Diversity and Life Processes from Ocean and Land 105 - 113, 2007
Editors: P V Desai & R Roy

Changes in renal clearance under the influence of monocrotophos in mice

S N Desai and P V Desai

Keywords : *Renal , Monocrotophos, Phosphatases, Urea, Creatinine*

Monocrotophos [O, O - dimethy - O - (2 methyl carboxy – 1 methyl vinyl) phosphate], an organophosphate ester, is one of the organophosphate insecticides that has sufficient selective toxicity to insects and thus has been widely used. The mechanism of the toxicity of organophosphate compounds is mainly by blocking of acetylcholinesterase – an enzyme that decomposes acetylcholine [1,2].

Many investigators have reported organophosphate-induced nephrotoxicity[2-10]. Greater levels of organophosphates have been found to be localized in the kidney than in blood [11]. Kidneys, besides having important physiological functions also account for most important route of excretion of foreign compounds and their metabolites from the body. Renal excretion occurs by glomerular filtration, active tubular secretion and passive tubular reabsorption. The formation of urine begins at the glomerulus with the generation of plasma ultrafiltrate. The rate of formation of this ultrafiltrate (GFR) is reduced before the onset of symptoms of renal failure and is related to the severity of the structural abnormality in renal disease.

Guano *et al.*[12] reported decrease in glomerular filtration rate (GFR) of mongrel dogs on acute exposure to parathion. Webb [13] reported reduction in GFR along with glomerular and tubular defects in sheep on exposure to single acute doses of paraquat. However, despite the wide spread application of monocrotophos as pesticide, only limited studies appear to have been done to evaluate extensive responses of mammalian renal system to its toxicity in terms of renal clearance with respect to acid phosphatase, alkaline phosphatase, proteins and urea. Therefore, present study was undertaken to investigate the changes in renal clearance in mouse treated with monocrotophos.

Ten week old male swiss albino mice, *Mus musculus* weighing 22-25g (N=120) were procurred from the animal house facility of, Vaccine

Institute, Belgaum, India, and were housed in polypropylene cages (dimensions 290 x 220 x 140 mm) bedded with paddy husk and free access to commercial feed (Pranav Agro industries, Sangli, India) and water. They were allowed to acclimatize to laboratory conditions (temperature: 29-31°C; relative humidity: 66-69%; photoperiod; 14L: 10D) for a period of 10 days before use. The experiments were performed according to guidelines of Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA) of India and were approved by the Animal Ethics Committee of Goa University, India.

Suspension of Monocrotophos (Technical grade purity 90%; source; Ciba-Geigy, Goa) was used as pesticide toxicant. Suspension of pesticide was made in physiological saline (0.85%). LD_{50} for intraperitoneal route of administration of monocrotophos for different exposure periods was determined by using probit analysis[14]. Based on this LD_{50} data, 1ppm, 5ppm and 10ppm sub-lethal doses were selected for present work. Intraperitoneal injection of 1 ml volume of either 1ppm or 5 ppm or 10 ppm per animal served as a single acute dose. Five animals were used for each treatment as well as for a control set. Animals administered with identical dose volume of physiological saline served as control. Twenty-four hours prior to termination of exposure period, 0.5 ml of 0.05% inulin was administered to each mouse intravenously via tail vein [15] and the urine sample was collected for twenty-four hours before the termination of exposure period. At the end of exposure period animals were decapitated after collection of blood (plasma analysis) from hind paw and were then dissected to take out the kidneys for biochemical and histological analysis.

Plasma and urine, samples were diluted with respective pH buffers for estimation of acid phosphatase (ACP), alkaline phosphatase (ALP) and protein. For estimation of creatinine urea, and uric acid from plasma and urine, samples were diluted with buffered mammalian saline. For the measurement of ACP, the method of Linhart and Walter [16] was employed. Briefly, 1.0 ml of assay volume containing 0.9 ml of 1.0 M acetate buffer (pH 4.8), 0.05 ml of 0.0055M p-nitrophenyl phosphate (PNPP) and 0.05 ml of plasma/urine sample, was incubated for 30 minutes at 37°C. Reaction was terminated by addition of 0.1 N NaOH. Yellow colour developed was spectrophotometrically read at 405 nm.

