

## EFFECT OF TARTRAZINE ON ACID AND ALKALINE PHOSPHATASES IN THE LIVER OF ALBINO RATS

Veena B. Kushwaha\* and Geeta Bharti

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273008, U.P., India

Article Received on  
19 Aug 2015,

Revised on 09 Sep 2015,  
Accepted on 29 Sep 2015,

### \*Correspondence for Author

**Veena B. Kushwaha**  
Department of Zoology,  
D.D.U. Gorakhpur  
University, Gorakhpur-  
273008, U.P., India

### ABSTRACT

Tartrazine (E102, Trisodium (4*E*)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl) hydrazono]-3-pyrazolecarboxylate) is a synthetic lemon yellow azo dye, water soluble and commonly used as food coloring agent all over the world. Hence, this study is aimed on estimation of the variation in the activity of enzyme acid and alkaline phosphatase in the liver of both male and female albino rats after exposure to sub lethal oral doses (100, 200 and 400mg/kg body weight) for 28 days. A non-significant increase in the activity of acid phosphatase after 7 and 14 days, while a significant ( $p < 0.05$ ) increase in the activity of acid phosphatase after 21 and 28 days in female and male rats treated with 100 and 200 mg/kg body weight of Tartrazine in

comparison to control was observed. A significant ( $p < 0.05$ ) increase in the activity of acid phosphatase was observed in both male and female rats in comparison to control rats, on exposure to 400 mg/kg body weight of the Tartrazine. A significant decrease in activity of alkaline phosphatase after 14, 21 and 28 days in animals treated with 100 mg/kg body weight, after 7, 14, 21 and 28 days in animals treated with 200 and 400 mg/kg body weight of Tartrazine in comparison to control animals was observed. Variation in the strength of dose and exposure time significantly influences the activity of acid and alkaline phosphatase in both male and female rats, suggesting induction of liver dysfunction and change in permeability of the membrane of hepatocytes.

**KEYWORDS:** Food additive, Color additive, Tartrazine, Hepato-toxicity, Acid Phosphatase and Alkaline Phosphatase.

### INTRODUCTION

Tartrazine (E102, FD&C Yellow 5) is a synthetic lemon yellow azo dye used as a food colorant. It is water soluble. It is water soluble and has a maximum absorbance at  $427 \pm 2$  nm.

Among food items Tartrazine is commonly used as colourant in confectionery, candies, colored drinks, ice creams, ice pops, flavor corn chips, cereals, yellow popcorn, gelatin, marmalade, mustard, horseradish, yogurt, noodles and Kraft dinner, pickles, many foods together with glycerin, lemon and honey products, bakery industries etc.

In non food products, soaps, cosmetics, shampoos, and other hair products, moisturizers, crayons, green hand sanitizer, nail polish, inks for writing instruments and stamp dyes contain Tartrazine as colorant. Tartrazine is also used in vitamins, antacids, medicinal capsules and various other commonly prescribed medicines (Kushwaha and Bharti, 2013). Therefore, an attempt to measure its effect on the activity of enzymes acid and alkaline phosphatase in rat liver after exposure to sub lethal dosage is made in this paper.

## **MATERIAL AND METHODS**

### **Experimental animals**

Healthy adult male and female albino rats weighing approximately 60-160 g were selected for the experiments. The albino rats were procured, with the help of local animal supplier. They were kept in cage, with polypropylene coated wire gauze on all sides, at room temperature  $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . Rats were exposed to photoperiod of 12 hours per day. The cages were cleaned regularly to avoid rat smell and to maintain proper hygienic conditions. The rats were acclimatized to laboratory conditions for 10 days and fed on rat pellets and water ad libitum. Each rat was weighed and assigned a number for convenience prior to the onset of experiment. The protocol for these experiments was approved by the Ethical Committee of the D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India, 273009.

