EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
666-669
Year: 2017

STATISTICAL ANALYSIS OF MARKER ENZYMES OF SWISS ALBINO RAT EXPOSED TO ENDOSULPHAN

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Article Received on 19/05/2017 **Article Revised on 09/06/2017** **Article Accepted on 29/06/2017**

ABSTRACT
Acid phosphatase (ACP) and alkaline phosphatase (AKP) are lysosomal enzymes, which catalyse the splitting of phosphoric acid from certain phosphoric esters and commonly found in most tissues of the body. They are generally located on absorptive or secretory surface of cells as membrane bound enzymes. ACP which hydrolysis the ester linkage of phosphate esters at acidic pH 5 to 6 and helps in autolysis of the degenerated cells. AKP which splits phosphorus esters at alkaline pH (10) and mediates membrane transport and is intimately associated in protein synthesis Interaction of sublethal dose of endosulphan with the enzyme acid phosphatase and alkaline phosphatase in liver and kidney of albino rat was studied for different period of exposure. The enzyme activity was increased due to biliary hindrance and renal failure due to nephrotoxic and hepatotoxic effect. Increase in Acid phosphatase and Alkaline phosphatase activity is due to leakage of lysosomal enzymes and renal and hepatic injury. Endosulphan effects the biomarker of albino rat.

KEYWORDS: Sublethal, autolysis, hepatotoxic, biomarker.

INTRODUCTION

Endosulphan is an important environmental pollutant which is a pesticide of organochlorine group. Primary site of organochlorine storage in the body is adipose tissue. It is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by mono-oxygenase systems which cause oxidative stress.

Pesticide may pollute water through run-off from the treated areas; air drift during aerial application, industrial effluents etc. Water pollution is the cause of death of several interdependent aquatic forms of life and also a source of biomagnifications of persistence pesticide. Several reports of large fish kill due to pesticides particularly endosulphan is on the record. Endosulphan is of great ecological significance that residues of endosulphan are transported from agricultural field to aquatic system mainly through run off. In water endosulphan has a half life of 35 to 150 days.

Endosulphan is used extensively for the control of agricultural pests. Its metabolites have strong tendencies to get accumulated in different organs and tissues of the body *e.g*., adipose tissue, liver and food items (Winter and Street, 1992; Thao *et al*., 1993). Endosulphan induce metabolic changes in liver, which are indicators of toxicity. A positive correlation between changes in liver structure and biochemical constituents of the liver and serum has been shown in a number of studies on different mammals exposed to various pesticides (Boulechbache and Spries, 1974; Gertig and Nowakzyk, 1975; Ali and Shakoori, 1999). However, a considerable variation in nature, magnitude and direction of changes in response to pesticide exposure are evident from these studies. It has also been detected in the air, soils, sediments, runoff waters, ground and surface waters (Funari *et al*., 1995; Schultz *et al*., 2001; Ayati, 2003;Chorom and Shrifi, 2010). According to researcher's reports, levels of agrochemicals, in particular, diazinon, DDT and endosulfans, are a major cause for concern in the Caspian Sea and Anzali Lagoon (UNDP, 2004; FAO, 1990). Exposure to pesticides may have significant impacts on aquatic animals and human health.

Several underlying mechanisms have been invoked in past to explain the nature, of changes in liver under given conditions of pesticidal exposure and dosage (Meany and Pocker, 1979). The suggested mechanisms include elevated biosynthetic activity associated with parallel regeneration of liver tissue, concomitant curtailment of leakage of enzymes from it and elevated synthesis of liver enzymes. This leakage of enzymes is obviously due to impaired functions of plasma membrane and it has been reported that administration of lindane significantly decreases the brush border sialic acid content of the membrane, which alters membrane permeability (Labana *et al*., 1997).

MATERIAL AND METHOD

Fourty albino rats weight 38±2 gm of 5 week age was taken and divided into two groups. One served as test and other as control. The tested animals were injected intra dermal injection of endosulfan sublethal (13.01ppm/kg^{-1}) dose and the rats was scarified on 15, 30 and 45 day by decapitation without using any anesthesia, in the morning hours, in order to avoid diurnal fluctuation in enzyme level. The liver and kidney were dissected out and homogenized in distilled water using glass mortar and pestle. This was followed by centrifugation at 1000 rpm for 30 min in a laboratory centrifuge. The supernatant containing crude enzyme extract was used for the biochemical estimation of the enzyme acid and alkaline phophatase by U.V. Kinetic method (2008).

OBSERVATIONS

Acid and Alkaline phosphatase activity in liver and kidney shown in Table 1 and Table 2. The higher level of acid and alkaline phosphatase activity in liver of rat due to destruction of platelets and tissue injury leading to necrosis of renal and hepatic tissue.