One unit of enzyme was expressed as the amount of p-nitrophenol (mol) liberated in 30 minutes at 37°C. The reaction mixture of ALP assay contained 0.9 mL of 0.05 M glycine buffer (pH 10.2), 0.05 mL of 0.0055 M PNPP and 0.05 ml of urine or plasma sample, was incubated for 30 minutes at 37°C. Reaction was terminated by addition of 0.02 N NaOH. Optical density of yellow-coloured complex formed was spectrophotometrically read at 405 nm. One unit of enzyme was expressed as the amount of p-nitro phenol (mol) liberated in 30 minutes at 37°C.[17]. Protein concentration in plasma and urine samples was measured according to Lowry *et al.*[18] using bovine serum albumin as the standard. Creatinine was estimated by following alkaline picrate method of Bowers [19]. The reaction mixture for its assay contained 1.0 ml of creatinine reagent containing alkaline picric acid and 0.1 ml of plasma or urine sample. Mixture was then incubated at room temperature for 5 minutes and optical density of orange-coloured complex formed was read spectrophotometrically at 505 nm and was expressed in amount of creatinine in mg. For the measurement of urea in plasma and urine samples, the method of Wybenga *et al.*[20] was employed. Assay volume contained 1.5 ml of urea reagent with thiosemicarbazide, 0.015 ml diacetylmonoxime reagent and 0.1 ml of plasma/urine sample. The mixture was incubated for 10 minutes in boiling water bath. Rose-purple-colour developed was spectrophotometrically read at 525 nm and was expressed as the amount of urea in mg. Plasma and urine inulin clearances were spectrophotometrically determined by following the method of Roe *et al.* [21] for determination of glomerular filtration rate to evaluate renal tubular function. The rate of urine flow was measured on the basis of 24 h-urine-collection period. Renal clearance was determined by using following formula :

$$\text{Renal Clearence } (RC_x) = UFR \times U_x / P_x$$

where UFR is urine flow rate, U_x is concentration of x in urine and P_x is concentration of x in plasma.

The results represent as means ± standard deviation for the number of animals indicated. Significance of differences was determined by an unpaired students t-test. Differences were considered significant when $P < 0.05$.

Male swiss albino mice treated with single acute dose of monocrotophos exhibited many changes in the renal functions of mice. Renal clearance of ACP elevated after exposure to 1, 5 and 10 ppm doses of monocrotophos (Figure 1). The highest elevation was observed for 1ppm dose at the end of 24 hour exposure period (92.79 ± 0.056, $P < 0.001$).

ALP renal clearances also showed increments after exposure to monocrotophos doses Figure 2 The highest increment was observed after exposure to 1ppm dose at the end of 24 hours exposure period (0.55 ± 0.009, $P < 0.001$).

Renal clearance of proteins showed increase after exposure to 1, 5 and 10 ppm doses of monocrotophos (Figure 3). The highest renal clearance of protein was noted for 10/ppm dose at the end of 24 hours exposure period (0.049 ± 0.001, $P<0.001$). Alterations in renal clearance creatinine was also observed after exposure to monocrotophos doses (Figure 4).

