

Impact of acute iron and copper intoxication on biochemical liver function in Wistar rat

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Abstract: Various indispensable metabolic processes are reliant on minute amounts of essential trace elements i.e., Fe and Cu and on Fe-Cu association as well to maintain normal health. An excess of both elements can render abnormality in liver function. The aim of this work was to evaluate the acute hepatotoxicity of Fe & Cu by analyzing the biochemical functions of liver. Adult male Wistar rats (200g) were divided in three experimental groups (n=3) against a control group (G-I; 60mg/kg FeSO₄; G-II: 10mg/kg CuSO₄; & G-III: 30mg/kg FeSO₄ and 5mg/kg CuSO₄ while control group: distilled water). Doses were administered via intra-peritoneal route and animals were sacrificed after 24h. Blood samples were collected and processed to isolate serum. Serum samples were analyzed for liver function tests to determine ALT, AST, ALP, albumin, total protein and total bilirubin. Experimental groups showed statistically significant elevation in ALT, AST and ALP activity, Total bilirubin concentration and a significant decline in albumin and total protein concentration, as compared to the control. We can conclude that acute exposure to Fe & Cu and their co-administration caused noticeable alterations in liver biochemical functions, thereby signifying as indicators for the severe hepatotoxicity during acute Fe & Cu overload conditions.

Keywords: Copper, Iron, ALT, AST.

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INTRODUCTION

Various indispensable metabolic processes are reliant on minute amounts of essential trace elements i.e., iron (Fe) and copper (Cu) and on Fe-Cu association as well to maintain normal health. As liver holds central importance in both Fe and Cu homeostasis, therefore their exceeding quantities can render abnormality in liver function. As iron and copper ions are being essential for electron transport reactions^{1,2} but their unbound forms direct the generation of reactive oxygen species through Fenton and/or Haber-Weiss reactions³, which in turn causes oxidative damage to biomolecules^{3,4}, including cleavage of DNA and RNA molecules, direct oxidation of proteins and lipid peroxidation in membranes⁵.

Besides the pro-oxidant property of copper ions⁶, they also possess ability for the non-specific and irreversible binding to thiol proteins; ultimately modifying their functions⁷⁻⁹ and do variations in numerous hepatic enzymes³. On the other hand iron ions do have pro-oxidant activity¹⁰⁻¹² but their property of non-specific binding to thiol proteins are not fully acknowledged³.

The aim of this work was to evaluate the acute hepatotoxicity of Fe & Cu by analyzing the biochemical functions of liver.

MATERIALS AND METHODS

Adult male Wistar Rats (200g) were used and three experimental groups (n=3) were established against a control group i.e., Group-I: 60mg/kg FeSO₄; -II: 10mg/kg CuSO₄; and -III: 30mg/kg

FeSO₄ and 5mg/kg CuSO₄ while control group (Con) received only distilled water. Doses were administered via intra-peritoneal route and animals were sacrificed after 24 hours. Blood samples were left at room temperature for 2 hours in serum tubes. The serum was separated by centrifuging the blood samples for twenty minutes at 4000rpm. Sera samples were collected in the sterilized and labeled eppendorfs and were analyzed for liver function tests (ALT, AST, ALP, Albumin, Total protein and Bilirubin total) through using DiaSys Kits as per manufacturer's instructions.

One-way ANOVA with Tukey's post-test was performed for data analysis using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

RESULTS AND DISCUSSION

Significant changes in the liver biochemistry were recorded in response to the iron and copper intoxication (Table 1). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were significantly increased in the experimental groups as compared to the control group, (P<0.0001 and P=0.0012 respectively; Figure 1). In a study of acute iron intoxication, serum ALT and AST were found to be significantly higher¹³. High levels of ALT have been reported in the liver, while the heart, kidney, skeletal muscle, lung, spleen and pancreas have lower levels of ALT. Clinically, liver diseases relevant to some extent of hepatic necrosis including cirrhosis, toxic or viral hepatitis, carcinoma and obstructive jaundice are characterized by augmented

ALT levels. AST is found in higher amounts in the liver, heart, kidney, skeletal muscles and erythrocytes and any pathological conditions pertaining to these tissues such as liver necrosis, cirrhosis, myocardial infarction, viral hepatitis and muscular dystrophy are responsible for elevated levels of serum AST¹⁴. These transaminases are found within the hepatocyte cytosol and the mitochondria. Any injury or metabolic disturbance modifies the membrane permeability of hepatocytes; resulting in the efflux of these enzymes. In acute conditions, their plasma activity rises to an extent, indicating the amount of affected hepatocytes¹⁵.