### **Sub chronic treatment**

The rats were weighed and divided into eight groups. The groups of male and female rats, each containing sixteen rats were administered a sub lethal dose, 100 mg/kg body weight (I), 200 mg/kg body weight (II) and 400 mg/kg body weight (III) of Tartrazine for 28 days orally. The remaining group of both male and female rats was orally fed with vehicle of similar dilution without test material and they served as control.

### **Assessment of biochemistry**

The rats were reweighed and sacrificed. The liver was dissected out from control and sub chronically treated rats after 7, 14, 21 and 28 days. Liver was washed with distilled water, blotted with the help of blotting paper, weighed and processed for biochemical estimation.

**Estimation of phosphatase activity**

Activity of acid and alkaline phosphatase in the tissue was determined according to method of Bergmeyer (1967) as modified by Singh and Agarwal (1989) using p-nitro phenyl phosphate as substrate. Homogenates of tissue (2% w/v) were prepared in ice cold 0.9% NaCl solution and centrifuged at 5000 g at 0°C for 20 minutes. The supernatant were used as enzymes.

**Acid phosphatase**

Acid phosphatase activity was determined by adding 0.2 ml of enzyme source, 1.0 ml of acid buffer substrate solution (prepared by dissolving 0.41 g citric acid, 1.125 g sodium citrate and 165 mg p-nitro phenyl phosphate sodium salt to 100 ml of double distilled water), the mixture was mixed thoroughly and incubated for 30 minutes at 37°C. Then 4.0 ml of 0.1 N NaOH was added to the incubated mixture. A yellow color developed which was measured at 420nm using visible spectrophotometer. Standard curve was drawn using p-nitro phenol as substrate. Enzyme activity has been expressed as  $\mu$  mole substrate hydrolyzed/30 minute/mg protein in supernatant.

**Alkaline phosphatase**

Alkaline phosphatase was determined by adding 0.1 ml of enzyme source to 1.0 ml of alkaline buffer substrate. Alkaline buffer substrate was prepared by addition of 375 mg glycine, 10 mg MgCl<sub>2</sub>.6H<sub>2</sub>O and 165 mg p-nitro phenyl phosphate sodium salt in 42 ml of 0.1 N NaOH. The mixture was made up to 100 ml with double distilled water. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C. In the incubated mixture 10 ml of 0.02 N NaOH was added to stop the reaction. p-nitro phenol formed as a result of hydrolysis of p-nitro phenyl phosphate, gave a yellow color with NaOH. Optical density was measured at 420nm using visible spectrophotometer. Standard curve was drawn with different concentrations of p-nitro phenol. Enzyme activity has been expressed as  $\mu$  mole substrate hydrolyzed/30 minute/mg protein in supernatant.

**Statistical analysis**

The data is expressed as mean  $\pm$  S.E. of four replicates. Student't' test and Two-way ANOVA are applied for measurement of variation between control and treated groups.

## RESULTS

### Acid phosphatase

The mean value of activity of acid phosphatase was  $0.033\pm 0.0010$ ,  $0.0030\pm 0.0005$ ,  $0.031\pm 0.0004$  and  $0.038\pm 0.0010$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 100 mg/kg body weight of Tartrazine. The mean value of activity of acid phosphatase was  $0.035\pm 0.0006$ ,  $0.0032\pm 0.0007$ ,  $0.036\pm 0.0009$  and  $0.045\pm 0.0009$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 200 mg/kg body weight of Tartrazine. The mean value of activity of acid phosphatase was  $0.038\pm 0.0009$ ,  $0.0036\pm 0.0007$ ,  $0.044\pm 0.0007$  and  $0.059\pm 0.0004$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 400 mg/kg body weight of Tartrazine. While in control female rats the mean value of activity of acid phosphatase was  $0.032\pm 0.0005$ ,  $0.0028\pm 0.0009$ ,  $0.025\pm 0.0005$  and  $0.023\pm 0.0006$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively.