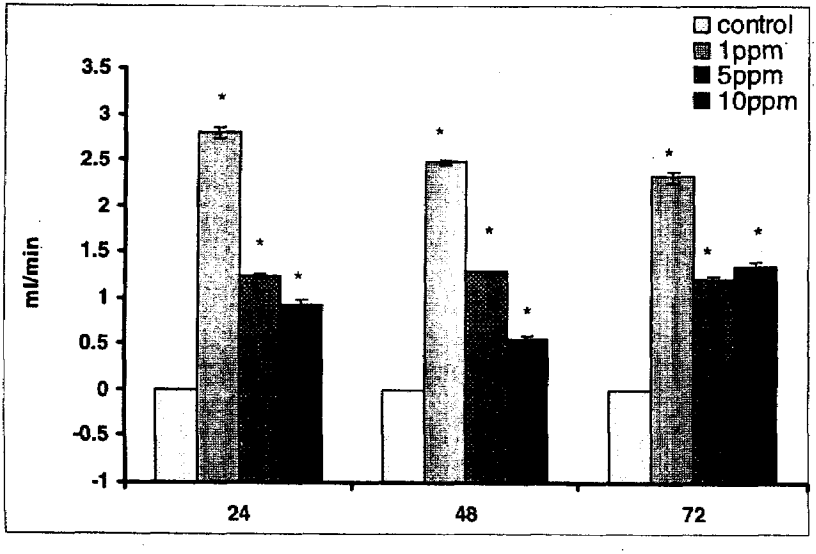

Figure 1 : Renal Clearance of acid phosphatase under the influence of monocrotophos. Data are mean ± SD for five independent experiments performed in pentuplate. Values are significantly different from the control set (* $P< 0.001$)

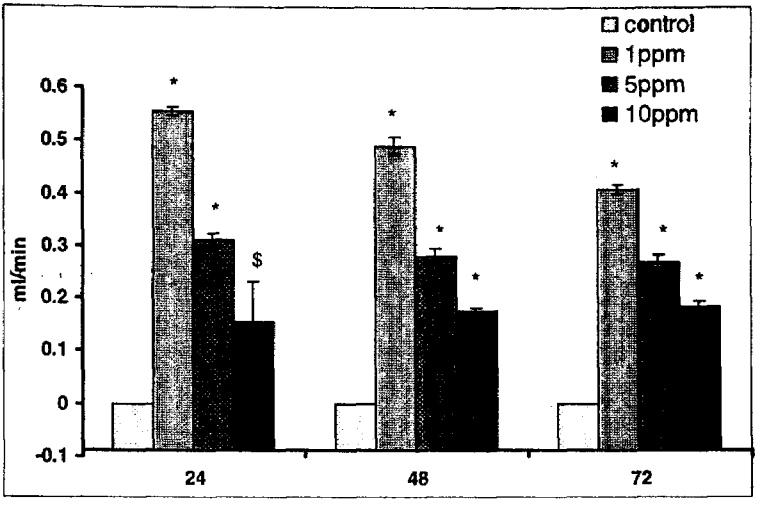

Figure 2 : Renal Clearance of alkaline phosphatase under the influence of monocrotophos. Data are mean ± SD for five independent experiments performed in pentuplate. Values are significantly different from the control set (* $P < 0.001$; \$, $P < 0.05$) .

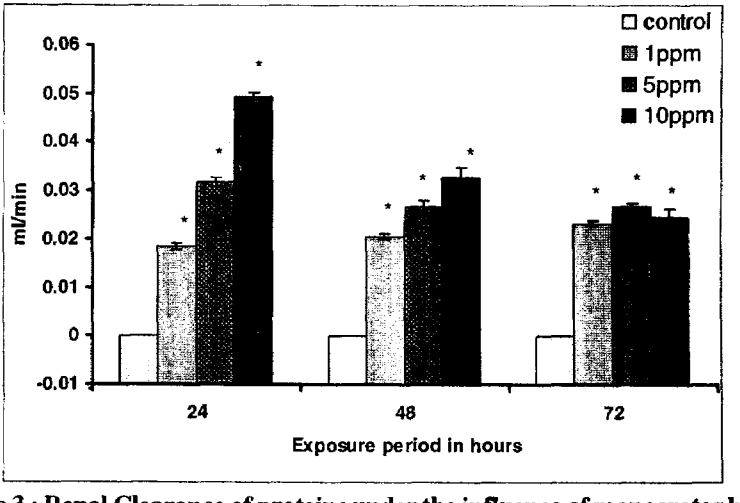

Figure 3 : Renal Clearance of proteins under the influence of monocrotophos. Data are mean ± SD for five independent experiments performed in pentuplate. Values are significantly different from the control set (* $P < 0.001$).

Renal clearance of urea showed reduction after exposure to monocrotophos doses (Figure 5). The highest reduction was observed after exposure to 1ppm dose at the end of 24 hours exposure period 90.074 ± 0.002, p<0.001).

