IN-VIVO DETERMINATION OF NO OBSERVED ADVERSE EFFECT LEVEL AND NO OBSERVED EFFECT LEVEL (NOAEL AND NOEL) OF FIPRONIL IN RATS USING HAEMATOLOGICAL INDICES AND LIVER MARKERS

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ABSTRACT

There is the need to determine the no observed adverse effect level and no observed effect level (NOAEL and NOEL) of fipronil using haematological indices and liver markers of albino rats as recent trend in development of resistance by pests may lead to increasing the quantity of fipronil required to control them. Acute toxicity of fipronil (Terminator®) was performed in rats using modified up and down method to determine the lethal dose fifty (LD_{50}). Sub-acute study of fipronil (Terminator®) was performed using twenty four rats assigned into four groups to determine the no observed adverse effect level and no observed effect level (NOAEL and NOEL) of fipronil. The rats were treated with 4.0, 1.0 and 0.25 mg/kg body weight, bw of fipronil (Terminator®) orally for groups A, B and C respectively. Rats in group D was treated with 10ml/kg water and serve as the control. Both the Terminator® and water were given daily for 28 days. Hematological indices (haemoglobin concentration, Hb; red blood cell counts, RBC; packed cell volume, PCV and total white blood cell counts, TWBC) and Liver markers (alkaline phosphotase, ALP; albumin, Albumin; Total protein, TP; total cholesterol, TCHOL) were assessed on days 0, 14 and 28. Results for acute toxicity tests revealed decreased feed consumption, decreased grooming, hind limb paralysis, prolonged recumbency, and death among rats. The LD_{50} was estimated at 62.98mg/kg. For the sub-acute study, results of PCV, Hb, TWBC and RBC of rats in group A showed a decrease ($P<0.05$) on day 14 and 28 compared to other groups. The NOAEL and NOEL of fipronil was estimated to be 4.0 and 1.0 mg/kg respectively.

INTRODUCTION

Fipronil is a broad spectrum insecticide belonging to the phenylpyrazole chemical family which is a pesticide family with only a few members. Fipronil was discovered and developed by a French scientist, Rhône-Poulenc, 1985 - 1987 and placed in the market in 1993. It is sold under a variety of brand names including Frontline®, Termidor®, Regent®, Combat®, Maxforce® and Terminator®. Fipronil is an extremely active molecule and is a potent disrupter of the insect central nervous system via interference with the passage of chloride ions through the gamma-aminobutyric acid (GABA) regulated chloride channel (Rhône-Poulenc, 1996), resulting to uncontrolled central nervous system activity and subsequent death of the insect. Fipronil is highly effective against a variety of arthropod pests, and so it is used widely in agriculture and pet care. Fipronil has been combined with other agents to produce even better and more effective formulations with long lasting effects and with a reduced tendency for insects to develop resistance. For instance, a study carried out on the comparative efficacy of a single topical treatment with Fipronil – Methoprene on flea infested dogs, confirms both the high efficacy and long duration of efficacy of this formulation with atleast six weeks of 100% efficacy (Beugnet et al., 2011). Although fipronil exhibits differential binding affinity for GABA receptor subunits with a lower binding affinity for mammalian receptors, it is considered moderately toxic by ingestion. Studies have proven that the past use of cyclodienes (e.g. lindane, chlordane, dieldrin) in the control of certain arthropod pests has given rise to present fipronil resistance by those pests (Schal et al., 2003). This resistance to fipronil is in reality cross-resistance to the cyclodienes because fipronil and the cyclodienes have a similar mode of action (Wafford et al., 1989; Cole et al., 1993) and because the GABA receptors of cyclodiene-resistant insects show decreased sensitivity to fipronil (Bloomquist, 1994). Moreover, in insect colonies where the phenomenon of secondary transfer of toxicity exists, translocated baits containing partially metabolized fipronil may serve as a powerful selective pressure for resistance

development. Furthermore, fipronil's popularity in the control of agricultural pests may contribute to the development of resistance in some ectoparasites of animals. This is because baits formulated for ants and termite control contains substantially less fipronil (as little as 0.001%) than formulations used against fleas and ticks. It is therefore likely that exposure to such sub lethal concentrations to fleas and ticks may result in increase rate of build-up of resistance in these ectoparasites (Georghiou and Taylor, 1986; Denholm and Rowland, 1992). This increase in the rate of buildup of resistance to fipronil by pests may necessitate the use of higher doses of fipronil to eradicate pests normally eliminated by lower dose. There is therefore the need to determine the NOAEL and NOEL using commercial preparation of fipronil (Terminator®) used in the eradication of pests.

