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OXIDATIVE STRESS FOLLOWING INTOXICATION OF COPPER SULPHATE AND POTASSIUM DICHROMATE IN ALBINO RATS

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ABSTRACT

Trace heavy metals are integral components of body cells and responsible for various metabolic reactions. In addition, they are also a part of broad variety of consumer products. Any alteration in their level with in the body of an organism can prove fatal. It is thus very important to use and release them within appropriate limits. Present study deals with the toxicity assessment in terms of oxidative damage, of two broadly used trace heavy metals, copper and chromium. Albino rats have been selected as experimental animals in this study. Adenosine triphosphatase (ATPase), lipid peroxidase (LPO), Glutathione-s-transferase (GST) and metallothioneins have been selected as parameters to assess the oxidative damage. ATPase has been found to decrease biochemically within the liver and serum of intoxicated rats, where as other selected parameters showed an increasing pattern. This may be a consequence of the oxidative stress caused by these two trace heavy metals respectively. These biochemically analyzed numerical values were positively correlated together.

KEYWORDS: Trace heavy metals, copper, chromium, rats, liver, serum, oxidative stress, biochemistry.

INTRODUCTION

Toxicity of trace heavy metals has gained considerable attention in recent past. Trace heavy metals are an integral part of cellular structure and functions. In addition, these trace heavy metals are a part of variety of consumer products. Pertaining to their biological role, they are considered comparatively safe and harmless and are thus used and released indiscriminately in the environment. Such practices can result in their (*vide supra*) increased levels at various levels in different ecosystems and thus related toxicities. Further it is also important to have a differentiation of their safe and unsafe levels. (Sinkovic *et al.*, 2008; Elshazly *et al.*, 2016; Gamakaranage, 2018; Tiwari *et al.*, 2020; Tiwari *et al.*, 2020).

Copper and Chromium have been selected as experimental compounds in the present study for toxicity assessment. Both these trace heavy metals are a part of variety of consumer products, are responsible for variety of vital cellular metabolic reactions as well as structures. In addition, both these experimental compounds can exist in different valance states, when present naturally (Mohammed *et al.*, 2014; Tiwari and Saxena, 2017; Tiwari *et al.*, 2019).

Excessive use of these trace metals as well as indiscriminate release in the environment can disturb the normal tolerable levels of these metals in the environment and can thus be fatal for various life forms inhabiting this planet. Such imbalances can lead to variety of unknown and unrealized health impacts (Balakrishnan *et al.*, 2013; Tiwari, 2007).

Therefore, oxidative stress potential of these two trace heavy metals has been assessed in the present study through estimation of adenosine triphosphatase (ATPase) and lipid peroxidase (LPO) in liver and serum, whereas glutathione-S-transferese (GST) and metallotheoneins in liver of intoxicated rats.

MATERIAL AND METHODS

1. Experimental animal- Rearing and maintenance

Present study was conducted on forty five male albino rats, *Rattus norvegicus* (Berkenhout). These animals were 110 ± 20 gm in weight, eight weeks in age and selected from an inbred colony. Experimental animals were provided standard conditions of temperature, humidity, photoperiod, food and water. After proper acclimatization for a period of two weeks to the laboratory conditions, these animals were then randomly divided as per experimental protocol. This

experimentation included one acute set and four sub-acute sets. In acute set, total nine albino rats were kept. Three of them were orally administrated distilled water only and no any heavy metal externally. Other three were orally administrated calculated acute dose of copper sulphate for one day only, rest three potassium dichromate acute dose. Similarly nine rats each were selected for rest of the four groups corresponding to sub-acute (7, 14, 21, and 28 days) treatments and administrated experimental compounds accordingly.

2. Experimental compounds

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

3. Dose administration and sample collection

Control: Albino rats corresponding to control sets were administrated distilled water only. None of the experimental compound was given to them externally.