Table 1: Changes in the Acid and Alkaline phosphatase activity in liver of albino rats

Enzyme Activity	Control	Experimental (Days of Exposure)		
		15 Days	30 Days	45 Days
ACP	3.68±0.06	4.12±0.04	4.52±0.05	4.98±0.07
ALP	1.30±0.09	1.34±0.06	1.40±0.07	1.49±0.09

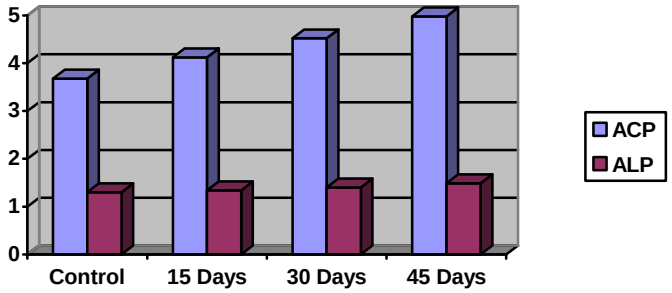

Figure 1 Changes in the Acid and Alkaline phosphatase activity in liver of albino rats

Table 2: Changes in the Acid and Alkaline phosphatase activity in kidney of albino rats

Enzyme Activity	**Control**	**Experimental (Days of Exposure)**		
		15 Days	30 Days	45 Days
ACP	1.96±0.04	2.11±0.06	2.32±0.07	2.39±0.09
ALP	0.32±0.01	0.34±0.03	0.38±0.04	0.44±0.06

Value are mean ± SE of 5 animals.

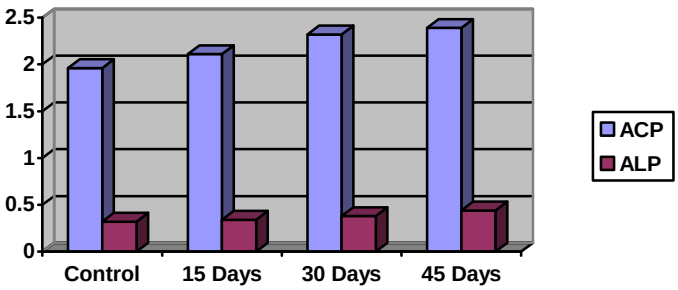

Figure 2 Changes in the Acid and Alkaline phosphatase activity in kidney of albino rats

RESULT AND DISCUSSION

Control rats showing normal integrity of the cortex and blood vessels. endosulfan induced congestion in capillaries in glomerulus and moderate cellular swelling with moderate inflammatory cell infiltration, tubular necrosis and glomerular atrophy, which is a degenerative phenomenon in 15 days of exposure in all endosulfan treated animals.

Acid phosphatase. (ACP) is a type of enzyme manufactured by the body. Like all enzymes, it is composed of specialized proteins that catalyze, or stimulate, certain biological reactions. ACP is classified as a hydrolase enzyme because its purpose is to catalyze the hydrolysis of a chemical bonds. Specifically Acid phosphatase targets and breaks the molecular bonds of phosphate groups. Alkaline Phosphatases are a group of enzymes found primarily in the liver and bone. There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). The total amount of alkaline phosphatases released from these tissues into the blood.

The increase in acid and alkaline phosphatase in albino rats may be due to the effect of endosulfan on absorptive or secretary surface of the cell membrane causing cellular leakage. Increased in alkaline phosphatase enzyme occur in acute renal impairment Kiran 1988 reported increased activity of acid and alkaline phosphatase due to interaction of endosulfan with enzyme molecules rather than with the tissue. It shows nephrotoxic effect on the kidney.

The higher level of acid and alkaline phosphatase activity in liver in comparison to kidney is due to destruction of platelets and chemical induced tissue injury along with necrosis of liver and prolong active hyperplasia.

Acid phosphatase plays an important role in carbohydrate metabolism. This enzyme can be found inside the membrane of lysosomes. So, any damage to the membrane of lysosomes can cause the release of this enzyme into muscle and increase its levels. In the present study, ACP activity in the liver of exposed animal was higher than control group on 30 days. These changes were observed in freshwater field crabs (Spiralothelphusa hydrodroma) which were exposed to Cypermethrin (Sreenivasan *et al*., 2011). Khan and Sharma (2012) reported an increase in the acid phosphatase activity in liver and kidney, tissue, the enzyme ALP activity was also increased. This is in conformity with the present study. Pesticide poisoning altered the activity of ACP and ALP. Acid phosphatase activity of liver of *albino rat* increased in all the three doses indicates increase in protease activity this may be due to the damage caused to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytosol.

The alkaline phosphatase plays a significant role in phosphate hydrolysis and in membrane transport as well as is a good bio-indicator of stress in biological systems. The importance of measuring alkaline phosphatase is to check the liver dysfunction (Banaee *et al*., 2011) and the cellular membrane health. A significant increase in the levels of ALP activity may indicate disorder in tissue cellular membrane of crayfish exposed to 65 µg L^{-1} of endosulfan after 30 days. Similar results were observed in freshwater field crab (*S.hydrodroma*) which was exposed to Cypermethrin (Sreenivasan *et al*., 2010).

In conclusion, the biochemical parameters measured liver and kidney in the present study was useful for monitoring the long-term effects of endosulfan, an organochlorine pesticide, on albino rat. One can infer that endosulfan was highly toxic to rats. Exposure to chronic sub-lethal concentrations of endosulfan resulted in significant biochemical alterations. These changes may be potentially disruptive for the survivability of the living beings and surrounding ecosystem. This fact should be taken into consideration when this pesticide is used for pest control in agricultural fields surrounding surface water.

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