

SOME EFFECTS OF AMETRYNE (GESAPAX-500 FW)[®] ON SOME BLOOD PARAMETERS IN COMMON CARP (*CYPRIVS CARPIO*)

BY

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(Received:

SUMMARY

Ametryne is a commonly used synthetic herbicide. It is used for eradication of water hyacinth. The present study was carried out to investigate the toxic effects of ametryne on common carp, *Cyprinus carpio*. Subchronic toxicity study was carried out by inoculation of 1 and 2 ppm dose for 4 weeks. Haematological and biochemical parameters were investigated during exposure to different sublethal concentrations of ametryne under controlled conditions. The following observed changes were statistically significant: decrease in total erythrocyte count, packed cell volume, haemoglobin concentration, total leucocytic count and total protein, and increases in glucose, GOT and GPT.

INTRODUCTION

The contamination of the aquatic ecosystems with the pesticides caused toxicity to the aquatic organisms. So, with the increasing and widespread use of such herbicides, it is becoming more important to determine the side effects of these chemicals on the fish.

In recent years, many herbicides have been found that attack the Hill reaction. These include triazines e.g. atrazine, simazine and ametryne. It is thought that these compounds act by preventing electron transfer between photosystem I and II (Duffus, 1980).

In the Delta region, chemical control method was used for aquatic weeds (specially water hyacinth) along the River Nile and its branches. ametryne in the herbicide Gesapax-500 FW was used as a herbicide.

Fish are in direct contact with the environment and are susceptible to any changes that may occur. It is expected that such changes would be reflected in the physiology of the fish and particularly in the values of haematological parameters (Bloxhall, 1972). Thus it should be possible to monitor the change in the physical and chemical properties of the water (Mawdesley-Thomas, 1991).

In experimental study, Assem et al. (1992) observed that the haematological parameters of *Clarias gariepinus* were changed during exposure to sublethal concentration of atazine. There were decreases in total erythrocyte count and plasma protein concentration, and increase in haematocrite. The changes were dose-dependent.

In the recent years, several approaches have been used to monitor deleterious effects of pesticide poisoning in fishes. A survey of literature, however, reveals a serious lack of information on the adverse effects of ametryne on the aquatic biota. This study was, therefore, undertaken to identify changes in the haematological and biochemical profile of a freshwater fish, common carp, exposed subchronically to ametryne.

MATERIAL AND METHODS

Ninety common carp fish ranging weight from 150-200 g. each, collected from Barseek farm, Behera Governorate were used in the present work. All fish were acclimatized prior to testing for at least 2 weeks. Fish were fed ad libitum a commercial fish food every 48 hrs. Feeding was discontinued 24 hrs. prior to transferring into experimental aquaria to empty the gut. Fish were

placed in experimental aquaria and acclimatized for 24 hrs. prior to adding ametryne. The carp were divided into 3 groups, 30 fish each. The first group was treated daily with 1 ppm and the second group was treated with 2 ppm for 4 weeks. The third group served as control. Control carp were kept in aerated tap water in aquaria identical to those occupied experimental fish. The aquaria were all glass basin with 60 litre capacity each. Ten fish from each group were sacrificed without being anaesthetized, and the blood samples collected. Blood was collected into a heparinized sample vial. After centrifugation, plasma samples were taken and immediately frozen for storage (-20°C).

Routine haematological procedures were followed to estimate the packed cell volume (PCV), haemoglobin concentration (Hb), erythrocytic count (RBCs), leukocytic count (WBCs), according to Schalm (1975) and Wintrobe (1967).

Plasma glucose, total protein and transaminases (GOT & GPT) were detected colorimetrically

according to Siest et al. (1981), Reitman and Frankel (1957) respectively using the chemical kits of Biomerieux company (France) and the colorimeter model Carlizeiss.

Gesapax-500 FW was obtained from Ciba-Gigy. The scientific name is ametryne and the chemical name is : 2-ethylamino-4- isopropylamino-6-methylthio-5-triazine.

Data from control and experimental groups were statistically compared by Student's t-test.