Copper sulphate treatment: Each albino rat corresponding to acute set was orally administrated 26.90 mg/kg b.wt. ($1/10^{th}$ of LD_{50}) of copper sulphate. The albino rats corresponding to the sub-acute treatment groups were orally administrated 26.90/7, 26.90/14, 26.90/21 and 26.90/28 mg/kg/day b.wt. of copper sulpahte for 7, 14, 21 and 28 days respectively.

Potassium dichromate treatment: 7.70 mg/kg b.wt. of potassium dichromate was orally administrated to albino rats corresponding to acute set. This dose was given only once and for a single day. 7.70/7, 7.70/14, 7.70/21 and 7.70/28 mg/kg/day b.wt. of potassium dichromate was administrated to albino rats corresponding to sub-acute groups for a period of 7, 14, 21 and 28 days respectively.

These rats were sacrificed at predetermined time intervals. Blood samples were collected and liver tissue was also excised out. Blood was immediately transferred to test tubes, mixed with EDTA. It was kept for 30 min at room temperature and centrifuged at 3000 rpm for 20 min to separate out serum. The serum so obtained was processed for the biochemical estimation of ATPase and LPO. Liver tissue on the other hand was immediately transferred into physiological saline (pH 7.4), cut into pieces and processed for biochemical analysis of ATPase, LPO, GST and metallothioneins (Seth and Tangari, 1966; Habig e*t al.*, 1974; Ohkawa *et al.*, 1976; Onosaka and Cherian, 1982; Bhushan *et al.*, 2013; Bhushan *et al.*, 2013).

Statistical analysis

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

RESULTS

Among various biochemical parameters selected and analyzed in the present study, lipid peroxidase revealed an increase in both the liver and serum of copper as well as chromium intoxicated rats. Similarly, values of GST and metallothioneins have also been found to increase biochemically in the liver of treated albino rats. However, there has been a significant decrease in the levels of ATPase enzyme activity, both in liver and serum of exposed groups in both the sets (Table 1-12). Correlation analysis was carried out between the serum and hepatic values of ATPase and LPO, hepatic and serum ATPase as well as LPO values separately (Table 13-16). Chromium has been found to alter these values comparatively more than that of copper.

Table 1: Hepatic ATPase (mg p/100g/hr) 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	1960-1982	1974±7.02	1964-1974	1970±3.05*
2	3	7	1959-1982	1973.66±6.06	1890-1920	1900±10.0***
3	3	14	1960-1986	1972±7.57	1940-1969	1959±9.50*
4	3	21	1961-1979	1972.33±5.69	1850-1880	1868.67±7. 7***
5	3	28	1962-1989	1973±8.18	1930-1970	1946.66±12.0**

Table 2: Hepatic ATPase (mg p/100g/hr) 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	1961-1980	1972.33±5.78	1780-1785	1782.7±1.45****
2	3	7	1962-1978	1972±5.03	1832-1834	1833.3±0.66****
3	3	14	1962-1979	1972.33±5.23	1848-1856	1853.3±2.66****
4	3	21	1958-1984	1972.66±7.68	1930-1960	1943.33±8.81*

5	3	28	1960-1986	1972±7.57	1930-1946	1935.33±5.3**

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

Table 3: Serum ATPase (mg p/100g/hr) 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	1966-1978	1972±3.46	1198-1208	1202±3.05****
2	3	7	1968-1977	1972.33±2.6	1200-1205	1202±1.45****
3	3	14	1969-1979	1973.33±2.96	1556-1562	1559.3±1.76****
4	3	21	1969-1978	1973±2.64	1738-1739	1738.3±0.33****
5	3	28	1971-1974	1972.33±0.88	1800-1802	1801±0.57***

Table 4: Serum ATPase (mg p/100g/hr) 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	1966-1977	1971.66±3.17	1292-1300	1297±2.51****
2	3	7	1969-1978	1973±2.64	1398-1406	1401±2.40****
3	3	14	1968-1978	1972.66±2.9	1502-1510	1505.6±2.33****
4	3	21	1969-1989	1976.66±6.22	1736-1757	1747±6.08****
5	3	28	1969-1977	1972.66±2.33	1800-1810	1807.7±1.45****

