ORIGINAL ARTICLE

ROLE OF EMBLICA OFFICINALIS IN COPPER INDUCED LIVER TOXICITY IN ADULT ALBINO RATS

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ABSTRACT

Background: Copper (Cu) is an important heavy metal used widely in all industries. However, its toxicity on various organs is established. *Emblica officinalis*, commonly known as Amla, is a potential antioxidant and can prevent Cu induced hepatotoxicity.

Objective: To evaluate the effects of *Emblica officinalis* extract on liver histology and Alanine Aminotransferase (ALT) levels after Cu induced toxicity in adult albino rats.

Methods: This was an experimental study of 28 days duration. 36 adult male albino rats were divided into three groups of twelve animals each. Group A was control group in which rats were given 1.5 ml normal saline while group B rats were given $CuSO_4$ 200mg/kg b.w./day. Rats in Group C were given both $CuSO_4$ (200mg/kg b.w/day) and *Emblica officinalis* fruit extract (300mg/kg bw/day). The agents were fed once daily via orogastric tube for four weeks and rats were sacrificed 24 hours after administration of last dose.

Results: Cu treated rats showed necrosis of hepatocytes and Increased serum ALT levels. *Emblica officinalis* co-treatment attenuated Cu-induced hepatic necrosis and variation in ALT levels. The difference among the groups was statistically significant with p-value less than 0.05.

Conclusion: *Emblica officinalis* fruit extract improves liver function in Cu induced hepatotoxicity by limiting oxidative damage.

Key words: Copper, Hepatotoxicity, Emblica officinalis, Adult albino rats, ALT.

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INTRODUCTION

Copper (Cu) is an essential trace metal required for normal metabolism of iron, synthesis of collagen and elastin, melanin production and proper functioning of nervous system and immune system.[1] It is one of the most used metals in electro-mechanical, agriculture, cosmetic and pharmaceutical industries. Recent advances in commercial and industrial usage of copper have led to contamination of dietary sources with copper.[2]

Copper induced cellular injury is mediated by free radical formation (ROS) and oxidative stress.[3] Cu also decreases the levels of Glutathione (GSH) and increases Nitrous oxide (NO) levels.[4] ROS along with decreased GSH levels cause lipid peroxidation and protein modification. Consequently, many degenerative diseases including malignancies, cardiovascular disease, metabolic syndrome, nervous disorders and chronic inflammatory processes can develop.[5] The main organs affected by copper are liver, kidneys and brain.[3]

Copper is a known hepatotoxic agent and exerts its effect by oxidative damage to cells. Local and international studies on human populations have linked increased amount of serum copper to hepatitis and chronic liver disease.[6] Excess copper leads to damage of membrane lipids by formation of per-oxy radicals and also causes peroxidation of the hepatic lysosomal membranes.[7] It also diminishes the activity of

cytochrome c oxidase and catalase.[8] It also impairs liver mitochondrial respiration.[9]

Emblica officinalis is found all over South Asia and is locally known as Amla. Traditional healers have used different parts of this plant as anti-pyretic, anti-inflammatory and revitalizing agents as well as in gastro-protective preparations and as diuretic. It is used to cure a number of diseases including anemia, sun stroke, jaundice, diabetes, leprosy and renal diseases.[10] It is also used as a tonic for healthy reproductive system and to treat female reproductive diseases. Its beneficial effects on brain, kidney and heart are now scientifically proven.[11]

Emblica officinalis is rich in anti-oxidants like polyphenols, flavonoids, tannins, and ascorbic acid.[12] These prevent tissue damage by inhibiting lipid peroxidation and scavenging free radicals, ROS and NO in a dose dependent manner. Amla also increases naturally occurring anti-oxidant enzymes including superoxide dismutase, GSH, catalase, GSH transferase and GSH reductase.[13] The protective effects of *Emblica officinalis* against metals like lead, cadmium and mercury have been reported in literature.[14]

Emblica officinalis exerts its hepatoprotective action by reducing free radical induced damage and by regulation of the expressions of various proteins including beclin-1, liver cytochrome P450, inducible nitric oxide synthase as well as Bcl-2 and Bcl-2 associated X protein. It also decreases the level of hepatic lipid peroxides.[15] Hepatoprotective effects of *Emblica officinalis* have been reported against alcohol, iron, arsenic and carbon tetrachloride (CCl_4) induced injuries[16] It prevents necrosis, vacuolization of hepatocytes and fatty changes in liver. It also attenuates derangement in levels of ALT, AST, total protein and albumin-globulin ratio.[17]

As there is a relative deficiency of hepatoprotective drugs, this study was conducted to assess the role of *Emblica officinalis* as a hepatoprotective agent against liver toxicity in general and in copper mediated liver toxicity specifically.