RESULTS

The results of haematological and biochemical parameters were recorded in Table (1 and 2). RBCs count, WBCs count, haemoglobin and PCV were decreased significantly in fishes exposed to 1 or 2 ppm of ametryne (Table 1). A sharp decline in the total protein and increase in glucose level and transaminases was observed in both treated groups (Table 2).

Table (I) : Showing haematological changes of fishes due to Ametryne treatment.

	RBCs 10/mm	HB g/100 ml	PCV %	WBCs 10/mm
Control	1.464±0.199	7.56±0.27	27.4±1.673	3.676±0.11
1ppm 2 weeks	1.236 ±0.077*	6.5 ±0.158**	25.2 ±0.836*	3.16 ±0.151**
2ppm	1.18±0.0836*	6.22±0.084**	24.4±1.14*	3.04±0.055
1ppm 4 weeks	0.994 ±0.0089**	5.96 ±0.114**	22.6 ±0.548**	2.79 ±0.074**
2ppm	0.964 ±0.0054**	5.56 ±0.0895	20.4 ±0.548**	2.46 ±0.55
1ppm 8 weeks	0.87 ±0.0273**	4.66 ±0.055**	18.4 ±1.14**	2.34 ±0.055**
2ppm	0.78 ±0.273**	4.42 ±0.045**	16.4 ±0.5485**	2.24 ±0.055**

* Significant at P < 0.05

** Highly significant at P < 0.01

Table (2): Showing Biochemical changes of fishes due to Ametryne treatment.

	Glucose mg/dl	T. Protein g/dl	GOT I.U./L	GOT I.U./L
Control	54.8±2.178	3.608±0.25	24.88±0.455	7.1±0.141
1ppm 2 weeks	57.18 ±0.785	3.342 ±0.0828	27.46 ±0.336**	7.56 ±0.089**
2ppm	60.84±0.559**	3.188±0.016**	27.72±0.259**	7.84±0.055**
1ppm 4 weeks	61.8 ±0.071*	3.154 ±0.005**	30.5 ±0.187**	8.96±0.055**
2ppm	62.26 ±0.239**	3.136 ±0.005	31.26 ±0.055**	10.0 ±0.187**
1ppm 8 weeks	65.64 ±0.416**	2.998 ±0.071**	34.62 ±0.259**	12.5±0.071**
2ppm	68.1 ±0.447**	2.84 ±0.055**	36.64±0.0351*	15.44±0.89**

* Significant at P < 0.05

** Highly significant at P < 0.01

DISCUSSION

The common carp fish has been chosen among others as this species plays an important role in the research programme of fish culture in Egypt on an economic basis.

Monitoring of blood parameters, both cellular and noncellular, may have considerable diagnostic value in assessing early warning signs of pesticide poisoning.

Changes in haematological values occur in relation to physiological stress, disease, toxic environmental conditions and husbandary practice (Barnhart, 1969; Blaxhall, 1972; Wedemeyer and Yasutake, 1977 and Aldrin et al., 1979).

If toxicants raise to a level producing stress, this will be reflected in one or more of the haematological parameters. The temperature and photoperiod in the holding aquaria and tanks were constant throughout our experiments which eliminated the influence of temperature as demonstrated by various authors (Smit et al., 1981 and Munkittrick and Leatherland, 1983). Seasonal changes did occur in haematological parameters of fish even when the temperature remains constant (Van Vuren and Hattingh 1976). The

result obtained in the present study could not be attributed to seasonal changes since experiments were conducted during the same season. The water used for experiments was obtained from tap water. Any change in water quality was thus due to the addition of chemicals; thus, differences found in the values of haematological and biochemical parameters were caused by ametryne.

Haematological results revealed severe anaemia as remarked by decrease haemoglobin concentration, lowering of PCV and marked fall in erythrocytic count, Table (1). Our results are in agreement to those describing decrease haematocrit values of *Clarias lazera* during acute and chronic stress induced by nitrite (Hilmy et al., 1987).

Acute haemolytic crisis sometimes results in severe anemia in higher vertebrates, as well as some species of fish exposed to different environmental contaminations (Gromysz-Kalkowska et al., 1985; Van Vuren, 1986 and Hilmy et al., 1987).