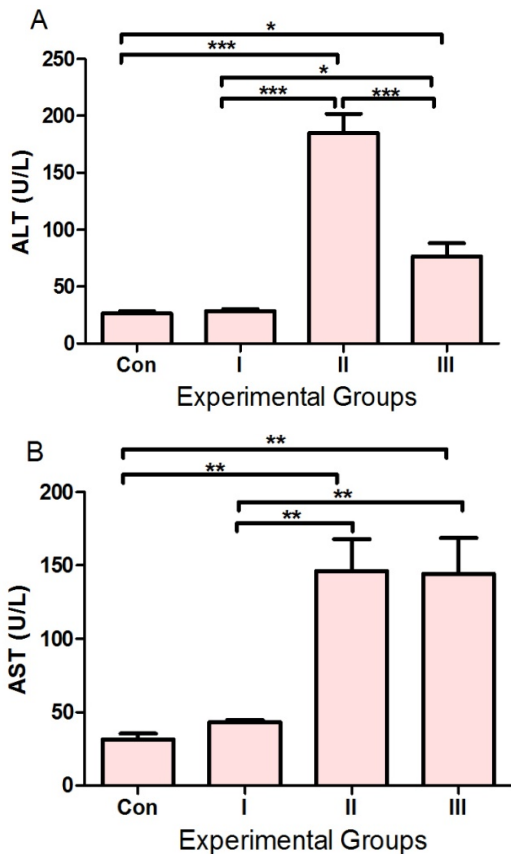


Figure 1: Serum ALT (A) and AST (B) activity (U/L) in the experimental animals as compared to control animals [I, FeSO₄ treatment (60mg/kg); II, CuSO₄ treatment (10mg/kg); III, FeSO₄ (30mg/kg) & CuSO₄ (5mg/kg) treatment for 24 hours; (Con, control)].

Serum alkaline phosphatase (ALP) activity was significantly elevated in the experimental groups in comparison to the control group, ($P=0.0001$; Figure 2A). In normal sera, ALP originates from the liver and biliary tract. ALP test is required for the diagnostic evaluation of obstructive jaundice and cholestasis and its augmented levels are associated

with diseases such as malignancy, hepatitis, cirrhosis and bone diseases^{16,17}. The initial increased ALP activity reflects the release of cell membrane fragments in plasma as a result of injury or damage, and subsequent elevation in its activity is due to local cholestasis pertinent to hepatic damage¹⁸.

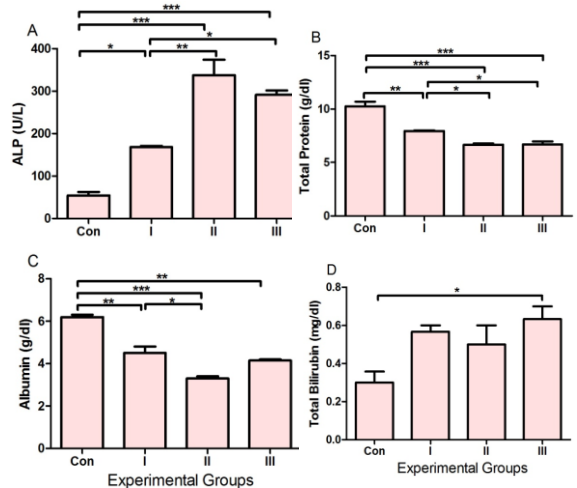


Figure 2: Serum ALP activity (A), total protein (B), albumin (C) and total bilirubin (D) in the experimental animals as compared to control animals [I, FeSO₄ treatment (60mg/kg); II, CuSO₄ treatment (10mg/kg); III, FeSO₄ (30mg/kg) & CuSO₄ (5mg/kg) treatment for 24 hours; (Con, control)].

