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ORIGINAL ARTICLE

Assessment of Phosphatase Activity in Liver and Serum of Albino Rat under Stress of Heavy Metals

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ABSTRACT

When man and other animals began their life on earth, there was very little amount of pollutants that were present in the ecosystem. With the increase in population and the knowledge utilizing natural resources for human welfare, the amount of pollutants has gradually increased and presently they are increasing at a faster rate causing serious problems. In the present era of science and technology, albeit steps are utilized for elimination of various pollutants that are generated by the industries, household and other anthropogenic activities, yet pollution has become an unbroken companion of human activities. In the light of present scenario where pollution specially heavy metals overwhelming everywhere and every aspect of human being, it is necessary to assess enzymatic activity in liver and serum of albino rat to highlight the extent and magnitude of problem.

***Key words**: Phosphatase Activity, Albino Rat, Heavy Metals*

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INTRODUCTION

Pollution has become an inevitable part of the industry. Pollution takes its toll when the contaminant reaches to various levels of food chain and the delicate balance of nature gets disturbed. In nature, the aquatic organisms are first to receive the pollutants, as the water resources that are in near vicinity of the industrial cities act as a dumping ground for the wastes generated by the industries and various anthropogenic activities. Any material becomes waste when it is discharged in the environment beyond certain level of degradation. The wastes that are discharged in the environment include substances like smoke, chemicals of factories and are generally waste products or byproducts. The wastes that need considerable attention in the present era are chemicals, pesticides and mineral elements. The metals are unique among mineral elements that cause adverse effects in that they occur naturally and in most instances are ubiquitous in the environment. Regardless of how heavy metals are used in consumer products or in industrial processes, some level of human exposure is, in most instances, inevitable. Furthermore, many are biologically essential but become toxic with increasing dosage. Increasing technologic use of metals is one measure of man's progress since the emergence from Stone Age. This has posed thousands of hazards to the health from the time metals fashioned into spears of present-day exposure to space-age metals, alloys or salts. High natural concentrations of metals in food or water could have led to the first exposures. Metals leached from eating utensils or metallic cookware increased the risk of exposure. Intentional use of compounds containing toxic metals as pesticides or as therapeutic agents increased the opportunity for hazardous exposures. The action of metals could be further divided as the

biological action required for sustenance of optimum health, pharmacological actions where their supplements are used and toxicological action where a dose exceeds the biochemical need.

The albino rat has been selected for the present investigation to assess the effect of copper and chromium on mammals since it is easy to rear in the laboratory. Moreover, it has been noted that the studies conducted on human population had revealed similar findings as reported by investigations conducted on rats and other mammals like rabbits. The liver's unique metabolism and relationship to the gastrointestinal tract makes it an important target for toxicity of drugs and xenobiotics. However, hepatic drug metabolism, often with an imbalance between the generation of toxic metabolites and detoxification processes can influence the degree of hepatotoxicity. The liver undergoes dramatic changes during development that determine the rate and metabolic pathways used in the disposition of drugs and other xenobiotics. However, liver's main function is to synthesize an array of body proteins and to act as the detoxifying center for both, the multiple toxic metabolic byproducts endogenous to the body and the toxins ingested daily by the organisms, thereby acting as the main site for detoxification and biotransformation that involves two broad categories, phase– I (oxidations– reductions and hydrolysis) and phase– II (synthetic conjugations with sulfate, glutathione, acetate and glycine) mechanisms. Serum, which is a component of blood, has been chosen for the present investigation since it reveals the details of physiological status of the experimental animal.

MATERIALS AND METHODS

Rearing and Maintenance of Experimental Animals:

45 Adult individuals of albino rats of almost equal size and weight representing both the sexes were selected from inbred colony. The rats were kept in polypropylene cages of sizes 45 cm X 27 cm X15 cm at the temperature of 25 $\pm 5^0$C and relative humidity 60 $\pm$ 5% and photoperiod of 10 hours a day. The rats were fed on feed obtained from Hindustan Antibiotic Ltd. In addition to its water was provided *ad libitum*.

Experimental Heavy Metallic Compounds:

Copper sulphate and Potassium dichromate have been selected for present study to assay the biochemical changes in the serum and liver of albino rat after 1, 7, 14, 21 and 28 days.