The mean value of activity of acid phosphatase was  $0.021\pm 0.0006$ ,  $0.0021\pm 0.0006$ ,  $0.027\pm 0.0006$  and  $0.036\pm 0.0004$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 100 mg/kg body weight of Tartrazine. The mean value of activity of acid phosphatase was  $0.022\pm 0.0007$ ,  $0.0023\pm 0.0009$ ,  $0.031\pm 0.0006$  and  $0.046\pm 0.0004$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 200 mg/kg body weight of Tartrazine. The mean value of activity of acid phosphatase was  $0.024\pm 0.0012$ ,  $0.0026\pm 0.0006$ ,  $0.038\pm 0.0008$  and  $0.058\pm 0.0007$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 400 mg/kg body weight of Tartrazine. While in control male rats the mean value of activity of acid phosphatase was  $0.020\pm 0.0010$ ,  $0.0019\pm 0.0007$ ,  $0.022\pm 0.0006$  and  $0.025\pm 0.0006$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively (Table 1).

**Table.1. Effect of different doses of Tartrazine on the activity of acid phosphatase ( $\mu$  mole substrate hydrolyzed/30 min/ mg protein) in the liver of the rats.**

Sex of rats	Day	Control Mean $\pm$				Change in %		
			I	II	III	I	II	III
Female	7 <sup>th</sup>	0.032 $\pm$ 0.0005	0.033 $\pm$ 0.0010	0.035 <sup>+</sup> $\pm$ 0.0006	0.038 <sup>+</sup> $\pm$ 0.0009	$\uparrow$ 3.125	$\uparrow$ 9.38	$\uparrow$ 18.75
	14 <sup>th</sup>	0.028 $\pm$ 0.0009	0.030 <sup>+</sup> $\pm$ 0.0005	0.032 <sup>+</sup> $\pm$ 0.0007	0.036 <sup>+</sup> $\pm$ 0.0007	$\uparrow$ 7.15	$\uparrow$ 14.29	$\uparrow$ 28.60
	21 <sup>st</sup>	0.025 $\pm$ 0.0005	0.031 <sup>+</sup> $\pm$ 0.0004	0.036 <sup>+</sup> $\pm$ 0.0009	0.044 <sup>+</sup> $\pm$ 0.0007	$\uparrow$ 24.00	$\uparrow$ 44.00	$\uparrow$ 76.00
	28 <sup>th</sup>	0.023 $\pm$ 0.0006	0.038 <sup>+</sup> $\pm$ 0.0010	0.045 $\pm$ 0.0009	0.059 <sup>+</sup> $\pm$ 0.0004	$\uparrow$ 65.23	$\uparrow$ 95.70	$\uparrow$ 156.53
Male	7 <sup>th</sup>	0.020 $\pm$ 0.0010	0.021 $\pm$ 0.0006	0.022 <sup>+</sup> $\pm$ 0.0007	0.024 <sup>+</sup> $\pm$ 0.0012	$\uparrow$ 5.00	$\uparrow$ 10.00	$\uparrow$ 20.00
	14 <sup>th</sup>	0.019 $\pm$ 0.0007	0.021 <sup>+</sup> $\pm$ 0.0006	0.023 <sup>+</sup> $\pm$ 0.0009	0.026 <sup>+</sup> $\pm$ 0.0006	$\uparrow$ 10.60	$\uparrow$ 21.06	$\uparrow$ 36.85
	21 <sup>st</sup>	0.022 $\pm$ 0.0006	0.027 $\pm$ 0.0006	0.031 <sup>+</sup> $\pm$ 0.0006	0.038 <sup>+</sup> $\pm$ 0.0008	$\uparrow$ 22.80	$\uparrow$ 40.91	$\uparrow$ 72.73
	28 <sup>th</sup>	0.025 $\pm$ 0.0006	0.036 $\pm$ 0.0004	0.046 <sup>+</sup> $\pm$ 0.0007	0.058 <sup>+</sup> $\pm$ 0.0007	$\uparrow$ 44.00	$\uparrow$ 84.00	$\uparrow$ 132.00

