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Hepato-biochemical changes under stress of Copper sulphate and Potassium dichromate in albino rats

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Abstract

Heavy metals contamination poses serious health threat to various life forms present on the earth. The condition is still more complex in case of trace heavy metals where it is very difficult to ascertain whether they are toxic or not. In order to find the answer, present study has been undertaken to assess the hepatotoxicity if any, of two vital trace heavy metals, copper and chromium. Both these trace heavy metals are vital to body in terms, are integral component of cellular structures as well as functions. Hepatotoxicity has been assessed on the basis of biochemical estimation of the levels of enzymes AST and ALT in the liver and serum of the experimental albino rats. Data so analyzed has been statistically analyzed using Student's 't' test followed by varied correlation analysis. Results revealed enhancement in the levels of these enzymes both in liver and serum of exposed albino rats compared to those of control groups. These values were highly correlated together. Both these experimental trace heavy metals are thus serious hepatotoxic compounds and can cause generalized hepatocellular damage extending upto altered gene expression.

Keywords: Trace heavy metals, copper, chromium, rats, liver, biochemistry.

Introduction

Heavy trace metals play an important role in human life, as an integral component of body structure and functions, as well as of various natural and manmade products. Excessive use of such heavy trace metals, probably as a consequence of enhanced anthropogenic activity, can induce toxicities at cellular, tissue and organ levels in various organisms(Mohammed *et al.*, 2014; Tiwari and Saxena, 2017; Gamakaranage, 2018; Tiwari *et al.*, 2020).

Copper and Chromium are among the trace heavy metals which have received considerable attention in recent past due to their biological functions and equal chances of non-target toxicity. Broad role in these two trace heavy metals in various biological reaction and structures as well as wide spread and indiscriminate use in variety of consumer products is continuously increasing their levels in the environment. Moreover the production of various metabolic forms from them, whenever such metals are exposed to environment can worsen the condition much. This fact makes it considerably important to assess their toxicity (Sinkovic *et al.*, 2008; Elshazly *et al.*, 2016; Tiwari *et al.*, 2019).

It is with this reason that present study has been undertaken to ascertain the hepatotoxic potential of both these trace heavy metals copper and chromium in the albino rats. Hepatotoxicity has been ascertained on the basis of estimation of important hepatocellular enzymes Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the liver and serum of experimental albino rats.

Materials and Methods

1. Experimental animal- Rearing and maintenance:

Forty five male albino rats, *Rattus norvegicus* (Berkenhout), weighing 110 ± 20 gm, eight weeks old, selected from an inbred colony were used in the present study. Experimental albino rats were kept under standard conditions of photoperiod, temperature, humidity, food and water. These animals were properly acclimatized to laboratory conditions for two weeks prior to the experimentation. These animals were then randomly divided into five sets each containing nine rats. Within each such set, three albino rats were kept as control animals, next three administrated copper sulpahte and left there potassium dichromate, as per the experimental protocol. These five sets were corresponding to acute (1day) and sub-acute (7, 14, 21 and 28 days) treatments respectively.

2. Experimental compounds:

Technical grade of Copper sulphate and Potassium dichromate (~95%purity) were obtained from Sigma chemicals Ltd., Mumbai. Their calculated LD_{50}came out to be 269.0 and 77.0 mg/kg b.wt. respectively (Finney, 1971; Tiwari *et al.*, 2020).

3. Dose administration and sample collection:

Both the experimental compounds were orally administrated to experimental albino rats. Controls were given distilled water only. 26.90 mg/kg b.wt. ($1/10^{th}$ of LD_{50}) of copper sulphate was orally administrated to each rat corresponding to the acute treatmentset. This dose was administrated for one day only. The albino rats corresponding to the sub-acute treatment groups were orally administrated doses of copper sulpahte at the rate of 26.90/7, 26.90/14, 26.90/21 and 26.90/28 mg/kg b.wt. for 7, 14, 21 and 28 days respectively.

Similarly,7.70 mg/kg b.wt. of potassium dichromate was orally administrated for one day only to the albino rats corresponding to acute set, whereas an amount of 7.70/7, 7.70/14, 7.70/21 and 7.70/28 mg/kg b.wt. for a period of 7, 14, 21 and 28 days respectively was administrated to sub-acute groups. Controls were run simultaneously.

These rats were then after, sacrificed at predetermined time intervals (*Vide supra*) blood was taken out from the ventricle of the heart through syringe, then put into test tubes and mixed with EDTA. It was kept for 30 min at room temperature and further was centrifuged at 3000 rpm for 20 min to separate out serum. The serum so obtained was processed for the biochemical estimation of AST and ALT (Reitman and Frankel, 1957; Bhushan *et al.*, 2013).