Increase in blood glucose levels are also indicative of a stressed condition (Hattingh, 1976), Grant and Mehrle (1973) reported hyperglycemia and elevated glycogen reserves in

the rainbow trout fed endrin over and extended period. These biochemical effect of endrin toxicosis were ascribed to inhibition of glycogenolysis or increase in glycogenesis or gluconeogenesis. In the catfish, *Heteropneustes fossilis*, acute methyle parathion poisoning induced hyperglycemia followed by increase in hepatic glycogen content (Srivastava and Singh, 1980).

The effect of ametryne on plasma protein was pronounced. Gluth and Hanke (1983 and 1984) and Assem et al. (1992) reported similar changes in serum protein concentration in carp, *Cyprinus carpio*, exposed to phenol or atrazine. Neucomb (1974) also found a decrease in serum protein in juvenile steelhead trout (*Salmo gairdneri*) exposed to nitrogen supersaturation for 35 days, which he attributed to hypoxia. On the contrary, Scott and Rogers (1980) recorded no significant changes in plasma protein values during prolonged sublethal hypoxia of the channel catfish (*Ictalurus punctatus*).

The reduced plasma protein concentration recorded in the literature and by ourselves could be attributed to several pathological changes including in vivo plasma dissolution, renal damage and elimination in the urine (Pfeifer and Weber, 1979), alteration in hepatic blood flow (Gingerich et al., 1978) and/or haemorrhages into the peritoneal cavity and intestine.

A number of factors could be responsible for changes in enzymatic activity in plasma; changes in the enzymatic of synthesising organs, alterations in the rate of synthesis or changes in catalytic properties are all possible mechanisms (Bell, 1980 and Sauer and Haider, 1977). Changes in blood ammonia levels in fish exposed to hepatotoxicants (D'Appallonia and Anderson, 1980) may be responsible for observed increases in plasma transaminases activity.

Although the hepatic response is of prime importance in toxicological studies the possibility that damage to other tissues, such as the heart or kidney, contributed to increases in activity in the plasma cannot be entirely discounted.

Results are compatible with the degree of damage observed by El-Swak et al. (1992).

REFERENCES

- Aldrin, J.F.; Messenger, J.L. and Mevel, M. (1979): *Essai sur le stress de transport chez le saumon coho juvenile (Oncorhynchus kisutch)*. (A study of transport stress in juvenile coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 17: 279-289 (English abstract).
- Assem, H.; Abo-Hegab, S. and Belal, I. (1992): Comparison of Haematological effects of some toxicants on *Clarias gariepinus*. *J. Egypt Ger. Soc. Zool.* 9 (A): 33-50.
- Barnhart, R.A. (1969): Effects of certain variables on haematological characteristics of rainbow trout, *Salmo gairdneri* (Richardson). *Trans. Am. Fish. Soc.*, 98: 411-418.
- Blaxhall, P.C. (1972): The haematological assessment of the health of freshwater fish. *J. Fish Biol.*, 4:593-605.
- Bell, G.R. (1968): Distribution of transaminases, (aminotransferases) in the tissue of pacific salmon (*Oncorhynchus*, with the emphasis on the properties and diagnostic use of glutamic oxalacetic transaminase. I. *Fish. Res. Board Can.*, 25: 1247-1268.
- D'Appallonia, A. and Anderson, P.D., (1980): Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from rainbow trout, *Salmo gairdneri*. *Can. J. Fish. Aquat. Sci.* 37:153-169.
- Duffus, J.H. (1980): *Environmental toxicology, Resources and Environmental Sciences Series*. Edward Arnold, Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh.
- El-Wwak, A.; Hussein, Y. and Soliman, M. (1992): Histopathological changes in Carp (*Cyprinus Carpio L.*) Intoxicated with Ametryne (Gesapax-500). *Egypt. J. Pathol. Clin. Patho-5* (1): 151-161.
- Gingerich, W.H.; Weber, L.J. and Larson, R.E. (1978): The effect of carbon tetrachloride on hepatic accumulation, metabolism and biliary excretion of sulfobromophthalen in rainbow trout. *Toxicol. Appl. Pharmacol.* 43: 159-167.
- Gluth, G. and Hanke, W. (1983): The effect of temperatures on physiological changes in carp, *Cyprinus carpio* induced by phenol. *Ecotoxicol. Environm. Safety*, 7: 373-389.
- Gluth, G. and Hank, W. (1984): A comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotoxicol. Environ-Safety*, 9: 179-188.
- Grant, B.F. and Mehrle, P.M. (1973): Endrin toxicosis in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* 30 31-40.
- Gromysz-Kalkowska, K., Szubartowski, E., Sulikowska, J. and Trociewicz, K. (1985): Periodical blood changes of the Japanese quail *Coturnix coturnix japonica* following repeated small doses of trichloroform. *Bull. Environ. Contam. Toxic.*, 35: 757-766.