For female- $F_1=6.29^+$ ,  $F_2=4.18^*$  and  $n_1=3$ ,  $n_2=3$

For male- $F_1=6.79^+$ ,  $F_2=3.86^*$  and  $n_1=3$ ,  $n_2=3$

\* indicates significant ( $p<0.05$ ) difference between control and treated groups when student 't' test is applied between treated and control groups

<sup>+</sup> indicates significant ( $p<0.01$ ) effect of variation in dose and time on treated rats when Two-way ANOVA is applied between control and treated groups

I -100 mg/kg body weight, II -200 mg/kg body weight, III -400 mg/kg body weight

**Table.2. Effect of different doses of Tartrazine on the activity of alkaline phosphatase ( $\mu$  mole substrate hydrolyzed/30 min/ mg protein) in the liver of the rats.**

Sex of rats	Day	Control rats Mean $\pm$				Change in %		
			I	II	III	I	II	III
Female	7 <sup>th</sup>	0.051 $\pm$ 0.0009	0.046 $\pm$ 0.0006	0.041 <sup>+</sup> $\pm$ 0.0007	0.036 <sup>+</sup> $\pm$ 0.0010	$\downarrow$ 9.81	$\downarrow$ 19.61	$\downarrow$ 29.42
	14 <sup>th</sup>	0.048 $\pm$ 0.0009	0.031 <sup>+</sup> $\pm$ 0.0007	0.028 <sup>+</sup> $\pm$ 0.0009	0.027 <sup>+</sup> $\pm$ 0.0006	$\downarrow$ 35.42	$\downarrow$ 41.67	$\downarrow$ 43.75
	21 <sup>st</sup>	0.050 $\pm$ 0.0007	0.031 $\pm$ 0.0011	0.029 <sup>+</sup> $\pm$ 0.0006	0.027 <sup>+</sup> $\pm$ 0.0009	$\downarrow$ 38.00	$\downarrow$ 42.00	$\downarrow$ 46.00
	28 <sup>th</sup>	0.047 $\pm$ 0.0006	0.028 <sup>+</sup> $\pm$ 0.0012	0.026 <sup>+</sup> $\pm$ 0.0012	0.025 <sup>+</sup> $\pm$ 0.0012	$\downarrow$ 40.43	$\downarrow$ 44.70	$\downarrow$ 46.81
Male	7 <sup>th</sup>	0.065 $\pm$ 0.0006	0.062 $\pm$ 0.0012	0.060 <sup>+</sup> $\pm$ 0.0010	0.047 <sup>+</sup> $\pm$ 0.0006	$\downarrow$ 4.62	$\downarrow$ 7.70	$\downarrow$ 27.70
	14 <sup>th</sup>	0.062 $\pm$ 0.0007	0.056 <sup>+</sup> $\pm$ 0.0004	0.054 <sup>+</sup> $\pm$ 0.0006	0.040 <sup>+</sup> $\pm$ 0.0009	$\downarrow$ 9.68	$\downarrow$ 12.91	$\downarrow$ 35.49
	21 <sup>st</sup>	0.057 $\pm$ 0.0007	0.046 <sup>+</sup> $\pm$ 0.0008	0.039 <sup>+</sup> $\pm$ 0.0009	0.035 <sup>+</sup> $\pm$ 0.0010	$\downarrow$ 19.29	$\downarrow$ 31.58	$\downarrow$ 38.60
	28 <sup>th</sup>	0.063 $\pm$ 0.0010	0.040 <sup>+</sup> $\pm$ 0.0007	0.032 <sup>+</sup> $\pm$ 0.0010	0.029 <sup>+</sup> $\pm$ 0.0006	$\downarrow$ 36.51	$\downarrow$ 49.21	$\downarrow$ 53.97