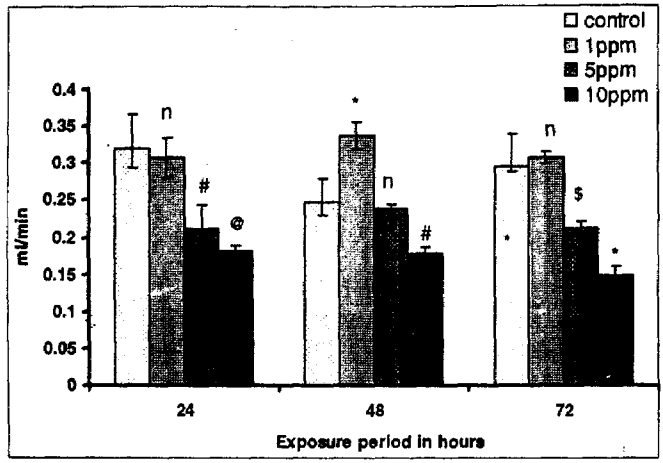

Figure 4 : Renal Clearance of creatinine under the influence of monocrotophos. Data are mean ± SD for five independent experiments performed in pentuplate. * Values are significantly different from the control set (*$P <$ 0.001; #, P <0.01; $, P <0.05 ; in, insignificant).

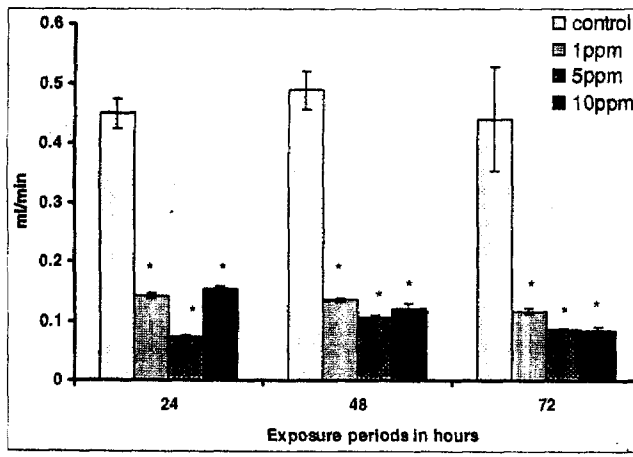

Figure 5 : Renal Clearance of urea under the influence of monocrotophos. Data are mean ± SD for five independent experiments performed in pentuplate. Values are significantly different from the control set (* P< 0.001).

Overall elevated renal clearances of enzymes suggests the tubular dysfunction. The kidney is sensitive to the action of toxicants because of its intensive metabolic activity and multiple functions namely those of excretion and pollutants concentration. Previous researchers attempted to show the effect of organophosphate-pesticides which induce tubular dysfunction or a tubulo-interstitial nephropathy which can be due to a renal deficiency [10]. These organophosphate pesticides are generally eliminated through urine [22-24]. However, during elimination process they can cause damage to glomeruli as observed in present study.

Elevation of renal clearances of ACP indicates activation of lysosomal system in renal tissue due to toxic effect of monocrotophos. Increased renal clearances of ALP suggested an altered membrane transport system of proximal convoluted tubule (PCT) cells especially towards the brush border which is the renal home-site of this enzyme. Increased renal clearances of total protein suggests impairment of the glomerulus (i.e. structural changes in filtering unit) which bring about clearance of more proteins. Necrosis of tubules of nephrons also account for total protein rise in filtrate which is proved by increased clearance of ACP and ALP, that confirms the tissue necrosis. Increased renal clearance of protein reflects on increased glomerular permeability. Lowered renal clearances of urea indicates reduction in protein metabolism. Thus, it appears that acute intraperitoneal administration of sub-lethal doses of monocrotophos altered renal clearances in mice. Reduction in creatinine clearance under the influence of 5 and 10 ppm monocrotophos indicate altered creatine metabolism.

