Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (3):** 30-34 (2014)

Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Effect of Sub-Lethal Exposure Of DDT on Mid-Gut Lipid Peroxidation and Some Nutritional Parameters of the Earthworm *Pheretima Peguana* (Rosa)

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ABSTRACT

Healthy earthworms of the species Pheretima peguana (Rosa) were exposed to 5ppm and 10ppm of DDT respectively (Groups-II & III) against normal control group (Group-I), in a rearing media prepared by thorough mixing of sundried and powdered cow-dung, fine crushed rotted pseudo-stem of banana plant, silted soil and distilled water in 5:3:1:2 by weight. Prior to experiment the LD_{50} value of DDT for this species was determined as13.66±0.102 ppm (mg/kg of media) for 7 days. After the study period of 7 days it had been revealed that, DDT is very toxic to the earthworms even though in sub-lethal amount. It leads to lipid-peroxidation of the main metabolic organ "the mid-gut" of these animals, resulting in lowering of nutrient levels in body fluid including Glucose and Total Protein. Total Protein–Albumin ratio and Total Cholesterol-Very Low Density Lipoproteins ratio were drastically changed. Enhancements of Triglycerides, Low Density Lipoproteins and Very Low Density Lipoproteins were also marked.

Keywords: *- DDT, earthworm, lipid peroxidation, nutrient.*

INTRODUCTION

DDT ("dichlorodiphenyltrichloroethane") is a colorless, crystalline, tasteless and almost odorless organochloride known for its insecticidal properties. DDT has been formulated in almost every conceivable form, including solutions in xylene or petroleum distillates, emulsifiable concentrates, water-wettable powders, granules, aerosols, smoke candles and charges for vaporisers and lotions[1].

First synthesized in 1874, DDT's insecticidal action was discovered by the Swiss chemist Paul Hermann Müller in 1939. It was then used in the second half of World War II to control malaria and typhus among civilians and troops. After the war, DDT was made available for use as an agricultural insecticide and its production and use duly increased[1]. Müller was awarded the Nobel Prize in Physiology or Medicine "for his discovery of the high efficiency of DDT as a contact poison against several arthropods" in 1948.[1] However, widespread agricultural use accelerated resistance among insect populations, in many cases reversing early successes against malaria-carrying mosquitoes.

In 1962, the book *Silent Spring* by American biologist Rachel Carson was published. It catalogued the environmental impacts of indiscriminate DDT spraying in the United States and questioned the logic of releasing large amounts of chemicals into the environment without a sufficient understanding of their effects on ecology or human health. The book demonstrated that DDT and other pesticides had been shown to cause cancer and that their agricultural use was a threat to wildlife, particularly birds. Its publication was a seminal event as regards the environmental movement and resulted in a large public outcry that eventually led, in 1972, to a ban on the agricultural use of DDT in the United States.[1] A worldwide ban on its agricultural use was later formalized under the Stockholm Convention, but its

limited use in disease vector control continues to this day and remains controversial,[1] because of its initial effectiveness in reducing deaths due to malaria, as well as the pesticide resistance among mosquito populations it engenders after several years of use.

Along with the passage of the Endangered Species Act, the US ban on DDT is cited by scientists as a major factor in the comeback of the bald eagle (the national bird of the United States) and the peregrine falcon from near-extinction in the contiguous United States.[1] DDT is a persistent organic pollutant that is readily adsorbed to soils and sediments, which can act both as sinks and as long-term sources of exposure contributing to terrestrial organisms.[1] Depending on conditions, its soil half life can range from 22 days to 30 years. Routes of loss and degradation include runoff, volatilization, photolysis and aerobic and anaerobic biodegradation. Due to hydrophobic properties (non polar characteristics), in aquatic ecosystems DDT and its metabolites are absorbed by aquatic organisms and adsorbed on suspended particles, leaving little DDT dissolved in the water itself. Its breakdown products and metabolites, DDE and DDD, are also highly persistent and have similar chemical and physical properties.[1] DDT and its breakdown products are transported from warmer regions of the world to the Arctic by the phenomenon of global distillation, where they then accumulate in the region's food web[1].

Because of its lipophilic properties, DDT has a high potential to bioaccumulate, especially in predatory birds.[2] DDT, DDE, and DDD magnify through the food chain, with apex predators such as raptor birds concentrating more chemicals than other animals in the same environment. They are very lipophilic and are stored mainly in body fat. DDT and DDE are very resistant to metabolism. In humans, their half-lives are 6 and up to 10 years, respectively. In the United States, these chemicals were detected in almost all human blood samples tested by the Centers for Disease Control in 2005, though their levels have sharply declined since most uses were banned in the United States.[3] Estimated dietary intake has also declined,[3] although FDA food tests commonly detect it.[1] Marine macro-algae (seaweed) help reduce soil toxicity by up to 80% within six weeks[4].