For female- $F_1=5.59^+$ ,  $F_2=5.31^*$  and  $n_1=3$ ,  $n_2=3$

For male- $F_1=6.32^+$ ,  $F_2=4.89^*$  and  $n_1=3$ ,  $n_2=3$

\* indicates significant ( $p<0.05$ ) difference between control and treated groups when student 't' test is applied between treated and control groups

<sup>+</sup> indicates significant ( $p<0.01$ ) effect of variation in dose and time on treated rats when Two-way ANOVA is applied between control and treated groups

I -100 mg/kg body weight, II -200 mg/kg body weight, III -400 mg/kg body weight

Student 't' test indicates a non-significant increase in the activity of acid phosphatase after 7 and 14 day, while a significant ( $p < 0.05$ ) increase in the activity of acid phosphatase after 21 and 28 days in female and male rats treated with 100 and 200 mg/kg body weight of Tartrazine in comparison to control. A significant ( $p < 0.05$ ) increase in the activity of acid phosphatase was observed in both male and female rats in comparison to control rats, on exposure to 400 mg/kg body weight of the all three food additives. Two-way ANOVA test indicates that variation in the strength of dose and exposure time, significantly ( $p < 0.01$ ) influences the activity of acid phosphatase of both male and female treated rats.

### **Alkaline phosphatase activity**

The mean value of activity of alkaline phosphatase was  $0.046 \pm 0.0006$ ,  $0.031 \pm 0.0007$ ,  $0.031 \pm 0.0011$  and  $0.028 \pm 0.0012$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 100 mg/kg body weight of Tartrazine. The mean value of activity of alkaline phosphatase was  $0.041 \pm 0.0007$ ,  $0.028 \pm 0.0009$ ,  $0.029 \pm 0.0006$  and  $0.026 \pm 0.0012$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 200 mg/kg body weight of Tartrazine. The mean value of activity of alkaline phosphatase was  $0.036 \pm 0.0010$ ,  $0.027 \pm 0.0006$ ,  $0.027 \pm 0.0009$  and  $0.025 \pm 0.0012$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in female rats treated with 400 mg/kg body weight of Tartrazine. While in control female rats the mean value of activity of alkaline phosphatase was  $0.051 \pm 0.0009$ ,  $0.048 \pm 0.0009$ ,  $0.050 \pm 0.0007$  and  $0.047 \pm 0.0006$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively.

The mean value of activity of alkaline phosphatase was  $0.062 \pm 0.0012$ ,  $0.056 \pm 0.0004$ ,  $0.046 \pm 0.0008$  and  $0.040 \pm 0.0007$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 100 mg/kg body weight of Tartrazine. The mean value of activity of alkaline phosphatase was  $0.060 \pm 0.0010$ ,  $0.054 \pm 0.0006$ ,  $0.039 \pm 0.0009$  and  $0.032 \pm 0.0010$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 200 mg/kg body weight of Tartrazine. The mean value of activity of alkaline phosphatase was  $0.047 \pm 0.0006$ ,  $0.040 \pm 0.0009$ ,  $0.035 \pm 0.0010$  and  $0.029 \pm 0.0006$  ( $\mu$  mole substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively in male rats treated with 400 mg/kg body weight of Tartrazine. While in control male rats the mean value of activity of alkaline phosphatase was  $0.065 \pm 0.0006$ ,  $0.062 \pm 0.0007$ ,  $0.057 \pm 0.0007$  and  $0.063 \pm 0.0010$  ( $\mu$  mole

substrate hydrolyzed/30 minutes/mg protein) after 7, 14, 21 and 28 days, respectively (Table 2).

Student 't' test indicates a significant ( $p < 0.05$ ) decrease in activity of alkaline phosphatase in both treated female and male rats in comparison to control rats after exposure of Tartrazine. Two-way ANOVA test indicates that variation in the strength of dose and exposure time, significantly ( $p < 0.01$ ) influence the activity of alkaline phosphatase of both male and female treated rats.