METHODS

This experimental study was carried out in Anatomy Department, SZPGMI, Lahore in collaboration with Animal house, Anatomy Department, Punjab PGMI, Lahore. 36 Healthy, adult male rats of 3-4 months of age with average weight of 190-220g were procured from University of Veterinary and Animal Sciences, Lahore. Rats showing lethargy, decreased appetite and sleep time, sneezing, nasal or eye discharge, breathing problems and unexplained bleeding were excluded from the study. After 1 week of acclimatization, rats were labelled and placed in three separate cages (A, B, C). They were kept in animal house of PGMI following ethical considerations.

The agents were fed to the rats by orogastric tube for 28 days. Group A rats were given 1.5ml Normal saline. Copper Sulphate was administered in a dose of 200 mg/kg once daily to group B and C and *Emblica officinalis* extract was given in dose of 300 mg/kg once daily to group C.

Blood samples were taken from the tail vein of the rats for ALT levels of all three groups before the start of experiment and also 24 hours after the last dose of the agents.

SPSS 22 software was used to analyze the obtained data. The data for hepatic necrosis is stated by using frequency and percentages in each group. Comparison among groups was made by using Chi-square test. p-value of ≤ 0.05 was considered significant. ALT levels is presented as Mean ± S.D. Group wise comparison was performed by using One-way ANOVA. For post hoc analysis Tukey's test was used where required.

RESULTS

Focal necrosis of hepatocytes was observed in 8 (66.7%) rats from group B (Cu treated). The necrotic area showed collapsed stroma with cellular lysis, absent or pyknotic nuclei and lymphocytic infiltrate (Figure 1). Necrosis of hepatocytes was absent in 10 (83.3%) rats of group C (Cu+EO treated) and in all rats of control group A (Figure 2).

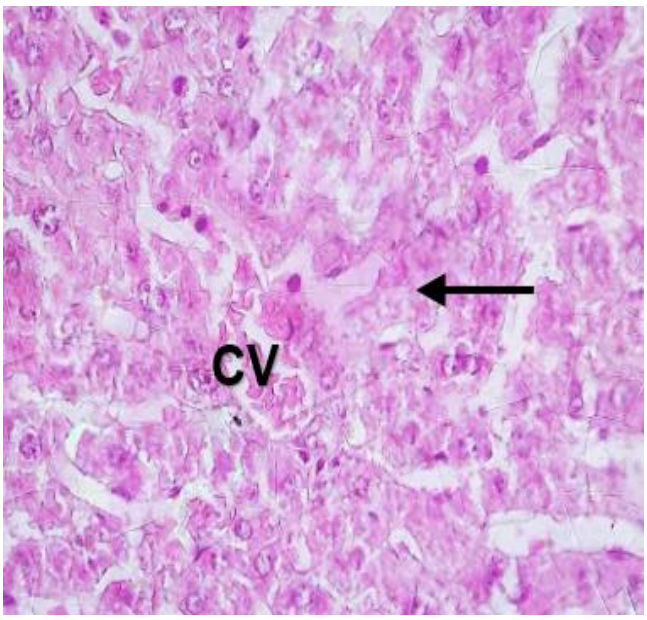

Figure 1: Photomicrograph of liver of adult albino rat of group B (Cu treated) showing areas of necrosis (black arrows) around central vein (CV). (H&E stain, 40X).

Fisher's exact test showed that there was an association between necrosis of hepatocytes and groups. The p-value among groups was statistically significant with value <0.001 (Table 1).

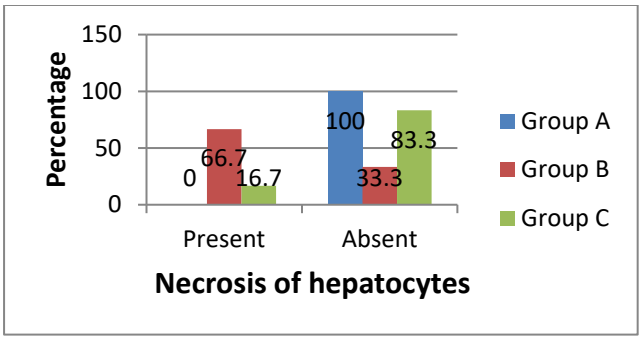

Figure 2: Bar chart showing distribution of necrosis of hepatocytes among groups.

Table 1: Necrosis of hepatocytes in control and experimental groups.

