



Changes of fish liver (*Tilapia nilotica*) made by herbicide (Pendimethalin)

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ABSTRACT:

Objectives: This study was carried out in August 2010 and it was aimed to investigate the effect of the herbicide pendimethalin (used as a herbicide in Kenana Sugar Company, KSC) on fish (*Tilapia nilotica*) serum alanine amino transferase (ALT) and its influence on the food chain at Kenana site.

Methodology and results: Fish samples were collected from four locations. Location 1: Blue Nile Stream (Singa area free from pendimethalin), location 2: Recycled – water (Kenana area - contaminated with pendimethalin), location 3: White Nile Stream (Kenana area - contaminated with Pendimethalin and location 4: Drainage – water (Kenana area - contaminated with pendimethalin). Pendimethalin was extracted from fish fat extracted edible tissues

Conclusion and application: 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.

INTRODUCTION

Pendimethalin has been determined to be a systemic toxicant (U.S. E.P.A, 1984)). An Acceptable Daily Intake (ADI), defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect, for Pendimethalin is .005 (mg/kg body weight/day) by oral exposure (U.S.E.P.A 1984, Kidd and James (1991). Pendimethalin is a selective herbicide used to control most annual grasses and certain broadleaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts, and sunflowers. It is used both pre-emergence (that is before weed seeds have sprouted) and early post-emergence. Incorporation into the soil by cultivation or irrigation is recommended within 7

days following application (Kidd and James, 1991). Pendimethalin is available in emulsifiable concentrate, wettable powder, or dispersible granule formulations. Pendimethalin is slightly to practically nontoxic by ingestion, with reported oral LD50 values of 1050 mg/kg to greater than 5000 mg/kg in rats (Kidd and James, 1991). It is slightly to practically nontoxic by skin exposure, with reported dermal LD50 values of greater than 2000 mg/kg. It is not a skin irritant or sensitizer in rabbits or guinea pigs, but it causes mild eye irritation in rabbits (Weed Science Society, 1994). The inhalation 4-hour LC50 for technical pendimethalin in rats is 320 mg/L, indicating practically no toxicity via this route (Weed Science Society, 1994). Some formulated products (e.g., Prowl® may show slight

toxicity by inhalation, and may have a greater capacity to cause skin irritation. Inhalation of dusts or fumes may be mildly to moderately irritating to the linings of the mouth, nose, throat, and lungs (U.S. E P A (1985). Increases in alkaline phosphates level and liver weight were produced in dogs fed 50 mg/kg/day for 2 years, but not at a dose of 12.5 mg/kg/day (U.S. E. P. A, 1987). In a 90-day feeding study of rats, no effects were observed at doses of 40 mg/kg/day (U.S. E P A, 1987). Pendimethalin is highly toxic to fish and aquatic invertebrates. The reported 96-hour LC50 for pendimethalin in bluegill sunfish is 199 ug/L, in rainbow trout is 138 ug/L, and in channel catfish is

420 ug/L (Kidd and James, 1991). The 48-hour LC50 in a small freshwater crustacean is 280 ug/L (5). The bioconcentrate factor for this compound in whole fish is 5100, indicating a moderate potential to accumulate in aquatic organisms. Elevations in serum ALT activity are considered to be relatively specific for liver disease (Stockham and Scott, 2002). Pendimethalin can bioaccumulate, or build up to toxic levels in humans that chain. The objectives of this study are to assess the effect of pendimethalin on fish by using serum alanine aminotransferase enzyme (ALT) as an indicator in the White Nile Province (Kenana area, Sudan) and Blue Nile Province.

MATERIALS AND METHODS

Biological Experiment: Fishes of different sex, age (25 – 35 days) and weight (170 gm – 1.0 kg) were collected from the four locations (Blue Nile Stream, White Nile Stream, Recycled – water and Drainage - water). Blood samples of a live fishes were collected from fish heart and stored at 5 °C until analysis. The blood was centrifuged at 30000 rpm for separation. Then pendimethalin was extracted from fish samples separately according to following method:

Extraction of pendimethalin from fish tissues: The analysis required 50 gm of the edible part of fish from each sample which was homogenized. Extraction was performed using 100 to 150 ml of acetonitrile. The samples were filtered and rinsed twice with 25 ml of the solvent. The combined extract was concentrated using a rotary vacuum and evaporated over a hot water bath (less than 50°C) 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 and 100 ml of n-hexane. The combined n-hexane layer was passed through anhydrous sodium sulphate and concentrated to near dryness and taken in about 10 ml n-hexane as described by (Paula et al, 2007).