Statistically significant decline in the serum total protein concentration and albumin concentration was observed in the experimental groups, ($P=0.0002$ and 0.0011 respectively, Figure 2B, 2C) when compared with control. Liver mainly synthesizes plasma proteins; responsible for maintaining water and acid-base balance between blood and tissues. Hypoproteinaemia is a consequence of dietary protein deficiency and due to pathological situations including bleeding, nephritic syndrome and salt retention¹⁹. In normal individuals, albumin forms the major portion of the serum and is involved in binding and solubilizing many compounds including bilirubin and calcium. Hypoalbuminaemia is associated with many diseases such as liver diseases, kidney diseases, cancer, severe burns, infections and some genetic abnormalities²⁰.

Total bilirubin levels revealed the increasing trend in the experimental groups while the elevation in group III was only statistically significant as compared to the control ($P=0.0245$; Figure 2D). In reticuloendothelial system, mainly the spleen, involves in breaking down the RBCs that reach at their end of circulating life and the resultant non-iron-bound haem forms the bilirubin²¹. The main

reasons behind the bilirubinaemia involve the bile duct obstruction, hepatitis, cirrhosis and deficiencies of some enzymes. Elevation in bilirubin can be caused by haemolytic disorders or liver injury at periportal sites involving degenerative cell swelling with disturbance of cell membranes integrity, ultimately resulting in bile pigment retention in circulation²².

CONCLUSION

It is deduced from the above investigations that acute exposure to Fe and Cu and their co-administration caused noticeable alterations in liver biochemical functions, thereby signifying as

indicators for the severe hepatotoxicity during acute Fe & Cu overload conditions.

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Table 1: Serum biochemistry: Results indicate mean value $\pm$ S.E.M. (Level of Significance $P < 0.05^*$; 0.01^{**} ; 0.001^{***} analyzed by one-way ANOVA with post-hoc Tukey test).

Groups	ALT(U/l)		AST (U/l)		ALP (U/l)	
	$\bar{x} \pm s.e.m$	Significance	$\bar{x} \pm s.e.m$	Significance	$\bar{x} \pm s.e.m$	Significance
Con	37.67 $\pm$ 1.20		38.00 $\pm$ 3.21		54.00 $\pm$ 8.50	
G-I	28.67 $\pm$ 1.66		29.67 $\pm$ 4.09		168.0 $\pm$ 3.00	a*
G-II	185.0 $\pm$ 17.0	a***,b***	146.3 $\pm$ 21.65	a**,b**	337.3 $\pm$ 36.59	a***,b**
G-III	76.50 $\pm$ 11.5	a*,b*,c***	144.3 $\pm$ 24.54	a**,b**	291.0 $\pm$ 10.69	a***,b*
Groups	Albumin (g/dl)		Total Protein (g/dl)		Bilirubin Total (mg/dl)	
	$\bar{x} \pm s.e.m$	Significance	$\bar{x} \pm s.e.m$	Significance	$\bar{x} \pm s.e.m$	Significance
Con	6.20 $\pm$ 0.10		10.25 $\pm$ 0.45		0.30 $\pm$ 0.57	
G-I	4.50 $\pm$ 0.30	a**	7.95 $\pm$ 0.05	a**	0.56 $\pm$ 0.03	
G-II	3.30 $\pm$ 0.10	a***,b*	6.66 $\pm$ 0.12	a***,b*	0.50 $\pm$ 0.10	
G-III	4.15 $\pm$ 0.05	a**	6.70 $\pm$ 0.26	a***,b*	0.63 $\pm$ 0.06	a*

a: significance level when compared to control. b: significance level when compared to Group I. c: significance level when compared to Group II.

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