## DISCUSSION

Acid and alkaline phosphatase are non specific phosphomonoester have pH specificity and are capable for splitting of phosphate from organic phosphate, at different pH. According to de Duve (1959), a number of hepatic enzymes are concentrated in small sub cellular fraction which sediment between the usual mitochondrial and microsomal fractions. These enzymes are presumed to be enclosed within a lipoprotein membrane, the lysosome, acid phosphatase is one of them. De Lamirande, *et. al.* (1967) found this enzyme bound to the lysosome, endoplasmic reticulum, mitochondria, nuclei and as a soluble form in the cytoplasm. Acid phosphatase breaks the ester linkage in the molecules and also helps in the autolysis of the cells (Novikoff, 1961).

Increased level of acid phosphatase activity in liver may be attributed to the pathophysiological changes in the liver and increase in the relative liver weight by food additives in the present study. Change in membrane permeability may cause labilization of lysosomal membrane with the release of enzyme, thus elevating the levels of acid phosphatase in the liver of treated rats as reported by Abou-Donia (1978) and Kushwaha and Maurya (2012). The findings further gain support by the observations of Kiran, *et. al.* (1985) and Bhatnagar and Jain (1986) as they too observed elevation in the level of acid phosphatase in the liver of rats on exposure to pesticides.

In the liver, alkaline phosphatase is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes (Thapa and Walia, 2007). Alkaline phosphatase, plays a critical role in protein synthesis (Pilo, *et. al.*, 1972). It also plays an important role in the transport of metabolite across the membrane (Vorbrodt, 1959). Alkaline phosphatase is a brush border enzyme. Srivastava (1966) established that the alkaline phosphatase is concerned with phosphorylation and dephosphorylation processes during glucose absorption.

A significant fall in the activity of alkaline phosphatase in hepatocyte of rats after oral administration of food additives were observed in present study. Since alkaline phosphatase is excreted through liver via bile juice (Keele and Neil, 1971), a decline in the level of alkaline phosphatase in liver can be reasoned to the biliary obstruction. This is also evident by the increase in the level of the cholesterol and relative weight of liver in the treated rats (Kushwaha and Bharti 2013). The mechanism by which alkaline phosphatase reaches the circulation is uncertain, leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions (Rosalki and McIntyre, 1999 and Kaplan, 1986). Laboured breathing and sustained muscle fasciculations, during convulsion and tremor observed and reported Kushwaha and Bharti (2013), probably leads to oxygen insufficiency causing impairment in the cell membrane permeability of hepatocytes in the rats after treatment (Kushwaha and Maurya, 2010 and Gupta, *et. al.*, 1991). Mull, *et. al.* (1972), Enan, *et. al.* (1987), Ramalingam and Vimaladevi (2002) Moreno, *et. al.* (1995), Inuwa *et. al.* (2011) and Aniaga *et. al.* (2005) also observed a decrease in the activity of alkaline phosphatase in liver, increase in serum alkaline phosphatase activity and relative liver weight of animals on exposure to avicide 3-chloro p-toludine, profenophos, lindane and food additives respectively. Hence it may be concluded that the observed alteration in the activity of alkaline phosphatase enzyme in the liver of treated rats with food additives might be due to liver dysfunction and change in liver permeability.

## CONCLUSION

The present investigation indicates that Tartrazine induces liver dysfunction in rat leading to a significant increase in acid phosphatase activity and decrease in alkaline phosphatase activity that further affects the physiology of the animal.