	Necrosis of hepatocytes		Total
	Present	Absent	
Group A	0 (0.0%)	12 (100.0%)	12 (100.0%)
Group B	8 (66.7%)	4 (33.3%)	12 (100.0%)
Group C	2 (16.7%)	10 (83.3%)	12 (100.0%)
p-value		0.001*	

*Statistically significant difference (p-value < 0.05)

The ALT levels at the start of experiment were measured and recorded. The mean ALT levels in group A (Control), B (Cu treated) and C (Cu+EO treated) were 31.92 ± 1.97 u/l, 31.58± 1.56 u/l and 30.92± 2.27 u/l respectively. No significant difference was found in mean ALT levels among groups on one-way ANOVA test. The mean ALT levels at the end of experiment in group A (Control) was 31.8± 2.25 u/l. The mean ALT levels in group B (Cu treated) and C (Cu+EO treated) were 48.9± 5.28 u/l and 32.9 ± 2.07 u/l respectively. One-way ANOVA test was applied to compare the ALT levels among groups. It was found that there was statistically significant difference in mean ALT levels at the end of experiment among the groups with p-value less than 0.001 (Table 2).

Table 2: Comparison of ALT levels (u/l) at the end of experiment among groups by using One Way ANOVA.

	Sum of Squares	**Df**	**Mean Square**	**F**	**p-value**
Between Groups	2196.056	2	1098.028	88.486	< 0.001*
Within Groups	409.500	33	12.409		
Total	2605.556	35			

* Statistically significant p-value (≤ 0.05)

For multiple comparisons, post hoc Tukey test was used which showed that an ALT level in group B was significantly higher as compared to group A and C (p-value < 0.001). However, no significant difference was found in ALT levels between group A and C (p- value = 0.734).

DISCUSSION

The present study was designed to assess the ameliorative role of *Emblica officinalis* extract on copper induced hepatotoxicity in adult albino male rats. Liver was selected as the organ of study as it is the main site of metabolization for both Cu and *Emblica officinalis*.

The livers of rats in Cu treated group (group B) were grossly enlarged and congested as compared to Control (group A) and Cu+EO treated group (group C). Multiple hemorrhagic spots were noted in the surface of liver along with irregular contour of liver borders. These gross changes can be attributed to alteration in normal liver functioning due to copper toxicity.

Fatty degeneration and vacuolization were also noted in hepatocytes of rats from group B (Cu treated). Yousif et al explained that these vacuolizations appeared because of accumulation of free fatty acids in hepatocytes coupled with the inability of liver to utilize or excrete these fatty acids. Also, due to failure of protein synthesizing machinery, liver cannot couple fatty acids with proteins to make lipoproteins which are the mean by which liver excretes fatty acids.[1]

Minimal alterations were present in hepatocytes of rats of Cu+EO treated group C. This can be attributed to anti-oxidant and anti-inflammatory potential of *Emblica officinalis* as reported by Yadav.[17] Protective effects of *Emblica officinalis* were also reported by Reddy et al against alcohol induced hepatotoxicity. They suggested that the near normal appearance of hepatocytes was due to protection against ROS induced damage.[18]

In the current research, the gross and microscopic observations in various study groups correlated with the liver function tests biochemically which confirms that Cu is hepatotoxic while *Emblica officinalis* acts as a potent hepatoprotective agent. Copper overload leads to alterations in liver functions which are manifested clinically by deranged liver function tests.

The biochemical analysis of the liver was done by analyzing the ALT levels before the start of experiment and at the end of experiment. No statistical difference was found between the levels of ALT at the start of experiment (p-value 0.454). However, at the end of experiment the mean ALT level in group B (Cu treated) was 48.9 u/l which was significantly raised as compared to that in group A (Control) and group C (Cu+EO treated). Cu destabilizes the hepatocyte plasma membranes leading to leakage of liver enzymes in blood.[19] Kumar et al reported increased serum bilirubin, AST and ALT levels in Cu treated rats.[20]

Emblica officinalis exerts its hepatoprotective effects by restoring the hepatocyte membrane and thereby preventing leakage of liver enzymes.[21] Similar

mitigating effects of *Emblica officinalis* on ALT levels have been reported by Deori et al and Chaphalkar et al against carbon tetrachloride induced and ethanol induced hepatic damage.[21, 22]

CONCLUSION

The current research establishes the ameliorative role of *Emblica officinalis* against copper induced hepatotoxicity in rats. It may be used as a hepatoprotective agent to prevent hepatic damage from environmental toxins and infectious agents. Its use may also be recommended in patients suffering from liver damage as it is cheap, easily available, and is a safe dietary supplement.

Ethical Approval: *Submitted*
Conflict of Interest: *Authors declare no conflict of interest.*
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