Statistical analysis

The numerical data so obtained was statistically analyzed for significance level if any through Student's 't' test followed by correlation analysis by SPSS 20 for windows (Fisher and Yates, 1950).

Results

Biochemical estimation of AST and ALT levels in the liver and serum of experimental albino rats sowed an increase following the intoxication of copper and chromium, both (Table 1-8). Correlation analysis was carried out between the serum values of AST and ALT and also between hepatic values of same enzymes separately. Hepatic and serum AST and ALT were also assessed for correlation between them within liver as well in serum and serum and liver collectively (Table 9-12). Correlation analysis showed a strong association between the values of both these enzymes together in the liver, the serum as well as liver and serum. Comparatively chromium has been found to alter these values more than that of copper.

Table 1: Hepatic AST following Copper sulphate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Copper sulphate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	24-30	28±2.0	27-34	31.33±2.18*
2	3	7	24-34	28.66±2.90	34-38	35.33±1.33*
3	3	14	26-32	30±2.0	37-40	38.0±1.0***
4	3	21	26-33	29.66±2.02	42-45	43.66±0.88***
5	3	28	23-38	29.66±4.40	54-66	60.66±3.53****

Table 2: Hepatic AST following Potassium dichromate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Potassium dichromate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	24-30	27.66±1.85	34-36	35.0±0.57**
2	3	7	24-34	28.66±2.91	38-39	38.33±0.33*
3	3	14	24-33	28.33±2.66	44-48	46.66±1.33***
4	3	21	26-33	29.66±2.03	50-55	52.66±01.45****
5	3	28	23-40	29.66±5.23	65-68	66.66±0.88****

Significance level: *: p>0.05, **: P<0.05, ***: p<0.01, ****: p<0.001

Table 3: Hepatic ALT following Copper sulphate intoxication

Sr no.	No. of rats	Treatment time(in days)	Test group			
			Control		Copper sulphate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	23-27	24.33±0.88	27-29	27.66±0.66**
2	3	7	23-25	24.0±0.57	28-37	33.66±2.84***
3	3	14	23-27	24.33±1.33	34-48	43.0±4.50****
4	3	21	24-26	24.66±0.66	34-67	55.0±10.5****
5	3	28	23-27	24.66±1.20	50-83	63.33±9.52****

Table 4: Hepatic ALT following Potassium dichromate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Potassium dichromate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	23-25	24±0.57	28-39	38.33±0.33***
2	3	7	24-27	25±1.00	35-54	43.6±5.54****
3	3	14	23-26	24.66±0.88	44-68	53.33±7.42****
4	3	21	22-32	24.0±1.00	52-78	65.33±7.51****
5	3	28	28-38	24.66±1.76	62-88	76.0±7.59****

Significance level: *: p>0.05, **: P<0.05, ***: p<0.01, ****: p<0.001

Table 5: Serum AST following Copper sulphate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Copper sulphate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	40-45	43.0±5.27	44-58	49.0±4.50*
2	3	7	41-45	43.33±1.20	48-60	53.33±3.52**
3	3	14	41-46	43.66±1.45	58-64	60.66±2.40****
4	3	21	41-45	43.33±1.44	58-78	66.0±6.11****
5	3	28	42-45	43.0±1.0	64-78	71.33±4.05****

Table 6: Serum AST following Potassium dichromate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Potassium dichromate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	40-48	43.33±2.40	49-55	52.0±1.73**
2	3	7	39-48	43.0±2.64	52-56	54.0±1.15**
3	3	14	40-44	42.0±1.20	52-66	58.0±4.16****
4	3	21	41-46	43.3±1.45	60-66	62.66±1.76****
5	3	28	40-44	42.33±1.20	56-70	64.66±4.37****

Significance level: *: p>0.05, **: P<0.05, ***: p<0.01, ****: p<0.001

Table 7: SerumALT following Copper sulphate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Copper sulphate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	43-45	44.0±0.57	46-49	46.66±0.33*
2	3	7	40-45	43.0±1.52	49-58	54.66±2.84**
3	3	14	43-44	43.0±0.33	52-66	60.33±4.25***
4	3	21	43-46	44.0±1.15	56-72	62.66±4.84****
5	3	28	43-45	44.0±0.57	60-88	75.0±8.14****

Table 8: Serum ALT following Potassium dichromate intoxication

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Control		Potassium dichromate intoxicated	
			Range	Mean ± SEm	Range	Mean ± SEm
1	3	1	43-45	44.0±0.57	48-54	49.66±1.66*
2	3	7	42-46	44.0±1.15	48-60	56.0±5.29**
3	3	14	42-44	43.0±0.57	60-78	68.0±5.29****
4	3	21	42-45	43.33±0.88	74-77	75.0±1.00****
5	3	28	42-44	44.33±1.20	70-90	82.66±0.33****