In insects it opens sodium ion channels in neurons, causing them to fire spontaneously, which leads to spasms and eventual death. Insects with certain mutations in their sodium channel gene are resistant to DDT and other similar insecticides. DDT resistance is also conferred by up-regulation of genes expressing cytochrome P450 in some insect species,[5] as greater quantities of some enzymes of this group accelerate metabolism of the toxin into inactive metabolites—in essence, these enzymes are natural antidotes to the poison.

Pheretima peguana (Rosa) of the Order: Oligochaeta, Family: Megascolicidae is an earthworm of Oriental distribution and native to Assam and Bengal[6]. It has role in decomposition of biological wastes by which converting and simplifying these materials to manures[7]. As DDT is a residual insecticide, on mixture to soil where earthworms reside may be accumulated in the body (especially in fat portions), generally occurs[8,9]. This may be a major cause of loss of diversity of the earthworms.

This study is aimed to investigate the effect of sub-lethal exposure of DDT on mid-gut lipid peroxidation and some nutritional parameters (Glucose, Total Protein, Albumin, Total Cholesterol, Triglyceride, HDL, LDL and VLDL) in the body-fluid of earthworm *Pheretima peguana* (Rosa).

MATERIALS AND METHODS

Sundried cow-dung was powdered and mixed well with fine crushed rotted pseudo-stem of banana plant, silted soil and distilled water in 5:3:1:2 to make slurry where earthworms were reared. *Pheretima peguana* (Rosa) were procured from a local Vermicompost Farm and kept in media for acclimatization. At the very beginning the LD_{50} of DDT was determined as 13.66±0.102 ppm (mg/kg of media) for 7 days in the rearing media.

Three sets terracotta tubs (of 5kg capacity each) were filled up with rearing media. 1st set was for Normal Control Group (Group-I). Media of 2nd and 3rd sets were made containing of 5ppm and 10ppm of DDT respectively and these were for Experimental Groups (Groups-II & III). Then healthy earthworms were inoculated to each set. Not less than 10 worms were inoculated and 5 worms from each group were taken

for experimental assays. Treatment was done for 7 days. Body fluids of the experimental worms were collected under light ether anesthesia with micro-haemocrit capillaries and kept in separate labeled micro-centrifuge tubes. Collected body fluids were centrifuged at 5000 RPM for 10 min and supernatants were collected in separate labeled micro-centrifuge tubes and kept in deep freeze for assays of Glucose, Total Protein, Albumin, Total Cholesterol, Triglyceride and HDL Cholesterol. After assays fractionation of LDL Cholesterol, VLDL cholesterol was done from the data of Total Cholesterol, Triglyceride and HDL Cholesterol.

Mid guts of the experimental earthworms were collected after body fluid collection, washed properly in deionized water to remove gut contents and homogenized in deioniged water maintaining a ratio of 10 mg of mid-gut tissue per ml of deionized water and after centrifugation respective supernatants were kept in separate labeled micro-centrifuge tubes in deep freeze for the assays for Lipid Peoxide.

Measurement of Mid-gut Lipid Peroxidation was done by the photometric evaluation of molar extinction co-efficient of thiobarbituric acid[10]. Glucose content in body fluid is estimated by GOD-POD, End point Assay Kit[11] Total Protein content in body fluid is estimated by Modified Biuret, End Point Assay Kit[12,13]. Albumin content in body fluid is estimated by Bromocerol Green, End Point Assay Kit[14,12]. Total Cholesterol content in body fluid is estimated by CHOD-PAP, End Point Assay Kit. [15,16] Triglyceride content in body fluid is estimated by GPO-PAP, End Point Assay Kit[15,16]. HDL Cholesterol content in body fluid is estimated by PEG- CHOD-PAP, End Point Assay with Lipid Cleaning Factor (LCF) Kit[15,16]. Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL), Chylomicron Fats are precipitated by addition of Propylene Glycol 6000 (PEG). After centrifugation, High Density Lipoproteins (HDL) fraction remains in supernatant and is determined with CHOD-PAP method[15,16] (by the kit for Total Cholesterol). From the results of Total Cholesterol, Triglycerides and HDL fractionation of LDL Cholesterol and VLDL Cholesterol is done by using "Friedewald's Equation"[17]. The reagent kits for Total Cholesterol, Triglycerides and HDL were procured from Span Diagnostics (India) Ltd. Thiobarbituric acid is procured from Research Fine Chem (India) Ltd. The 4,4′-DDT (98%) is procured from Sigma-Aldrich Inc. (USA). The other regents and chemicals were procured from Ranbaxy-Ranchem LTD.

All the photometric observations and biochemical assays were done in a semi automated biochemistry analyzer ("Lab Life Chem-Master" manufactured by Ranbaxy- Diagnova LTD). Necessary kit specifications and dilution factors were preprogrammed in the machine.

