Original Article
Basic Science

Effect of Pendimethalin Herbicide on Fish (*Tilapia nilotica*) Skeletal Muscles, Gills and its Influence on Human

Muawia Ibrahim ABD-ALGADIR [1,*], Omer Fadul IDRIS [2], Murwan Khalid Sabah ELKHIER [2]

ABSTRACT [ENGLISH/ANGLAIS]

This study was aimed at assess the contamination and effect of the herbicide (pendimethalin) on fish (Tilapia nilotica) in Blue Nile River and White Nile River , Recycle and Drainage water in Kenana area by using serum aspartate amino transferase (AST) as indicator for this study. It was carried out in December 2009. Water and fish samples were collected from four locations;: Blue Nile River (Singa area): White Nile River White : Recycled and Drainage water (Kenana area). Pendimethalin was extracted from both water and fish edible tissues. Compared to Blue Nile River the sudden increase in serum aspartate amino transferase was observed in location 2, 3 and 4. These findings are indicated that White Nile River, Recycle and Drainage water in Kenana are most likely more contaminated by pendimethalin due to heavy uses of this herbicide for control growth of the weed in White Nile State.

Keywords: Pendimethalin, pesticide, herbicide, skeletal muscle cells, carcinogen

RÉSUMÉ [FRANÇAIS/FRENCH]

Cette étude visait à évaluer la contamination et l'effet de l'herbicide (pendiméthaline) sur le poisson (Tilapia nilotica) dans le Nil bleu et blanc de la rivière du Nil, de recyclage et de l'eau de drainage dans la zone à l'aide de sérum Kenana aspartate amino transférase (AST) comme indicateur de cette étude. Elle a été réalisée en Décembre 2009. Des échantillons d'eau et de poissons ont été prélevés à quatre endroits;: Blue Nile River (Région de Singa): Blanc du Nil Blanc: recyclé et de l'eau de drainage (zone Kenana). Pendiméthaline a été extrait de l'eau et les tissus des poissons comestibles. Par rapport à Blue River Nile l'augmentation soudaine dans le sérum aspartate amino transférase a été observée dans l'emplacement 2, 3 et 4. Ces résultats sont indiqués que White Nile River, recycler et de l'eau de drainage dans Kenana sont probablement plus contaminés par la pendiméthaline en raison d'utilisations lourdes de cet herbicide pour contrôler la croissance des mauvaises herbes dans l'État du Nil blanc.

Mots-clés: Pendiméthaline, de pesticides, d'herbicides, les cellules du muscle squelettique, cancérigène

Affiliations:

[1] Faculty of Education, University of Bakht El – Ruda, Eld – Dueim, Sudan

[2] Department of Biochemistry and molecular biology, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan.

* Email Address for Correspondence/ Adresse de courriel pour la correspondance: emoawia@yahoo.com

Accepted/Accepté: March, 2011

Full Citation: ABD-ALGADIR Muawia Ibrahim, IDRIS Omer Fadul, ELKHIER Murwan Khalid Sabah. Effect of Pendimethalin Herbicide on Fish (Tilapia nilotica) Skeletal Muscles, Gills and its Influence on Human. World Journal of Life Sciences and Medical Research 2011;1:5-10

INTRODUCTION

The monitoring of the fish for chemical contamination in the Kenana area is a critical activity for protecting human health because this area is important for sport fishing and other recreational activities. The term pesticides refer to a broad class of crop-protection chemicals. Herbicides are the most widely used chemicals in agriculture [1]. Pesticides help or control hundreds of weed species, more than one million species of harmful insects and some 1,500 plant diseases [2] Pendimethalin is considered as moderately persistent herbicide that can give rise to long-lasting metabolites. It contains dinitroanilines, which reportedly it could result in the formation of carcinogenic nitrosamines [3]. Aspartate aminotransferase (AST) is an enzyme that found in high amounts in skeletal muscle cells [4]. Pendimethalin is highly toxic to fish and aquatic invertebrates [5]. The chemicals also have the ability to bioaccumulate and biomagnify, and can bioconcentrate upto 70,000 times their original concentrations [6]. Pendimethalin widely used herbicide, has been classified as a group C possible human carcinogen by [7]. The gill of the fish is the main organ for different functions, such as gas exchange, ion regulation and excretion of metabolic waste products [8]. Its complexity and constant contact with the external environment make the gill to be the first target for waterborne pollutants [9]. The pollutants are not only entering the organism through the gills, but also exert

their primary toxic effects on the branchial epithelium [10] which in turn, may influence the general gill functions [11]. Pesticides may continue to poison non-target organisms in the environment and increase risk to humans[12]. By disruption its poison on the endocrine, reproductive, and immune systems and causes cancer; neurobehavioral disorders [6]. The objectives of this study are assessed the pollution of water and fish with herbicide (Pendimethalin) by using serum aspartate amino transferase (AST) as indicator in White Nile State (Kenana area, include recycle and drainage water) and Blue Nile State (Singa area).

