IMPACT OF REPEATED ORAL DOSE OF MONOCROTOPHOS ON BLOOD ACETYLCHOLINESTERASE ACTIVITY IN GOAT (*Capra hircus* Linn)

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ABSTRACT

Study on blood acetylcholine (AChE) inhibition due to monocrotophos poisoning on goats was carried out by repeated oral dose followed by blood collection. Twelve yearling local male goats were fed daily with 120 gram of commercial concentrate (17% CP), and Napier grass ad *libitum*. The animals were treated orally with 0.006 mg/kg body weight of monocrotophos for 36 days dosing period. Blood samples from each animal were collected before dose treatment and after every 3 days during the dosing period. It was observed that even with repeated daily oral dose of monocrotophos, there was no apparent adverse effect on the state of the animals' health as well as the occurrence of any toxic symptoms, although the blood AChE inhibition was noticed. Observation on blood acetylcholinesterase indicated that its activity was inhibited by 27.59 ± 4.16% (from $3.3 \times 10^{-6} \pm 1.05 \times 10^{-6}$ to $2.4 \times 10^{1-6} \pm 7.0 \times 10^{-7}$ mole substrate/ minute/liter blood). The blood acetylcholinesterase inhibition rate gradually increased up to day 9 of the dosing period followed by a relatively constant fluctuation.

Key words: Repeated oral dose, Monocrotophos, Blood acetylcholinesterase activity, Goat

INTRODUCTION

Organophosphate pesticides are widely used to control agricultural pests and disease vectors. These pesticides are preferred to organochlorine for field application because they are more effective, have relatively short half-lives and do not accumulate in the food web (Stickel, 1974). Even if organophosphate is considered as a non-accumulated pesticide in the food web, the study of Tejada (1993) indicated that in rice field application, monocrotophos (organophosphate pesticide) as high as 0.03 mg/kg was found in the rice straw, whereas FAO/WHO gave the maximum residue limit (MRL) for agricultural by-products to be as high as 0.05 mg/kg.

Most organophosphates, monocrotophos included, are potent neurotoxic agents that inhibit acetylcholinesterase activity, causing an accumulation of acetylcholine at the nerve synapse. This disturbance leads to the subsequent disruption of the neural transmission in both the central and peripheral nervous system. Based on this potency in inhibiting AChE activity, researchers are using the suppression of blood AChE activity as an indicator of contaminant stress and exposure. In USA, investigation is required when the blood AChE activity drops below 70% from the baseline, whereas WHO recommended the removal of an individual from the workspace if red blood cells (RBC) AChE activity falls below 70 or 75% from the baseline (Environmental Health Criteria 63, 1986).

This work emphasizes the influence of ingested monocrotophos on blood AChE activity in goat (*Capra hircus* Linn. 1738), since like other ruminants in Asian agriculture, this animal is raised exclusively by small-scale, marginal or landless producers, and those that are highly dependent on agricultural by-products as source of roughages (Sasaki, 1992). Therefore, this animal could be unintendedly exposed to the toxicant through the contaminated roughages, since pesticides are

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still considered a critical input in crop production.

MATERIALS AND METHODS

Locally yearling goats purchased from Tanauan public market in Batangas were used in this study which consisted of 12 male goats with body weights ranging from 10 to 11 kg. The animals were adjusted to the experimental ration for 20 days and dewormed before the start of the experiment using 1 ml/20 kg body weight of 11.25% albendazole suspension at the Institute of Animal Science, University of the Philippines Los Banos (UPLB).

The animals were fed with as much as 120 grams commercial concentrate (17% CP) and Napier grass given *ad libitum*. A repeated oral dose (through the concentrate) of monocrotophos was given to the experimental animals during the 35 days experimental period. A freshly prepared dose of monocrotophos of 0.006 mg/kg body weight was mixed with a small portion of concentrate and packed into a capsule size # 2. To ensure that the animals will consume all of the insecticide, the capsule was force-fed by manual insertion through the mouth directly into the esophagus. This was done every morning at 6 A.M. before the concentrate was offered.

Blood samples were taken from the jugular vein before treatment and after every 3 days during the dosing period. The samples were immediately brought to the National Crop Protection Center, UPLB for acetylcholinesterase activity analysis. The assay of acetylcholinesterase activity was carried out using rapid colorimetric technique (Ellman *et al.*, 1961).

The whole blood was suspended in phosphate buffer (PBS, pH 8.0, 0.1M) by adding 10 µl blood into 6 ml buffer. As much as 20 µl suspension was added into the cuvette filled with 3.0 ml of PBS, then 20 µl dinithiobisnitrobenzoic acid (DTNB) and 20 µl acetylthiocholine iodide (ACTI) were added one after another. The change in absorbance was measured using Secomam S-250 spectrophotometer equipped with S250 PC program and was read in 412 mµ wavelength for 1000 seconds.

The acetylcholinesterase activity expressed as the number of moles substrate or ACTI hydrolyzed/minute/liter blood was calculated using the formula : Moles substrate hydrolyzed per minute per liter blood = $(4.41)(10^{-6})(300.5)(\Delta A)$, where, $(4.41)(10^{-6})(300.5)$ is the factor for dilution and extinction coefficient, while the ΔA is the change in absorbance/ minute.