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

Table 5: Hepatic Lipid Peroxidase (IU/L) 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	28-29	28.33±0.33	28-34	31.33±1.76**
2	3	7	28-30	28.66±0.66	29-44	36.33±4.33****
3	3	14	27-29	28±0.57	38-50	42.66±3.71***
4	3	21	26-32	28.66±1.76	44-56	49.33±3.52*
5	3	28	27-32	29±1.53	42-56	50.66±4.37***

Table 6: Hepatic Lipid Peroxidase (IU/L) 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	28-32	29.66±1.20	32-55	44±6.65**
2	3	7	28-30	29±0.57	46-54	49.33±2.40***
3	3	14	29-30	29.33±0.33	49-56	53±2.08**
4	3	21	25-32	29.33±2.2	48-66	57.33±5.20***
5	3	28	28-32	29.66±1.20	54-64	60.66±3.33***

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

Table 7: Serum Lipid Peroxidase (IU/L) 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	27-29	28±0.57	36-44	39±2.66*
2	3	7	28-29	28.66±0.33	40-44	42±1.15*
3	3	14	26-32	29±1.73	42-48	44.66±1.76**
4	3	21	26-32	29.33±1.76	38-54	46±4.62***
5	3	28	26-30	28.66±1.33	44-58	50.33±4.1***

Table 8: Serum Lipid Peroxidase (IU/L) 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	26-32	29.33±1.76	44-58	48.66±4.66*
2	3	7	26-32	29.66±1.86	44-58	51.66±4.09***
3	3	14	28-32	29.66±0.88	48-66	54.33±5.84****
4	3	21	28-31	30±1.00	49-66	57±4.93***
5	3	28	27-32	29.66±1.45	43-69	59.33±4.91****

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

Table 9: Hepatic metallothioneins (IU/L) 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	5.6-5.9	5.76±0.08	7.8-7.9	7.80±0.03**
2	3	7	5.3-5.9	5.63±0.16	7.2-9.7	8.56±0.73**
3	3	14	5.4-5.8	5.62±0.11	7.5-14.0	11.8±2.05***
4	3	21	5.3-6.4	5.70±0.35	10-15.8	13.16±1.20****
5	3	28	5.4-6.3	5.76±0.22	15.7-27.6	21.86±3.44****

Table 10: Hepatic metallothioneins (IU/L) 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	5.4-5.9	5.69±0.14	8.6-13.0	10.73±1.15****
2	3	7	5.4-5.9	5.66±0.14	10.6-14.6	12.66±1.70****
3	3	14	5.5-5.9	5.69±0.11	12.0-27.4	18.3±4.66****
4	3	21	5.4-5.8	5.63±0.12	14.6-27.4	21.6±3.74****
5	3	28	5.4-5.8	5.66±0.08	18.4-31.1	25.7±3.78****

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

Table 11: Hepatic Glutathione-S-transferase (IU/L) 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	173-182	177±2.64	188-218	206.66±2.40*
2	3	7	175-182	177.66±2.18	208-248	222±13.01*
3	3	14	175-180	177±1.52	228-260	242.66±9.33***
4	3	21	175-180	177.33±1.45	224-280	254.66±16.38**
5	3	28	174-182	177.66±2.33	260-278	270±5.29****

Table 12: Hepatic Glutathione-S-transferase (IU/L) 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	173-188	178.66±4.70	185-198	191±3.78*
2	3	7	177-182	178.66±1.66	204-208	206±1.15****
3	3	14	169-192	179±6.80	206-244	223.33±11.09*
4	3	21	166-188	177±6.35	220-280	250±17.30**
5	3	28	176-178	177±0.57	244-294	264.66±15.1****

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

Table 13: Correlation analysis between serum ATPase and Lipid peroxidase.