MATERIALS AND METHODS

Biological Experiment

Water and fish of different sex, age (21 – 30 days) and weight (150 gm – 1.2 kg) was collected from four locations: White Nile River, Recycled and Drainage water and Blue Nile River.. Blood sample were collected from fish heart and stored at 5 oC until analysis, blood was centrifuged at 30000 rpm for serum separation. Serum AST concentrations were measured, then pendimethalin was extracted from both water and fish samples separately according to following method

Extraction of pendimethalin from water samples

250 ml of representative sample was partitioned with 50, 50, 25 ml mixture of n-hexane: diethylether (9:1). The combined extracts were dried through anhydrous sodium sulphate and concentrated, then taken in 2 ml of n-hexane [13].

Extraction of pendimethalin from fish tissues

The 50 gm of the edible part of fish from each sample was homogenized. Then extraction of pendimethalin was performed by using 150 ml and 100 ml of acetonitrile. Samples were filtered and rinsed twice with 25 ml of the solvent. The combined extract was concentrated by using a rotary vacuum and evaporated over a hot water bath (less than 50oC) to 50 ml. The liquid – liquid partitioning was taken as follows: the concentrated extract was taken in a 500 ml separator funnel, then diluted with 250 ml of 5% aqueous sodium chloride and partitioned into 150, 150 and 100 ml of n-hexane. The combined n-hexane layer was passed through anhydrous sodium sulphate and concentrated to near dryness and take in about 10 ml n-hexane [13].

Measurement of pendimethalin concentration by HPLC

A calibrated HPLC device was set for measurement of pendimethalin concentration as follows: Column: ODS (18), Flow rate: 1 ml / minute, Injection volume: 10 µL, Oven temp: 30 ^{0}C. Mobile phase: acetonitrile: water (80: 20) [7, 14].

Measurement of AST concentration

It was determined according to method described by [15]. Working solution was prepared by adding 2 ml from reagent 1 (buffer, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), L - aspartate, pH 7.8) and 500 µl from reagent 2 (substrate α-ketoglutarate). It was mixed and kept in 37^{0}C, 1 ml was taken from working solution, then 100 µl from serum was added, and then was mixed and incubated at 37oC for 1 minute. Initial absorbance was read at 1 minute intervals, the difference between absorbance were calculated.

The average absorbance difference per minute: Δ A / minute × 1750 (factor) = U/ L.

A calibrated spectrophotometer (Awareness Technology, model No. 1904 plus, serial No. 1904-5252) was set for measurement of AST concentrations.

Histopathologcial parameters

Skeletal muscles and gills were collected in clean, sterilized urine containers (from the autopsied fish), labeled and cleaned with distilled water, preserved in 10% formalsaline.

Sequence of steps which were carried according to method described by [16] for preparation the slides: As follows (1) Suspected skeletal muscles of were cut into small pieces and the gills was also cut into 4 lobes, the cut organs were dehydrated in solutions of 30% alcohol for two hours, then solutions of 50% alcohol for two hours, finally 70% alcohol for two hours to attain the preservation level. (2) Continuation of dehydration: 70% alcohol (twice 1/4 an hour, 1/4 an hour), 90% alcohol/2 hours, 95% alcohol/2 hours, 100% alcohol/1 hour, 100% alcohol/1 hour. (3) Clearing: Xylene 1/3/4 an hour, Xylene 2/1/2 an hour or Chloroform overnight. (4) Impregnation: Wax 1/1 hour, Wax 2/1 hour. (5) Embedding: Tissue is embedded in cassette. (6) Section: Microtome was used. (7) Mounting on slides: Formaldehyde and gelatin were used. (8) Wax fixation and tissue elongation: Slides were put on oven at temperature < 45º C. (9)Wax removal: Xylene 1/2 minutes, Xylene 2/2 minutes, Absolute alcohol 1/2 minutes, Absolute alcohol 2/2 minutes, 90 % Alcohol/2 minute, 70 % Alcohol/2 minutes, Distilled water/2 minutes. (10) Staining: Stain with iodine and

haematoxillin for 10 minutes (11) Blueing: Wash under running tab water if overstrained dip quickly in acid alcohol (3 drops of HCl in 70% alcohol), then distilled water/1/2 minute, Iodine/1/2 minute, Distilled water/1/2 minute, 70% alcohol/1/2 minute, 90% alcohol/1/2 minute, Absolute alcohol 1/1/2 minute, Absolute alcohol 2/1/2 minute, Xylene 1/1/2 minute and Xylene 2/1/2 minute. (12) Covering with Canada balsam/

Statistical Analysis

Three samples were taken, analyzed and averaged. Mean is average of thirty replicates. Data were assessed using Analysis of Variance (ANOVA) as described by Gomez and Gomez [17].