## REFERENCES

1. Abou-Donia MB. Increased acid phosphatase activity in hens following an oral dose of leptophos. *Toxicology Letters*, 1978; 2(4): 199-203.
2. Bhatnagar P, Jain, N. Morphological changes in the liver of male mice after chronic treatment with phosphamidon. *Bull Environ Contam Toxicol*, 1986; 37: 767-773.
3. de Duve C, Wattiauz R, Baudhuin P. Distribution of enzymes between subcellular fractions in animal tissues. *Advances in Enzymol*, 1962; 24, 291.
4. de Lamirande G, Morias R, Blackstein M. Intra cellular distribution of 5'ribonuclease and 5'phosphodiesterase in rat liver. *Arch Biochem Biophys*, 1967; 118(2): 347-51.

5. Enan E, Berberian IG, El-Fiki S, El-Masry M, Enan OH. Effects of two organophorus insecticides on some biochemical constituents in the nervous system and liver of rabbits. *J Environ Sci Health*, 1987; 22(2): 149-170.
6. Gupta RC, Goad JT, Kadel WL. Carbofuran induced alteration (in vivo) in high energy phosphates, creatine kinase (CK) and CK isoenzymes. *Arch Toxicol*, 1991;65: 304-310.
7. Kaplan MM. Serum alkaline phosphatase another piece is added to the puzzle. *Hepatology*, 1986; 6: 526-531.
8. Keele CA, Neil E. Samson Wright's applied physiology. 12<sup>th</sup> ed., Oxford University Press. Fly House, London, 1971, 563.
9. Kiran R, Sharma M, Bansal RC. In vivo effect of carbaryl on some enzymes of rat liver, kidney and brain. *Pesticides*, 1985; 19(5): 42-43.
10. Kushwaha VB, Bharti G. Tartrazine induced changes in rats. *Trends in Biosciences*, 2013; 6(4): 366-369.
11. Kushwaha VB, Maurya S. Toxicity study of ethanolic extract of *Parthenium hysterophorus* in rats. *Trends in Biosciences*, 2010; 3(2): 216-219.
12. Kushwaha VB, Maurya S. Toxic effect of ethanolic extract of *Parthenium hysterophorus* on mammalian blood biochemistry. *J Env Bio Sc*, 2011; 25(2): 155-160.
13. Jervis LE, Johnson FR, Sheff MF, Smyth DH. (1956) The effect of phlorhizin on intestinal absorption and intestinal phosphatase. *J Physiol*, 1956; 134: 675-688
14. Moreno M, Pellicer S, Fernandez Otero MP. Linden treatment alter both intestinal mucosa composition and brush border enzymatic activity in chickens. *Pestic Biochem Physiol*, 1995; 52: 212-221.
15. Mull RL, Giri SN, Peoples SA. (1972). Effects of an acutely toxic dose of the avicide 3-chloro-p-toluidine in chickens. *Toxicol Appl Pharmacol*, 1972; 22: 458-464.
16. Novikoff AB. Lysosome and related particals in *The cell biochemistry, physiology, morphology* Vol.2, J. Brachet and A.E. Mirsky (eds.) Academic press, New York, 1961; 423-488.
17. Pilo B, Asnani MV, Shah RV. Studies on wound healing and repair in pigeon liver III. Histochemical studies on acid and alkaline phosphatase activity during the process. *Journal of Animal Morphology and Physiology*, 1972; 19: 205-212.
18. Ramalingam V, Vimaladevi V. Effect of mercuric chloride on membrane bound enzymes in rats testis. *Asian J Androl*, 2002; 4: 309-311.

19. Rosalki SB, McIntyre N. (1999). Biochemical investigations in the management of liver disease. Oxford text book of clinical hepatology, 2<sup>nd</sup> ed. New York; Oxford University press, 1999; 503-521.
20. Srivastava AK. Alkaline phosphatase and Glycogen in intestine of certain fresh water teleosts. *Curr Sci*, 1966; 35: 154-155.
21. Thapa BR, Walia Anuj. Liver function test and their interpretation. *Indian J Pediatr*, 2007; 74(7): 663-667.
22. Vorbrodt A. The role of phosphate in intracellular metabolism, *Postepy. Hig. Med. DOSW*, 1959; 13: 200-206.