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- Hattingh, J., (1976): Blood sugar as an indicator of stress in the fresh water fish, *Labeo capensis* (Smith). *J. Fish Biol.*, 10: 191-195.
- Hilmy, A.M.; El-Domiati, N.A., and Wershana, K. (1987): Acute and chronic toxicity of nitrite to *Clarias lazera* comp. *Biochem. Physiolo.*, 86 C. 247-253.
- Mawdesly-Thomas, L.E. (1971): Toxic chemicals- the risk to fish. *New Sci.*, 49:74.
- Munkittrick, K.R. and Leatherland, J.F. (1983): Haematocrit values in feral goldfish, *Carrasino aevatus* L., as indicators of the health population. *J. Fish. Biol.*, 23: 153-161.
- Neucomb, T.W. (1974): Changes in the blood chemistry of Juvenile steelhead trout (*Salmo gairdneri*) following sublethal exposure to nitrogen supersaturation. *Res. Bd. Can.*, 31:1953-1957.
- Pfeifer, K.F. and Weber, L.J. (1979): The effect of carbon tetrachloride on the total plasma protein concentration of rainbow trout, *Salmo gairdneri*, *Comp. Biochem, physiolo.*, 64 c: 37-42.
- Peitman, S. and Frankel, S. (1957): A colorimetric method for determination of oxalacetic transaminase and serum glutamic pyruvic transaminase. *Am. J. Clin. Pathol.* 28:56.
- Sauer and Haider, (1977): Enzyme activities in the serum of rainbow trout, *Salmo gairdneri* Richardson; the effect of water temperature. *J. Fish Biol.*, 11: 605-612.
- Schalm, D. (1975): *Veterinary haematology*, 2nd Ed. Bailliere, Tindall and Cassel Ltd., London.
- Soott, A.L. and Rogers, W.A. (1980): Histological effects of prolonged sublethal hypoxia on channel cat fish, *Ictalurus punctatus*. *J. Fish. Dis.*, 3; 305-316.
- Siest, G.; Henny, J. and Schielf (1981): *Interpreation des examens de labororie* Karger ed. 206-223.
- Smit, G.L.; Hattingh, J. and Ferreira, J.T. (1981): The physiological respnses of blood ouing thermal edaptation in three fresh water fish species. *J. Fish Biol.* 19: 146-160.
- Srivastava, A.K. and Singh N.N. (1980): Effect of acute exposure to methyl parathion on carbohyarate metabolisms of Indian cat fish (*Hetero ustes fassilis*, *Acta phumacol toxical*, 4R: 26-31.
- Van Vuren, J.H. (1986): The effect of toxicants on the haematology of *labeo umbratus*. (teleostei cyprinidae)-comp. *Biochem. phusical.*, 83c: 155-159.
- Van Vuren, J.H.J. and Hatting, J. (1976): The seasonal haematology of the small mouth yellow fish (*Barous halubi*). *Zool. Afr.*, 11: 81-86.
- Wedemeyer, and Yasutake (1977): Clinical methods for the assessment of the effects of environmental stress on fish health. Technical Papers No. 89. U.S. Fish and Wildlife Service, Washington, DC, 19 pp.
- Wintrobe, M. (1967): *Clinical naematology*. 6th Ed. Lee, and Febiger Philadelphia.