RESULTS

Contamination of Fish and Water by Herbicide

Results (Table 1) showed that the concentration of pendimethalin in water of Blue, White Nile River, recycle and drainage water was 0, 25.5, 42.8 and 68.6 ppm, respectively, but concentration of pendimethalin in fish of Blue , White Nile River, recycle and drainage water was 0, 269.6, 451.1, 591.9 ppm, respectively. The serum of aspartate amino transferase in Blue ,White Nile River, recycle and drainage water was 21.3, 118.5, 280, 471.1 ppm, respectively. Plates A, B, C and D indicated sections of skeletal muscle of fish in Blue Nile River, recycle water, White Nile River and drainage water, respectively. Plates A-1, B-1, C-1 D-1 showed sections of gills of fish in Blue Nile River, recycle water, White Nile River and drainage water, respectively.

DISCUSSION

In Blue Nile River, the pendimethalin in tissues of fish and water is not found, but enzyme of AST is found in serum of the fish, but in White Nile River, the pendimethalin in fish is higher than in tissues of fish, but the concentration of serum aspartate aminotransferase is high compared with those values in Blue River. These results confirmed that White Nile River is more polluted by pendimethalin than Blue Nile River. In Kenana area, the pendimethalin in tissues of fish and water for the Recycle water became higher compared with White Nile River, but low compared with Drainage water. In addition concentration of serum aspartate aminotransferase is high in Drainage water compared with those values in Recycle water (Kenana area). These findings are indicated that drainage and recycle water in Kenana area are more contaminated with pendimethalin. Increase in aspartate aminotransferase associated with significantly increase of pendimethalin concentration in recycle and drainage water in Kenana area and consequently an increase fish tissues due to bioaccumulation of pendimethalin.

Plate A (a) indicated the skeletal muscle of fish in Blue Nile River is normal. Plate B (b) clearly observed that there is (n) muscle necrosis, with fragmentation of sarcoplasm in the right half compared with muscle on the left half. Plate (c) clearly noted that there is (n) muscle necrosis, with calcified muscles (ca) in White Nile River.

Plate D (d) noted that there is (n) muscle necrosis, with calcified muscles (ca), and mononuclear cell infiltration (m) in drainage water.. The finding is explained that there is gradual changes in skeletal muscle of fish in plates b, c and d compared plate a. Any diseases that affect on liver cells leads to increase AST levels and cause primary muscle changes [4, 18] reported that skeletal muscle is known to contain an isozyme of aspartate amino transferase that may be released into the blood stream following muscle necrosis. Whereas, Plate (A-1); indicated the gills of fish is shown nearly normal regular pattern of secondary filaments in Blue Nile River Plate (B-1): showed that there are secondary lamellae which indicating the hypertrophy (h) in recycle water. Plate (c-1): observed that there is swelling (s), curling (cu) and necrosis (n) of secondary lamellae in White Nile River. Plate (d-1) noted that there is thickening (t), clumping (cl), and necrosis (n) of secondary lamellae in drainage water.. The findings are supported the results that obtained by Playle et al. [10] and Monteiro et al. [11] whose reported that pendimethalin not only enter the organism through the gills, but also exert its primary toxic effects on the branchial epithelium and may influence the general gill functions.

CONCLUSION

The herbicide pendimethalin, is water pollutant and causes toxicity to fish and other aquatic invertebrates. Toxicity can end up in humans through the food chain. We recommend that water used in agriculture and industry should be completely recycled before reaching rivers and other sources of human drinking water or fishery activities.