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	0.59	P>0.05
2	3	7	0.94	P<0.01	0.84	P<0.05
3	3	14	0.92	P<0.01	0.94	P<0.01
4	3	21	0.86	P<0.05	0.98	P<0.001
5	3	28	0.35	P>0.05	0.68	P>0.05

Table 14: Correlation analysis between hepatic ATPase and Lipid peroxidase.

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	0.98	P<0.001	0.99	P<0.001
2	3	7	0.88	P<0.05	0.69	P>0.05
3	3	14	0.65	P>0.05	0.96	P<0.01
4	3	21	0.83	P<0.05	0.96	P<0.001
5	3	28	0.78	P>0.05	0.50	P>0.05

Table 15: Correlation analysis between hepatic and serum ATPase.

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	0.79	P>0.05	0.87	P<0.05
2	3	7	0.99	P<0.001	0.59	P>0.05
3	3	14	0.95	P<0.01	0.79	P>0.05
4	3	21	0.60	P>0.05	0.40	P>0.05
5	3	28	0.72	P>0.05	0.29	P>0.05

Table 16: Correlation analysis between hepatic and serum Lipid peroxidase.

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	0.82	P<0.05	0.82	P<0.05
2	3	7	0.99	P<0.001	0.90	P<0.05
3	3	14	0.98	P<0.001	0.75	P>0.05
4	3	21	0.98	P<0.001	0.85	P<0.05
5	3	28	0.85	P<0.05	0.78	P>0.05

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; Tiwari *et al.*, 2019).

ATPase is a class of transmembrane enzymes that catalyzes the decomposition of adenosine triphosphate into adenosine diphosphate and a free phosphate ion. This dephosphorylation reaction releases energy, which the enzyme harnesses to drive other chemical reactions that would not otherwise occur. In the present investigation, lowering of ATPase activity has been found following intoxication of either of the copper sulphate and potassium dichromate. The decrease in the activity of ATPase may be due to the binding of both metals to the sulphahydryl groups of the ATPase leading to membrane disorganization (Zhang *et al.*, 1990; Zimmerman, 1996; Rosenweig, 2002).

Lipid peroxidase is the enzyme that brings about lipid peroxidation process. Lipid peroxidation causes serious membrane damage and therefore leads to cell death. An increasing trend has been noted in the activity of lipid peroxidase under stress of copper and chromium both in the liver and serum. Further, correlation between lipid peroxidase and ATPase activity in the liver of intoxicated rats has been found to be positive and highly significant. The increased lipid peroxidase might have resulted in membrane breakdown through formation of free radicals. These free radicals in turn must have damaged the cellular membranes resulting in decreased levels of ATPase (Abe *et al.*, 2000; Banu and Sharma, 2005; El-Ashmawy *et al.*, 2006).

Glutathione-s-transferases (GST) forms a family of cytosolic, mitochondrial and microsomal proteins. GST is capable of multiple reactions with a multitude of substrates, both endogenous and xenobiotic. This class of enzyme is associated with phase-II of xenobiotic biotransformation. There has been an increase in the levels of this enzyme following heavy trace metal intoxication, probably due to their overexpression as they play an important role in the internal defense mechanism of the hepatocytes (Talalay *et al*, 2000; Sidhu *et al.*, 2004; Xu *et al.*, 2005).

Metallothioneins are the major intracellular proteins that have a profound role in metal detoxification. In the present investigation, a highly significant increase in the biochemical level of hepatic metallothioneins has been demonstrated under stress of both copper and chromium. This increase may be a consequence of their overexpression pertaining to their role in metal biotransformation (Florianczyk *et al.*, 2003; Rombach *et al.*, 2003; Das *et al.*, 2006).

Both copper and chromium are capable of transformation under various environmental and *in vivo* conditions, as well as formation of free radicals. In this study, chromium has been found to be at edge over copper in terms of toxicity. This difference in the toxicity of two trace heavy metals may be probably due to formation of much toxic metabolic intermediates and free radicals in case of chromium compared to copper (Tiwari and Saxena, 2017; Tiwari *et al.*, 2019; Tiwari *et al.*, 2020; Tiwari *et al.*, 2020).

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