RESULTS

Results obtained so far were analyzed statistically[18] with the help of Microsoft Excel and presented in the following table:

Table-1: Shows various study parameters of experimental earthworms during study period

Study parameters	Experimental Earthworm Groups		
	Group-I Normal-Control Group	**Group-II Earthworms exposed to 5ppm DDT**	**Group-III Earthworms exposed to 10ppm DDT**
Lipid Peroxide in Mid-gut (n mol/mg)	9.66± 0.099146	11.466± 0.094583 + 18.69565% *	13.69± 0.080436 + 41.71843% *
Glucose content in Body Fluid (mg/dl)	194.746±0.830166	165.63±1.182721 -14.9508% *	145.414±1.190511 -25.3315% *
Total Protein content in Body Fluid (mg/dl)	485.572±0.686305	375.358±1.040487 -22.6978% *	283.312±0.648933 -41.654% *
Albumen content in Body Fluid (mg/dl)	1.278±0.037868	1.948±0.006633 +52.42567% *	2.190±0.039623 +71.3615% *
Total Cholesterol in Body Fluid (mg/dl)	115.144±0.535319	98.086±0.216439 -14.8145% *	76.518±0.029223 -33.5458% *
Triglyceride content in Body Fluid (mg/dl)	31.608±0.064915	48.606±0.069253 +53.77752% *	52.594±0.066378 +66.39458% *
HDL Cholesterol in Body Fluid (mg/dl)	75.512±0.871656	42.878±0.549048 -43.217% *	15.964±0.344102 -78.859% *
LDL Cholesterol in Body Fluid (mg/dl)	34.388±0.117533	47.236±0.41256 +37.36187% *	51.32±0.256105 +49.23811% *
VLDL Cholesterol in Body Fluid (mg/dl)	6.262 ±0.013191	9.526±0.084652 +52.12392% *	10.464±0.176482 +67.10316% *
"*" indicates Significant at p<0.001, "+…%" and "-…%" indicate percent increase and decrease respectively.			

DISCUSSION

During this study it has been observed that Lipid Peroxidation increases in mid-gut, the major digestive organ of the earthworm up to + 18.69565% and + 41.71843% on exposure to 5ppm and 10ppm DDT respectively. Glucose content in Body Fluid decreases up to -14.9508% and -25.3315% on exposures to 5ppm and 10ppm DDT respectively. Total Protein content in Body Fluid decreases up to -22.6978% and -41.654% respectively on exposures to 5ppm and 10ppm DDT. But, Albumen content in Body Fluid increases +52.42567% and +71.3615% on exposures to 5ppm and 10ppm DDT respectively. Total Cholesterol in Body Fluid decreases up to -14.8145% and -33.5458% on exposures to 5ppm and 10ppm DDT respectively. Triglyceride content in Body Fluid increases up to +53.77752% and +66.39458% respectively on exposures to 5ppm and 10ppm DDT. HDL Cholesterol in Body Fluid decreases up to -43.217% and -78.859% respectively on exposures to 5ppm and 10ppm DDT. LDL Cholesterol in Body Fluid increases up to +37.36187% and +49.23811% respectively on exposures to 5ppm and 10ppm DDT. VLDL Cholesterol in Body Fluid increases up to +52.12392% and +67.10316% respectively on exposures to 5ppm and 10ppm DDT.

CONCLUSION

In this study it has been revealed that DDT is very toxic to the earthworms even though in a very trace amount in soil. In sub-lethal doses too it leads to lipid-peroxidation of the main metabolic organ “the mid-gut” of these animals. This results in lowering of nutrient levels (occasional mal-nutrition) including those of Glucose, Total Protein, and Total Cholesterol etc. in the body fluid. Total protein/Albumin ratio is also drastically changed. It has been observed that on exposure to DDT, the levels of High Density Lipoproteins (HDL) are decreased and on the other hand the levels of Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and Triglycerides are increased. As DDT is a non polar (lipophilic) substance it has higher affinity to saturated fatty acids viz. LDL, VLDL, Triglycerides etc. Hence, scope of accumulation of DDT and its byproducts is increases (biomagnifications or bioamplification) in these animals which may be a major cause of death of them. Application of DDT is already been banned in almost all developed countries but still this has been used for mosquito (malaria) control programme in India. Mosquitoes already developed resistance against DDT. Mainly seasonal application of DDT as residual insecticide to kill mosquitoes in villages, where application done inside residences including cattle shades, where scope of mixture with cow-dung is very high. Again villagers use cow-dung as manure in agricultural lands directly or after being composted with earthworms, or dump these in outskirts. This is why DDT spread through the habitats of earthworms and kills them. As earthworms are farmer’s friends and an integral part of ecosystem as decomposer, DDT should be totally banned for the safety of these individuals.

Acknowledgement

Authors are grateful to all the colleagues of Biodiversity and Ecological Research Centre, Department of Zoology, Bholanath College, Dhubri-783324, (Assam) for their support and technical assistance

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