Table 1

Table 1 shows concentration (ppm) of pendimethalin in fish, water and serum AST

Sample	Blue Nile stream	White Nile stream	Recycle - water in Kenana	Drainage - water in Kenana
Water	0	25.5 (± 4.03)	42.8 (± 5.1)	68.6 (± 6.6)
Fish	0	269.6 (± 43.6)	451.1 (± 43.6)	591.9 (± 46.5)
Serum AST	21.3 (± 10.5)	118.5 (± 41.5)	280 (± 43.8)	471.1 (± 42.9)

Value in parentheses is the standard error of the respective mean

PLATES

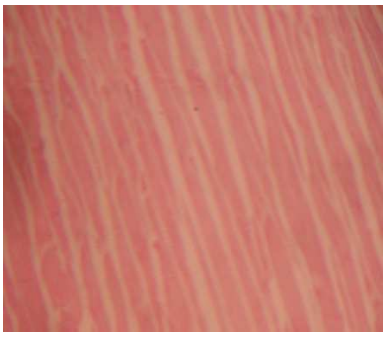

Plate A: Skeletal muscle section of fish collected from Station 1: as control, taken from the River Blue Nile Singa site , Normal fish skeletal muscle, (H&E × 250).

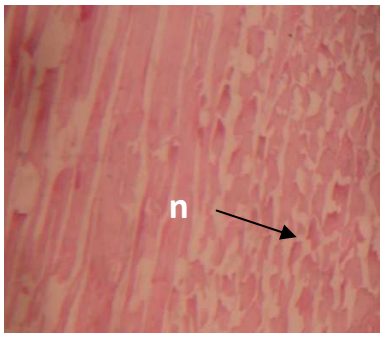

Plate B: Skeletal muscle section of fish collected from Station 2: recycled – water: (**n**) muscle necrosis, with fragmentation of sarcoplasm in the right half compared with muscle on the left half. (H&E × 100).

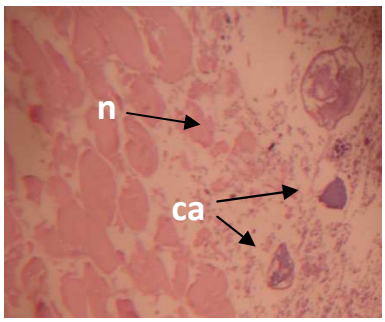

Plate C: Skeletal muscle section of fish collected from Station 3: the joining of drainage – water with the river White Nile: (**n**) muscle necrosis, with calcified muscles (**ca**). (H&E × 250).

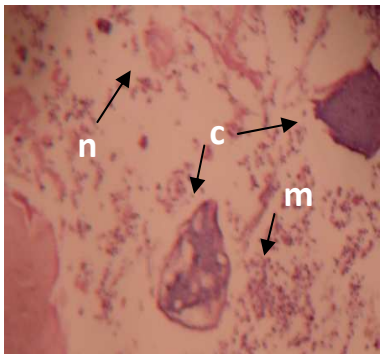

Plate D: Skeletal muscle section of fish collected from Station 4: drainage – water: (**n**) muscle necrosis, with calcified muscles (**ca**), and mononuclear cell infiltration (**m**). (H&E × 400).

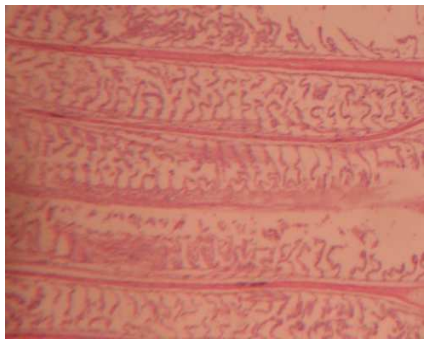

Plate A-1: Gills section of fish collected from Station 1: as control, taken from the River Blue Nile Singa site , nearly normal regular pattern of secondary filaments, (H&E × 250).

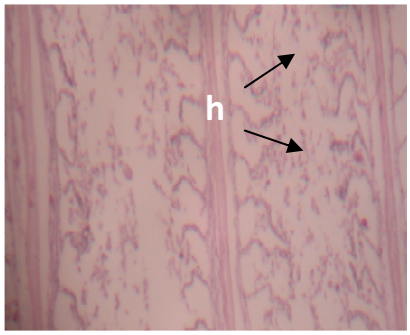

Plate B-1: Gills section of fish collected from Station 2: recycled – water: secondary lamellae showing hypertrophy (**h**) at places,(H&E × 100).

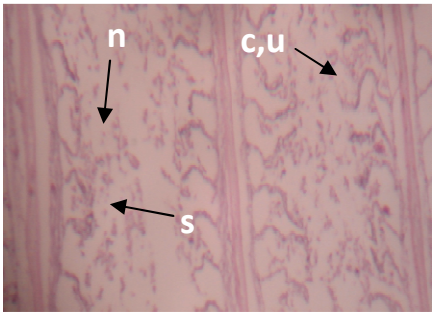

Plate C-1: Gills section of fish collected from Station 3: the joining of drainage – water with the River White Nile: Swelling (**s**), curling (**cu**) and necrosis (**n**) of secondary lamellae,(H&E × 250).