Significance level:*: p>0.05, **:P<0.05, ***:p<0.01, ****: p<0.001

Table 9: Correlation analysis between serum AST and ALT

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Copper sulphate intoxicated		Potassium dichromate intoxicated	
			Correlation	Test of significance	Correlation	Test of significance
1	3	1	0.95	P<0.01	0.92	P<0.05
2	3	7	0.81	P<0.05	0.50	P>0.05
3	3	14	0.99	P<0.001	0.99	P<0.001
4	3	21	0.99	P<0.001	0.94	P<0.01
5	3	28	0.99	P<0.001	0.99	P<0.001

Table10: Correlation analysis between hepatic AST and ALT

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Copper sulphate intoxicated		Potassium dichromate intoxicated	
			Correlation	Test of significance	Correlation	Test of significance
1	3	1	0.60	P<0.01	0.90	P<0.05
2	3	7	0.40	P>0.05	0.93	P<0.01
3	3	14	0.55	P<0.01	0.62	P>0.05
4	3	21	0.95	P<0.001	0.99	P<0.001
5	3	28	0.97	P<0.001	0.99	P<0.001

Table 11: Correlation analysis between hepatic and serum AST

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Copper sulphate intoxicated		Potassium dichromate intoxicated	
			Correlation	Test of significance	Correlation	Test of significance
1	3	1	0.65	P>0.05	0.50	P>0.05
2	3	7	0.94	P<0.01	0.86	P<0.05
3	3	14	0.69	P>0.05	0.72	P>0.05
4	3	21	0.98	P<0.001	0.95	P<0.01
5	3	28	0.96	P<0.01	0.97	P<0.001

Table12: Correlation analysis between hepatic and serum ALT

Sr no.	No. of rats	Treatment time (in days)	Test group			
			Copper sulphate intoxicated		Potassium dichromate intoxicated	
			Correlation	Test of significance	Correlation	Test of significance
1	3	1	0.99	P<0.001	0.67	P>0.05
2	3	7	0.97	P<0.001	0.99	P<0.001
3	3	14	0.99	P<0.001	0.99	P<0.001
4	3	21	0.30	P>0.05	0.84	P<0.05
5	3	28	0.99	P<0.001	0.85	P<0.01

Discussion

Trace heavy metals are an integral part of structural framework and metabolic pathways of cellular organisms. Altered levels of these metals inside the body of an organism can cause deleterious consequences. Liver is an important organ for metabolism, storage and transformation of xenobiotic substances (Bhushan *et al.,* 2010; Bhushan *et al.,* 2013; Tiwari *et al.,* 2019).

AST is a mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys.ALT whereas, is a cytosolic enzyme which is more specific for the liver. In the present investigation, marked elevation in AST and ALT in both the liver and serum of rat under stress of copper and chromium has been observed. Correlation studies reveal that there exists a positive correlation in between hepatic and serum ALT activity, which is found to be highly significant. Similar trend has been found to be true to AST under stress of copper and chromium. The reduction of copper (II) to copper (I) and chromium (VI) to chromium (III) has been noted to be involved in generation of reactive oxygen species (ROS) and reactive intermediates with ROS respectively. The increase in transaminase activity in the liver is indicative of the liver damage that must have occurred due to the formation of reactive oxygen species and reactive intermediates after absorption of copper and chromium in animals of respective groups. Thus ROS and related compounds must have interfered with the hepatocellular membranes and thereby causing considerable hepatic damage (Witmer *et al.,* 1994; Gaggelli *et al.,* 2002; Gazawat *et al.,* 2006; Tiwari *et al.,* 2019).

This increase in transaminase activity leads to cellular damage and releasing the enzyme in sinusoidal spaces to the intralobular vein. Further, increased ALP is an indicator of liver damage with hepatocellular lesions and parenchymal cell necrosis. Raised levels of these enzymes might have resulted from overexpression of these enzymes to reduce the copper or chromium induced oxidative stress respectively. Further, hepatocellular necrosis, breakdown of hepatocellular architecture and thereby leakage of the enzyme in the blood might be a possible reason for the enhanced level of these enzymes in the serum of intoxicated rats respectively (Tiwari, 2007; Balakrishnan *et al.,* 2013; Bhushan *et al.,* 2013; Bhushan *et al.,* 2013; Tiwari and Saxena, 2017; Tiwari *et al.,* 2019).

The greater toxicity of chromium can be assigned to the formation of comparatively stable covalent compounds with the hepatocytic macromolecules (Standeven and Wetterhahn, 1992; Ivanov *et al,* 2005; Tiwari *et al.,* 2019; Tiwari *et al.,* 2020).

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