Measurement of pendimethalin concentration by High Performance Liquid Chromatography (HPLC): A calibrated HPLC device was set for measurement of pendimethalin concentration as follows: Column: ODS, Flow rate: 1 ml / minute, Injection volume: 10 µL, Oven temp: 30 °C, Mobile phase: acetonitrile: water (80: 20) as described by (Oblinger et al, 1999).

Histopathological parameters: From the autopsied fish, liver tissues were collected in clean, labeled, sterilized containers. The liver tissues are cleaned with distilled water and preserved in 10% formal saline. Sequence of steps which were carried out according to method described by (Muawia I and Murwan K S, 2011) for preparing slides: Suspected sites of liver were cut into small pieces and were dehydrated in solutions of 30% alcohol for two hours, then solutions of 50% alcohol for two hours, finally in 70% alcohol for two hours to attain the preservation level. Continuation of dehydration in 70% alcohol for an hour, in 90% alcohol for 2 hours, in 95% alcohol for 1 hour and in 100% alcohol for 1 hour and in for in . Xylene for ¼ an hour, in Xylene for 1/2 an hour or in Chloroform overnight. For i in for 1 hour, in Wax for 1 hour. For embedding: tissue is embedded in cassette. For sectioning a microtome was used. To mounting tissues on the slides formaldehyde and gelatin were used. For wax fixation and tissue elongation the slides were put i in an oven of temperature < 45° C. For the wax removal slides were dipped in Xylene for 1 minute, Xylene minute, and Absolute alcohol 2 minutes, Absolute alcohol minutes, in 90 % Alcohol 2 minutes, in 70 % Alcohol 2 minutes and in distilled water for 2 minutes. Staining with iodine and haematoxylin was done for 10 minutes. Blueing: Washing slides under running tap water if over stained and then quickly dip in acid alcohol (3 drops of HCl in 70% alcohol), then in distilled water for a ½ minute, in Iodine for a ½ minute in for ½ a. Again in distilled water for ½ a minute, in 70% alcohol for ½ a minute, in 90% alcohol for ½ a minute, Absolute alcohol ½ a minute, Absolute alcohol a½ minute, in Xylene for ½ a minute,

Xylene for ½ minute. Slides were covered with Canada balsam.

Statistical Analysis: Three samples were taken, analyzed and averaged. Mean is average of thirty

RESULTS

Results(Table 1) indicates the concentration of pendimethalin in the liver of the fish collected from Blue Nile stream, White Nile stream, water –recycle and Water - drainage was 0.0, 281.6, 411.1 and 568.5 ppm, respectively. While concentration of ALT enzyme in blood serum of the fish that was collected from Blue Nile stream, White Nile stream, water –recycle and Water - drainage was 34, 144.2, 291.8 and 460.2 U/l.

replicates. Data were assessed using Analysis of Variance (ANOVA) as described by (Gomez TP and Gomez AA (1984). 10).

These findings clearly indicated that the fish of Blue Nile stream is not contaminated with the pendimethalin herbicide, but all fishes collected from White Nile stream, water –recycle and Water - drainage in Kenana area are contaminated with pendimethalin herbicide, These results are agreement with those findings obtained by (Muawia I and Murwan K S, 2011).

Table 1: show the concentration of pendimethalin and (ppm) ALT enzyme in fishes liver (U/L).

Province	Singa area	Kenana area		
Location	Blue Nile	White Nile	Water -recycle	Water – drainage
Pendimethalin (ppm)	0.0	281,6 ± 3.0	411.1± 23	568.5 ± 11
ALT enzyme (U/L)	34 ± 7.9	144.2 ± 5.4	291.8 ± 7.5	460.2 ± 9.8

Liver Dissection: Compared to the control plate (D), plates (A), (B) and (C) show gradual increase in liver changes, according to Blue Nile stream, White Nile stream, Recycled – water in Kenana and Drainage – water in Kenana, respectively). Plate (A) liver tissue shows extended extra-hepatic pancreas surrounding congested portal vessels. Plate (B) liver tissue shows pyknosis and portal vessel vein surrounded by extra hepatic pancreas; big part of the pancreas contains brown granules of haemosidrein (haemochromatosis). Plate (C) liver tissue shows areas of hepatocellular necrosis surrounded by thick fibrous tissue capsule. Plate (D) the control liver tissue shows extra-hepatic pancreas of nearly regular appearance. . The increased serum ALT activity can accompany hepatocellular injury or necrosis of striated muscle with cell injury or death

CONCLUSION

In order to reduce the amount of accumulated pendimethalin residues in fish and birds. Also that eat fish should be well cooked before human consumption. Consumers are advised to avoid fishing in chemical

(Figlio et al, 2004).Release of ALT from the cytosol can occur secondary to cellular necrosis or as a result of cellular injury with membrane damage and bleb formation (Stockham SL, Scott MA 2002:) also stated that elevations in serum ALT activity are considered to be relatively specific for liver disease.