Copper Sulphate-

Trade Name : *Blue vitrol, Blue stone*
Chemical name : Copper Sulphatepentahydrate
CAS Number : *7758 – 99 – 8*
Empirical formula : $CuSO_4.5H_2O$

Potassium Dichromate-

Trade Name : *Lopezite*
Chemical name : *Potassium dichromate*
CAS Number : *7778 – 50 – 9*
Empirical formula : $K_2Cr_2O_7$

Determination of Dose Response of Experimental Heavy Metallic Compounds:

All adult albino rats were divided into 5 groups. Each group consisted of 7 individuals. Standard solution of copper sulphate, the experimental test compound was prepared by dissolving it in distilled water. For copper sulphate, the doses administered orally include 100mg, 200mg, 300mg, 400mg and 500mg/kg body weight and for potassium dichromate, the doses administered orally include 25mg, 50mg, 75mg, 100mg and 125mg/kg body weight.. For each dose after 96 hours, the mortality and survival number of rats were recorded. Data was analyzed statistically by log-dose/probit regression line method (Finney, 1971). On the basis of two variables log dose and empirical probit the

regression line was drawn on a simple graph paper and used to determine the expected probit accuracy for LD50 determination.

Experimental Protocol:
To assay the effect of copper sulphate and potassium dichromate, the albino rats were grouped into five sets, one acute and four sub-acute sets and each set consisted of three rats. The control sets were run simultaneously for acute and sub-acute studies in all five groups.

Dosing and Experimentation:
The albino rats were given a sub-lethal dose of (1/10th of LD50) was 26.9 mg/kg body weight of copper sulphate for one day (24 hours). After one day the albino rats were dissected and liver was excised out. The blood was taken out by piecing the needle in the ventricle of the heart of albino rat and serum was separated by centrifugation at 2000 rpm for 10 minutes. Specific biochemical parameters were assessed from the liver and serum of albino rats. The albino rats were given a sub-lethal dose of (1/10th of LD50) was 7.7 mg/kg body weight of potassium dichromate for one day (24 hours). After one day the albino rats were dissected and liver was excised out. The blood was taken out by piecing the needle in the ventricle of the heart of albino rat and serum was separated by centrifugation at2000 rpm for 10 minutes. The liver and serum were assessed for specific biochemical parameters. All the rats of these groups (4 sets) were given sub-lethal dose divided by 7, 14, 21 and 28 days i.e., (1/70th of LD_{50})=3.84 mg, (1/140th of LD_{50})=1.92 mg, (1/210th of LD_{50})=1.28 mg and (1/280th of LD_{50})=0.96 mg/kg body weight of copper sulphate for 7, 14, 21 and 28 days of experimental duration, respectively. The rats were dissected after 7, 14, 21 and 28 days of experimental protocol, liver was excised out and kept in physiological saline (pH 7.4). The blood was taken out by piecing the needle in the ventricle of the heart of albino rat and serum was separated by centrifugation at 2000 rpm for 10 minutes. The liver and serum are then taken for biochemical estimations. All the rats of these groups (4 sets) were given sub-lethal dose divided by 7, 14, 21 and 28 days i.e., (1/70th of LD_{50})= 1.1mg, (1/140th of LD_{50})=0.55mg, (1/210th of LD_{50})=0.366mg and (1/280th of LD_{50})=0.275 mg/kg body weight of potassium dichromate for 7, 14, 21 and 28 days of experimental duration, respectively. The rats were dissected after 7, 14, 21 and 28 days of experimental protocol and liver was excised out and kept in physiological saline (pH 7.4). The blood was taken out by piecing the needle in the ventricle of the heart of albino rat and serum was separated by centrifugation at 2000 rpm for 10 minutes. The liver and serum are then taken for biochemical estimations. The control groups were run along with acute and sub acute treatments and liver was excised out after 1, 7, 14, 21 and 28 days. The blood was taken out by piecing the needle in the ventricle of the heart of albino rat and serum was separated by centrifugation at 2000 rpm for 10 minutes.

Estimation of Alkaline Phosphatase (EC- 3.1.3.1):
The estimation of alkaline phosphatase activity was done by Kind and King, (1954).

STATISTICAL ANALYSIS

All the data were subjected to standard statistical analysis with correlation studies with the help of computer software based on Fisher and Yates (1950).

RESULTS AND DISCUSSION

The data obtained from experimentation is arranged and analyzed statistically and discussed critically as shown in Tables 1-3 and Fig. 1-2 to revealmagnitude and significance of study.
Alkaline phosphatase (Orthophosphoric-monoesterphosphohydrolase) is a 68 to 82 kDa, Zn and Mg containing, plasma membrane bound glycoprotein localized on the outer extracellular surface (Thede, *et al.*, 1988). It includes a group of isoenzymes that brings

about the hydrolysis of organic phosphate present in the – 5 and – 3 positions from many types of molecules including nucleotides, proteins and alkaloids, principally occurring in bone, liver, kidney, intestinal wall, lactating mammary glands and placenta (Wasserman, *et al.*, 1996). Two, biochemically and immunochemically distinct forms of alkaline phosphatase are known in rat, an intestinal form and other bone/liver/kidney/placental form (BLKP).

