mg kg\(^{-1}\) once daily for three days orally. Skin scrapings were used to determine the therapy and the median number of treatments for demodicosis was three (range 2-5) and for sarcoptic mange was two (range 1-3). No failure or relapses occurred at 6-36 months after treatment.

5.8.2 Acaricidal efficacy of cypermethrin and amitraz combination pesticide against Ixodid ticks of cattle

The application of cypermethrin and amitraz combination pesticide at 3 ml/litre of water in cattle against *Boophilus microplus* ticks was found to be highly effective in causing mortality and detachment of ticks on the body of cattle. The cypermethrin and amitraz combination pesticide treated cattle did not show any significant changes in haematological or serum biochemical parameters or any clinical signs of toxicity or adverse reactions indicating that the cypermethrin and amitraz combination pesticide at the applied concentrations on tick infested cattle was highly effective and safe.

The cypermethrin and amitraz combination pesticide efficacy in cattle was found to be in accordance with Mekonen (2001), who reported that rapid detachment of all ticks form animal treated with the application of an aqueous emulsion of amitraz (Bovitraz, Bayer AH) when applied on eight cross bred heifer calves aged between six and eight months and heavily infested with ticks. Amitraz was hand sprayed on animals and ticks were counted and identified in the sites. 100% tick control was achieved on Day 3 after acaricide application and this was maintained for a further period of 18 days and the residual effects protected the animals from re-infestation for 21 days.

Trials conducted by Curtis (1985) indicated the elimination of mixed infection of *chorioptes spp. Psoroptes spp. and sarcopter spp.*, in cattle using spray applications of amitraz where in some cases organochlorine, organophosphorus and organotin compounds had failed. In a pilot study a heavy infestation of chorioptic mange was controlled in a calf using a pour-on formulation of amitraz.

Rothwell et al. (1998) who reported that zeta-cypermethrin pour-on at 2.5 mg/kg have good control of buffalo fly (*Haematobia irritans exigua*) for four weeks and the spray at 62.5 ppm gave 14 days control in groups of 20 cattle each indicating good control of *H. irritans exigua* for four weeks with 2.5 mg/kg zeta-cypermethrin pour-on.

The same researchers in 1999 reported that zeta-cypermethrin pour- given at 2.5 mg/kg is an effective treatment for cattle lice control in field trials in South-Eastern Australia to groups of 10 cattle each.
5.8.3 Efficacy of cypermethrin and amitraz combination pesticide against Ixodid ticks of sheep

Cypermethrin and amitraz combination pesticide sprayed at 3 ml/litre of water was found to be highly efficacious against *Hyalomma anatolicum anatolicum* ticks and sheep. The *H. anatolicum anatolicum* ticks were detached from the ears and found dead on the ground. Tick infected sheep did not show any significant changes in haematological or serum biochemical parameters or clinical signs of toxicity or adverse reaction to the application of cypermethrin and amitraz combination pesticide indicating that the cypermethrin and amitraz combination pesticide at the applied concentration on tick infested sheep was safe and highly effective.

The literature on the effect of either cypermethrin or amitraz efficacy in sheep is scanty. From the present study, it was found that cypermethrin and amitraz combination pesticide at the applied concentration was highly effective and safe against ticks in sheep.

**Conclusion**

The following conclusions were drawn from the present study.

1. Cypermethrin and amitraz combination pesticide used in the acute oral toxicity results in LD$_{50}$ values of 224.13 mg/kg body weight in male rats and 218.73 mg/kg body weight in female rats indicating that the female rats were more susceptible to the toxicity than the male rats. According to toxicity rating, the pesticide combination was considered very toxic (Clarke and Clarke, 1975).

2. In Repeated dose 28-day oral toxicity study, the signs of toxicity observed were depression, salivation, tremors, hyperexcitability, convulsions, depression, anaemia, weakness and decreased body weight gain at mid and high dose groups of both male and female rats.

   i. The decrease in TEC, Hb and Hct values in both the sexes at high dose indicated that the pesticide combination had an adverse effect on the haemopoietic system.

   ii. The increase in AST, ALT and ALP at mid and high dose groups of both male and female rats may indicate mild leakage of enzymes due to alteration of membrane permeability indicating mild hepatotoxicity, which was not enough to show in the histopathology of liver.

   iii. The increase in glucose level at mid and high doses groups of both male and female rats may be due to increased metabolic activity during convulsions and also struggling while blood collection.

   iv. The increase in serum BUN and creatinine at mid and high dose groups of both male and female rats may indicate mild nephrotoxicity. The increase in serum BUN and creatinine
might be due to the malfunctioning of kidneys which was not enough to show changes in
the histopathology of kidney.

3. Repeated exposure of cypermethrin (1%) and amitraz (1%) combination pesticide at 1, 2 and 4
ml/litre concentration dermally in dermal toxicity study in rats did not cause any toxic effects
on skin or systemically. Haematological and biochemical parameters did not show any
significant alterations compared to control groups. Histopathology of skin did not reveal any
changes in pesticide treated groups of both male and female rats.

4. Immunotoxicity study of cypermethrin and amitraz combination pesticide in rats showed
significant decrease in TSP, albumin and HAT at mid dose and high dose groups of both male
and female rats indicating suppression of humoral immune response.

5. Acute eye irritation study of cypermethrin (1%) and amitraz (1%) combination pesticide in
rabbits did not show any irritation or corrosive effects in ocular system at 0, 1, 2 and 4 ml/litre
of water. Only lacrimation was observed at higher concentration which was transient and
disappeared within a short period of time. Ocular gradings showed that cypermethrin and
amitraz combination pesticide at applied concentrations showed adverse effects in the form of
lacrimation and congested conjunctiva on rabbit eyes.

6. Skin sensitization study of cypermethrin (1%) and amitraz (1%) combination pesticide in
guinea pigs did not produce any cutaneous or systemic reactions at 0, 1, 2 and 4 ml/litre of
water. At higher concentration, there was mild patchy erythema which was transient and
disappeared shortly after sometime indicating that cypermethrin and amitraz combination
pesticide at applied concentrations did not produce any adverse cutaneous or systemic
reactions.

7. Cypermethrin (1%) and amitraz (1%) combination pesticide concentration at 2.0 ml/litre at
water was found to high effect against different stages viz., eggs, larvae, adult male,
unengorged female and engorged female ticks of *Rhipicephalus sanguineus* Immersion test.

8. Cypermethrin (1%) and amitraz (1%) combination pesticide concentration of 3.0 ml/ltr of
water was highly effective against ticks and fleas in dogs. In cattle and sheep, cypermethrin
and amitraz combination pesticide concentration of 3.0 ml/litre of water was highly effective
against Ixodid ticks. There was no significant change in haematological and biochemical
parameters in dog, cattle and sheep and it is suggested that cypermethrin and amitraz
combination pesticide is safe and efficacious against ticks and fleas control and treatment in
the livestock and pets.