The investigations proved that the pendimethalin – remains can be found in fish. The further accumulation of the results implies e.g. extended extra-hepatic pancreas surrounding congested portal vessels. pendimethalin for long- term may cause adverse effect because many pesticides are now suspected of being endocrine disruptors - chemicals that can lead to the increase in birth defects, sexual abnormalities and failure () Gwynne L. (2000).

polluted areas. Regular monitoring of fish and not eat birds that polluted with pesticide residues via fish is strongly recommended



Plate (A)

Fish source: White Nile stream; liver tissue showing extended extra-hepatic pancreas (eh) surrounding congested portal vessels (cv). (H&E × 100).

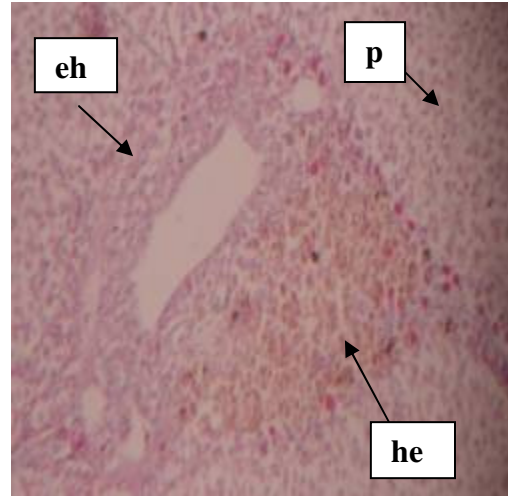


Plate (B)

Fish source: Recycled – water in Kenana; liver tissue showing probably pyknosis (p) and portal vessel vein surrounded by extra hepatic pancreas (eh); big part of the pancreas contain brown granules of haemosidrein (haemochromatosis) (he). (H&E × 250).

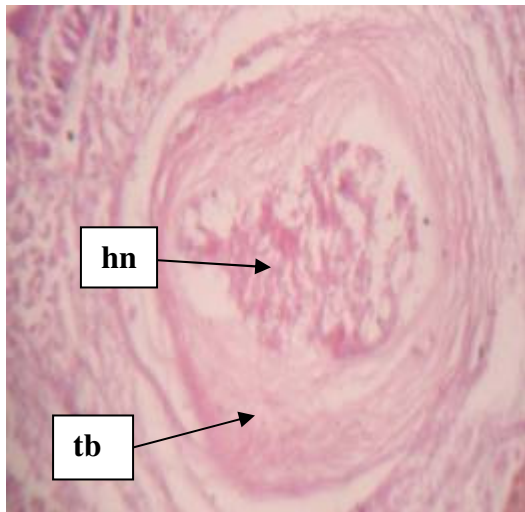


Plate (C)

Fish source: Drainage – water in Kenana; liver tissue showing area of hepatocellular necrosis (hn) surrounded by thick fibrous tissue capsule (tb). (H&E × 250).

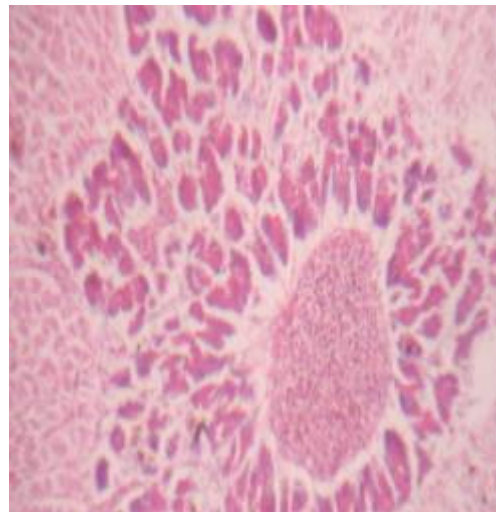


Plate (D)

Fish source: Blue Nile stream as control; liver tissue showing extra-hepatic pancreas of nearly regular appearance. (H&E × 400).

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