Table 1: Serum alkaline phosphatase after per os acute and subacute copper sulphateand potassium dichromate intoxication in albino rat

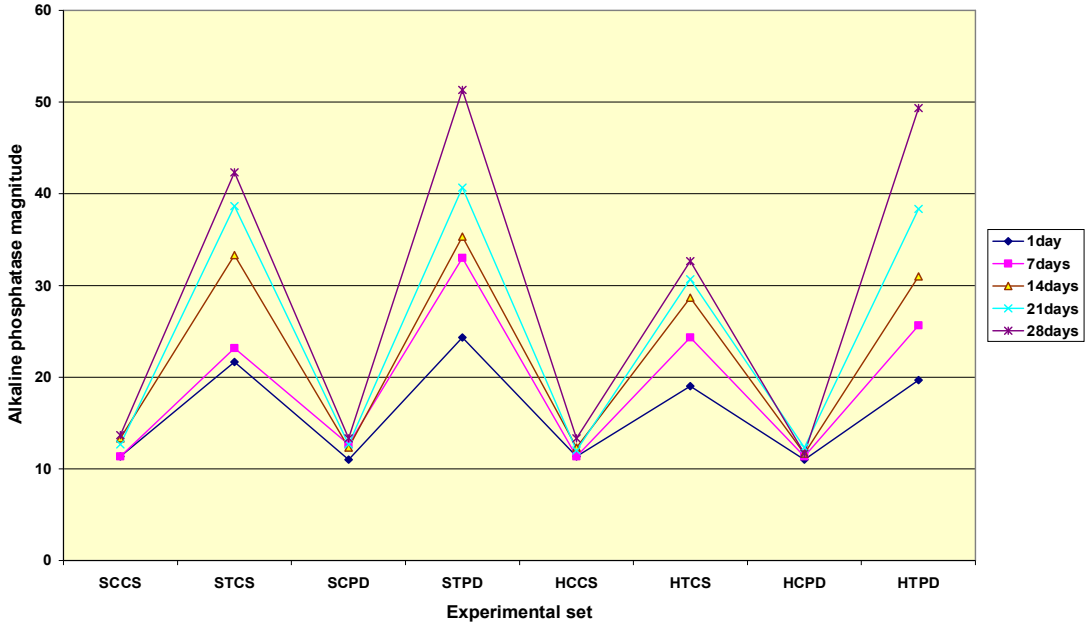
Treatment time (in days)	Alkaline phosphatase (IU/L)			
	Copper sulphate (Mean ±S.Em.)		Potassium dichromate (Mean ±S.Em.)	
	Control	Treated	Control	Treated
1	11.33±2.40	21.66±1.20****	11±2.5	24.33±1.45***
7	11.33±0.66	23.16±2.84****	12.66±2.18	33±1.73 ****
14	13.33±0.88	33.33±0.88****	12.33±0.88	35.33±1.76 ****
21	12.66±2.18	38.66±0.33****	12.66±0.66	40.66±1.76 ****
28	13.66 ±2.02	42.33±1.20****	13.33±0.33	51.33±1.76 ****

Table 2: Hepatic alkaline phosphatase after per os acute and subacute copper sulphateand potassium dichromate intoxication in albino rat

Treatment time (in days)	Alkaline phosphatase (IU/L)			
	Copper sulphate (Mean ±S.Em.)		Potassium dichromate (Mean ±S.Em.)	
	Control	Treated	Control	Treated
1	11.33±1.45	19±0.577 ***	11±1.527	19.66±1.20 ****
7	11.33±1.85	24.33±0.88 ***	11.33±1.45	25.66±3.84 ****
14	12.33±1.85	28.66±0.33 ****	11.66±2.18	31±4.35 ***
21	12 ±1.15	30.66±0.66 ****	12.33±1.15	38.33±5.17 ****
28	13.33±2.4	32.66±1.76 ***	11.66±0.33	49.33±5.81 ****

n.s. – (P>0.05); ** – (P<0.02); *** – (P<0.01); **** – (P<0.001); 3 replicates for each set

Fig. 1: Serum and hepatic alkaline phosphatase after per os acute and subacute copper sulphateand potassium dichromate intoxication in albino rat