Summary :

It is known that organophosphate pesticides are generally eliminated through urine and are supposed to cause damage to the nephrons. However, despite the wide spread application of Monocrotophos (an organophosphate ester) only limited studies appear to have been done on its toxicity to renal system. In the current series of experiments, total 120 number of male swiss albino mice were used to study effects of monocrotophos on kidney. Intraperitoneal injection of 1 ml volume of either 1ppm or 5 ppm or 10 ppm of monocrotophos per animal served as a single acute dose. The rate of urine flow was measured on the basis of 24 h-urine-collection period. Renal clearance was determined following the formula: $RC_x = UFR \times U_x / P_x$ Where UFR is urine flow rate, U_x is concentration of x in urine and P_x is concentration of x in plasma. Single acute dose of monocrotophos exhibited increase in renal clearance of enzymes such as acid phosphatase, alkaline phosphatase and nonspecific esterase. Total proteins, urea were also elevated whereas renal clearance of creatinine was lowered. Findings of the present study focus on the development of acute renal failure showing

abrupt and reversible changes induced in kidney by lower doses of monocrotophos at different exposure periods.

References :

1. Lewalter J and Korallus U : *Toxicol Lett*, 33, (1986) 153.

2. Harlin K S and Delllinger J A : *Vet Hum Toxicol*, 35 (1993) 201.

3. Prashad D N, Chamber, D and Beadle D J : *Gen Pharmacol*, 12 (1981) 291.

4. Webb D B : *J Toxicol Clin Toxicol*, 19 (1982) 911.

5. Swamy K V, Srinivas, T and Mohan P M : *Biochemistry International*, 24 (1991) 785.

6. Nagano N, Yogi M and Nishikori K : *Jpn J Pharmacol*, 59 (1992) 481.

7. Bosco C, Rodrigues R, Diaz S and Borax J : *Comp Biochem Physiol C : Pharmacol Toxicol Endocrinol*, 118 (1979) 247.

8. Mantle D, Sallem M A, Williams F M, Wilkins R M and Shakoori A R : *Clinica chimica Acta*, 262 (1997) 89.

9. Pal A K and Kushwah H S : *Indian Veterinary Medical Journal*, 21 (1997) 48.

10. Ben R A, Maillet M, Sescol L J and Guillam M C : *Arch Anat Cytol Pathol*, (1997) 45 : 199.

11. Poovala V S, Huang, H S, and Salahudeen A K : J *Am Soc Nephrol*, 10 (1999) 1746 .

12. Gauna H F, Domenechi C and Orias R : *Pest Biochem Physiol*, 18 (1982) 271.

13. Webb D B : *Toxicol Appl Pharmacol.*, 68 (1983) 282.

14. Finney D J : *Probit Analysis*, (1971). Cambridge University Press, Cambridge.

15. Ecker J L, Hook J B and Gibson J E : *Toxicol Appl Pharmacol*, 34 (1975) 178.

16. Linhart K and Walter K : In : *Methods of Enzymylogy,* (1965) pp 944. H V Bergmeryer (editor), Academic Press, New York.

17. King P J E and Armstrong A R : *Cand Med Assoc*, 31 (1934) 376.

18. Lowry O H, Rosebrough N J, Farr A Land Randall R J : *J Biol Chem,* 193 (1951) 265.

19. Bowers C D and Wong E I : *Clin Chem*, 26 (1980) 551.

20. Wybenga D R, Di Giorgio J and Pileggi V J : *Clin Chem*, 17 (1971) 891.

21. Roe J H, Epstein H and Goldstein N P : *J Biol Chem*, 178 (1949) 839.

22. Sharp C W, Ottolenghi A and Posner H S : *Toxicol Appl Pharmacol,* 22 (1971) 241.

23. Hughes R D, Milburn P and William R T : *Biochem J,* 136 (1973) 979.

24. Koertz J, Soffers A E M F, De-Kraker J W, Cnubben N H P and Reitzens J M C M : *Xenobiotica*, 27 (1997) 801.

Corresponding Address :
S N Desai
C/o, Dr. P V. Desai
Department of Zoology,
Goa University, Goa -403 206, India.