RESULTS AND DISCUSSION

Observations made during the treatment period indicated that a repeated dose of monocrotophos, as much as 0.006 mg/kg body weight for 36 days, produced no apparent adverse effects on the state of health of the animals. The absence of an apparent adverse effect on toxic symptoms might be due to the dose given (0.006 mg/kg body weight) which was too low to induce physical toxic symptoms because the oral lethal dose 50 (LD50) of monocrotophos for mammals is 18-21 mg/kg body weight (Kenanga and Morgan, 1978). This absence of adverse effect was also confirmed by the experiment of Qureshi *et al.* (1987) on lactating goats wherein they were not able to find any adverse effects on milk production, feed consumption, health, and behavioral responses when they applied a repeated oral dose of monocrotophos as high as 10 mg/17 kg or 5.9 mg/10 kg body weight for 4 days.

On the average, the blood acetylcholinesterase activity before dose application was significantly ($P<.01$) higher than after dose application. The acetylcholinesterase activity in the blood before dose application (mean ± standard deviation) was $3.3 \times 10^{-6} \pm 1.05 \times 10^{-6}$ mole substrate/minute/liter of blood which was reduced to $2.4 \times 10^{-6} \pm 7.0 \times 10^{-7}$ mole substrate/minute/liter blood after the animals received repeated daily oral doses of as much as 0.006 mg/kg body weight of monocrotophos. The reduction of acetylcholinesterase activity in the blood was $9.3 \times 10^{-7} \pm 3.97 \times 10^{-7}$ mole substrate/

minute/liter blood which is equivalent to 27.59 ± 4.16% (Figures 1 and 2).

Variations in blood AChE activity were noted before and after dose application. The values obtained were used as bases in detecting the individual variation in AChE activity among the experimental goats. The difference in AChE activity might be due to the genetic variation among these animals, since the animals used did not come from only one breeder but was purchased from different farmers. This result was in agreement with an experiment of Brock and Brock (1993) who reported that the inter-individual variation in human plasma AChE was related to ChE-1 phenotype U (genetic code for AChE biosynthesis), body weight, sex, and height.

Results of this experiment also agree with other studies (Lari *et al.*, 1994) on Japanese quail, (Elawar and Francis, 1988) chicken, (Johnson *et al.*, 1986) hen, (Anam and Maitra, 1995) Rosewinged parakeet, (Chamber and Chamber, 1989) rat, and (Rao *et al.*, 1994) humans. Lieske *et al.* (1984) and Johnson *et al.* (1986) reported that absorbed monocrotophos in the gastrointestinal tract is circulated in the bloodstream and interacts with AChE in the blood. These researchers proposed that the phosphorylation process inactivate the AChE exposed to an organophosphorus inhibitor. In this study, it was observed that 0.006 mg/kg body weight dose of monocrotophos also inhibited blood AChE activity in goats.

The average AChE inhibition, as high as 27.59% during the whole treatment period, did not manifest any observable symptom of toxicity in the animals as compared to the permissible maximum inhibition rate of blood AChE activity for humans which is 25 to 30% (Environmental Health Criteria 63, 1986). For further comparison, 50% inhibition of fish brain cholinesterase activity is generally used as the diagnostic threshold for acute avian poisoning (Ludke *et al.*, 1975) whereas, inhibition of enzyme as high as 40-80% or more was reported as the lethal poisoning for fish (Coppage *et al.*, 1975).

In this study, even though the toxic symptoms were not observed during the dosing period, this inhibition value (27.59%) was higher than the permissible percent inhibition for humans. Osman *et al.* (1987) also reported that the inhibition of erythrocyte AChE activity of as much as 60% on Egyptian lactating goat through dermal application of monocrotophos was not followed by an apparent adverse effect. Qureshi *et al.* (1987) likewise found that the remaining erythrocyte AChE activity, which was as low as 29% or 71% inhibition in lactating goat due to 0.59 mg/kg oral dose monocrotophos, was also not followed by clinical symptoms. Based on these observations, it could be inferred that the 27.59% inhibition of blood AChE activity caused by the oral dosing of monocrotophos was still tolerated by the goats, thus no symptoms were noted.

The increasing trend on the rate of inhibition of blood AChE was noted from day 3 up to day 12 of the dosing period. This trend was followed by fluctuations as reflected in Figure 2. The opposite observation was, however, noted by Chamber and Chamber (1989) when they applied a single dose of 2 mg of paraoxon/kg body weight into rats. They reported that the percentage of AChE inhibition in the cerebral cortex of rats decreased until the 4th day of observation; however, an opposite trend on the restoration of AChE activity was observed. Qureshi *et al.* (1987) on the other hand, observed that within 24 hours after a single dose of monocrotophos, about 50% of the pesticide was excreted in the urine whereas, the eliminated to the resistance developed by the body. The resistance mechanism may involve the saturation of the enzyme by an organophosphate inhibitor and the activation of some detoxifying enzymes. Resistance development of *Cocopsylla pyri* (an insect) to monocrotophos was found to be strongly related to the activity of AChE, mixed function oxidases, esterases, and glutathion S-transferase (Berrada *et al.*, 1994). They found that when AChE was saturated with monocrotophos, this enzyme was modified due to the rate constant for AChE phosphorylation that was increased by 70 fold. They also found that the activation of esterase, oxidase, and glutathion S-transferase were synergistically involved in detoxification. Yamano and Morita (1993)

reported that some organophosphate pesticides stimulate glutathion S-transferase and oxidase activity on the mitochondria and microsome of isolated rat hepatocytes. Furthermore, Moorhouse and Casida (1992) found that mouse liver microsomal glutathion S-transferase was activated by chloramil, EPTC sulfoxide, captan and acrolein.

CONCLUSION

Ingested monocrotophos through oral dose of as much as 0.006 mg/kg body weight in goats produced no apparent adverse effects on the state of health of the animals. Although there were no toxic symptoms exhibited, the total blood acetylcholinesterase activity was inhibited. This inhibition rate was gradually increased up to day 12 of the dosing period, followed by a constant fluctuation.

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