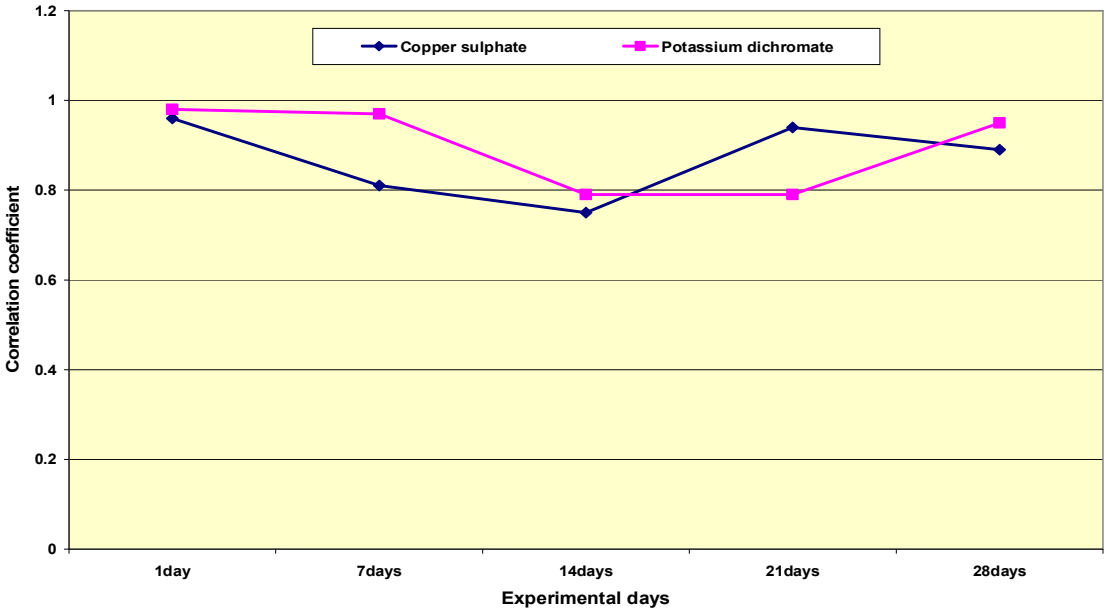


SCCS- Serum control copper sulphate; **STCS**- Serum treated copper sulphate; **SCPD**- Serum control potassium dichromate; **STPD**- Serum treated potassium dichromate; **HCCS**- Hepatic control copper sulphate; **HTCS**- Hepatic treated copper sulphate; **HCPD**- Hepatic control potassium dichromate; **HTPD**- Hepatic treated potassium dichromate

Table 3: Correlation between hepatic and serum alkaline phosphatase

Treatment time (in days)	Alkaline phosphatase (IU/L)			
	Copper sulphate		Potassium dichromate	
	Correlation	Test of significance	Correlation	Test of significance
1	0.96	(P<0.01)	0.98	(P<0.001)
7	0.81	(P<0.05)	0.97	(P<0.001)
14	0.75	(P>0.05)	0.79	(P>0.05)
21	0.94	(P<0.01)	0.79	(P>0.05)
28	0.89	(P<0.05)	0.95	(P<0.01)

Fig. 2: Correlation between hepatic and serum alkaline phosphatase



It is synthesized in many vertebrate cells and different isoenzymes are associated with different organisms (Claverie, 2000). In the present investigation, an increasing trend has been observed in the activity of alkaline phosphatase (BLKP) for both the heavy metallic compounds under investigation, in both serum and liver, on all treatment days. In the present investigation, an increasing trend has been shown in the hepatic and serum alkaline phosphatase activity following acute and sub-acute exposure of both the heavy metals. Highly significant increases have been observed in the hepatic and serum alkaline phosphatase activity under stress of copper sulphate and potassium dichromate intoxication on all treatment days. The activity of hepatic and serum alkaline phosphatase has been found to be positively correlated and highly significant also. Singh (2003) also revealed a highly significant increase in the liver under stress of mercuric chloride and noted that this may be due to destruction of cell membrane of lysosomes. Alkaline phosphatase is not organ specific, yet it is found in the liver primarily associated with lipid cell membrane of biliary canalicular zone and hence is an important marker for the state of organization of membranes, so that any hindrance with the bile flow, leads to membrane damage inturn increasing its concentration in the liver and serum probably due to the effect of accumulated acids on the lipid membranes and is in affirmation to

Deveki, *et al.,* (1992), Margeli, *et al.,* (1994), Theochris, *et al.,* (1994), El Daly (1996), Weshana(2001) and Endo, *et al.,* (2002).

Increased activity of ALP is an indicator of liver damage with hepatocellular lesions and parenchymal cell necrosis with their release in the blood stream from damaged tissue as affirmed by Stangl and Kirchgessner (1998) and Ramalingam, *et al.,* (1999), whereas Kumar and Kumar (2004) also revealed non-significant increase in activity of alkaline phosphatase in rat after treatment with copper and hypothesized that it neither indicates appreciable injury to membrane nor a change in the transport of phosphate ion through it but is due to the adaptive method to the changed environment of plasma membrane. The present findings are in contrast to the findings reported by Gayatri and Rao (1999), Adachi, *et al.* (2000), Todorovic and Vujanovic (2002) and Sidhu, *et al.,* (2004) who revealed marked decline in the activity of alkaline phosphatase under stress of chromium, mercury, vanadium, magnesium, cadmium, and zinc, respectively.

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