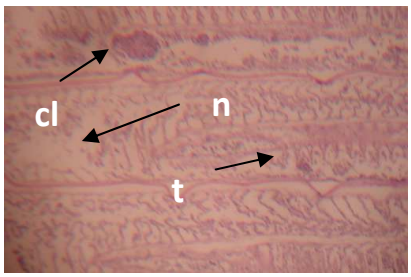

Plate D-1: Gills section of fish collected from Station 4: drainage – water: Thickening (**t**), clumping (**cl**), and necrosis (**n**) of secondary lamellae,(H&E × 250).

REFERENCES

[1] National Academy of Sciences Pesticides in the Diets of Infants and Children. National Academy Press, Washington DC 1993.

[2] National Agricultural Chemicals Association.. From Lab to Label. The Research, Testing, and Registration of Agricultural Chemicals. Washington DC 1993.

[3] Environmental Protection Agency Guidance for the Reregistration of pesticide products containing pendimethalin. OPTS, Washington DC 1985.

[4] Berk PD, Korenblat KM. Approach to the patient with jaundice or abnormal liver test results. In: Goldman L, Ausiello D, eds. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier 2007 chap 150.

[5] Meister RT. Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH 1992.

[6] Ritter L, Solomon KR, Forget J, Stemeroff M, O'Leary C. Persistent organic pollutants: An Assessment Report on: DDT, Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor, Hexachlorobenzene, Mirex, Toxaphene, Polychlorinated Biphenyls, Dioxins and Furans. Prepared for The International Programme on Chemical Safety (IPCS), 2007.

[7] U.S. Environmental Protection Agency; National study of chemical residues in fish: U.S. Environmental Protection Agency, Office of Science and Technology, EPA- 823-R-92-008b, v. 2, variously paged. Appendix 115, 1992.

[8] Wood C. M; Branchial ion and acid-base transfer in freshwater teleost fish: environmental hyperoxia as a probe. Physiol. Zool., 1991;64:68-102.

[9] Mallat J. Fish gill structural changes induced by toxicants and other irritants : A statistical review Con. J. Aguat. Sci., 1985; 42:630-48.

[10] Playle RC, Gensemer RW and Dixon D. Copper accumulation on gills of fathead minnows influence of water hardness : Complication and pH of the gill, 1992.

[11] Monteiro SM, Mancera JM, Fontaínhas-Fernandes A, Sousa M. Copper induced alterations of biochemical parameters in the gill and plasma of Oreochromis niloticus. Comp. Biochem. Physiol. 2005;141:375-83.

[12] Centers for Disease Control and Prevention. Pesticides. Accessed from: http://cdc.gov/ . Last Accessed: December 2007.

[13] Paula P, Michelangelo A, Dorothea M, Irina S, Bünyamin T, José O, Alberto B. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe, 2007

[14] Oblinger C, Foreman CJ, Conner WT, BF, and Maloney T.J. ; New reporting procedures based on long-term method detection levels and some considerations of interpretations of water-quality data provided by the U.S. Geological Survey National Water Quality Laboratory: U.S. Geological Survey Open-File Report 99-193, 1999.

[15] Murray R.. Kaplan MM., Gandolfo JV, Quaroni EG. Aspartate amino transferase. Clin. Chem. The C.V. Mosby Co. St Louis. Toronto. Princeton, 1112-116, 1984.

[16] Bancroft JD, Gamble C. Theory and Practice of Histological Techniques. 5th edition. Churchill Livingstone, London, Edinburgh, New York, Philadelphia, St Louis Sydney, Toronto 2002

[17] Gomez TP and Gomez AA. Statistical Procedure for Agriculture Research .John Wiley and Sons Inc. New York, USA, 1984.

[18] Janssen G.M.E., Kuipers H., Willems G.M., Does R.J.M.M., Janssen M.P.E.and Geurten P; Plasma activity of muscle enzymes. Quantification of skeletal muscle damage and relationship with metabolic variables. Int J Sports Med 1989;10:S123-S12.

ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Authors express their sincere gratitude to the administrative and the technical staff of Renal Dialysis Center, Ed-Dweim, Sudan and Central laboratory unit, Faculty of Science, University of Khartoum, Sudan and Agricultural Research Corporation, Wad Medani, Sudan, for their useful co-operation and guidance during the laboratory work. Lots of thanks for Prof. A. A. Jameel (of Faculty of Veterinary, University of Khartoum, Sudan) for his meaningful histopathologcial comments.

CONFLICT OF INTEREST

No conflict of interest was decleared by authors.