MATERIALS AND METHODS

Experimental animals: Male albino rats weighing between 180 ± 20 grams were used for the study. The rats were fed with grower mash and portable water ad lib. The rats were housed in galvanized metal cages. Upon arrival, the rats were allowed for a period of two weeks to acclimatize before commencement of the experiment. Acute Toxicity Study of Fipronil in Rats: The modified up and down procedure (Botham, 2004; OECD, 2001) and log-probit graph of Miller and Tainter, 1944 were used for the determination of median lethal dose (LD_{50}). The rats were treated with 12.0, 39.0, 125.0 and 0.0 mg/kg body weight bw of fipronil for group A, B, C, and D respectively for the acute study. The sub-acute study: The rats were treated with 4.0, 1.0, 0.125 and 0.0 mg/kg body weight, bw of fipronil for group A, B, C, and D respectively for the acute study. Parameters were assessed on days 0, 14 and 28 (three observations). The parameters assessed were hematology (PCV, Hb, RBC and WBC) and liver markers (serum alanine aminotransferase (ALT), alkaline phosphotase (ALP), total protein (TP), albumin, ALB; total cholesterol (TCHOL). Packed cell volume (PCV), red blood cells counts (RBC), total white blood cell counts (TWBC), differential white blood cell counts (DWBC), and haemoglobin concentration (Hb) were determined according to the methods of Coles (1986); Schalm et al. (1975) and Kachmar (1970) respectively. Assay kits for the estimation of (ALT, ALP, T P, ALB and TCHOL) were products of Quimica Clinica Applicada, Spain

RESULTS AND DISCUSSION

The acute toxicity of Fipronil in rats revealed decreased feed consumption, decreased grooming, hind limb paralysis, prolonged recumbency and death among rats. The LD_{50} was estimated to be 62.98mg/kg. This classifies fipronil in toxicity class 11 [(50 – 500 mg/kg oral toxicity of substances (WHO, 2001)]. However, this LD_{50} obtained is lower than that reported by Tomlin (2006), probably because of the toxic nature of the stabilizing agent, toluene. The fipronil used in this research is 0.25% and the stabilizing agent, toluene could have reduced the LD_{50} by synergy and potentiation of the two agents. The results of PCV shown in figure 1 is similar to that of Hb, RBC and TWBC (not shown) and revealed no difference ($P>0.05$) on day 0 among the groups. However, on day 14 and 28 the mean PCV, Hb, RBC and WBC of rats in Group A was lower ($P<0.05$) than that of rats in group D. There was no difference ($P>0.05$) among the mean PCV, Hb, RBC and WBC of rats in Group B, C and D. Though the mean PCV, Hb, RBC and WBC of rats in Group D was high in absolute values than those of group B and C, it was not found to be significant ($P>0.05$). The results of the mean ALT, ALP, T P and ALB of rats revealed no difference ($P>0.05$) among the groups throughout the experimental period. The NOAEL and NOEL of fipronil was estimated to be 4.0 and 1.0 mg/kg respectively. The reduction in the mean PCV, Hb, RBC and WBC of rats in group A suggests that higher doses of fipronil could cause anaemia. However, the fact that the mean values obtained for rats in group A fall within the reference ranges of rats (Mitruka 1977) suggests that the reduction in haematological parameters is not of clinical significance. This lack of overt toxicity by the fipronil in the red blood cell, haemoglobin, albumin, alkaline phosphotase and total protein in rats among all the groups throughout the experimental period could be attributed to the fact that fipronil exhibit differential binding affinity to GABA receptor subunits with higher binding affinity for insect receptors than mammalian receptors, thus increasing the margin of safety for use in animals (Jackson et al. 2009). Thus, for toxicity to be induced, higher doses than that used in this experiment are required. The overall dose administered in this experiment for group A in each animal is higher than the LD_{50} but no death was recorded. It therefore suggests that gradual increase in the dose of fipronil in the wake of development of resistance to it by pests

could be seen as good treatment approach since no adverse effect will be observed. This lack of toxicity observed with gradual increase of sub-lethal doses of fipronil may have led to enzyme induction, a phenomenon that made the toxic dose of LD_{50} tolerated without observable toxic effect after repeated administration.

Conclusion

It could be inferred that the NOAEL and NOEL of fipronil is 4.0 and 1.0 mg/kg respectively in this research. Hence, in the wake of development of resistance by pests to fipronil, gradual increase in the dose of this drug to achieve acaricidal effect could be practiced provided the increase does not exceed that used in this research.

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Figure 1: The packed cell volume of rats administered sub-acute oral sub-lethal doses of fipronil