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Emblica officinalis Reduces Copper Mediated Inflammation And Preserves Liver Morphology InThe Murine Model

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ABSTRACT

Introduction: Copper (Cu) is a metal widely used in agriculture and in industries. Its hepatotoxicity is established in literature. *Emblica officinalis,* locally known as Amla, has many beneficial health effects and is scientifically reported to be a powerful antioxidant.

Aim & Objective: To determine the role of *Emblica officinalis* fruit in reducing Cu induced inflammation and distortion of hepatic lobules of adult albino rats.

Place and duration of Study: The study was done in the Department of Anatomy, Shaikh Zayed Medical College, Lahore for a period of 4 weeks.

Material & Methods: 36 Adult male albino rats were equally divided into three groups (A, B, C) by randomization and acclimatized for 1 week. 1.5 ml of normal saline was given to rats of Group A (control) and $CuSO_4$ (200mg/kg b.w./day) was given to those in group B (Cu treated) once daily via orogastric tube. Rats in group C (Cu+EO treated) were given both $CuSO_4$ (200mg/kg bw) and *Emblica officinalis* fruit extract (300mg/kg bw) once daily by orogastric intubation. The animals were sacrificed after 28 days and livers were dissected out for histological study. Data was entered and analyzed using SPSS version 22.

Results: Cu treated rats developed significant distortion of liver architecture and inflammation while *Emblica officinalis* co-treatment lead to decreased inflammation with preservation of shape of hepatic lobule. These findings were further confirmed by difference in diameters of hepatic lobules between control, Cu-, Cu+EO- treated groups.

Conclusion: *Emblica officinalis,* by limiting oxidative damage, ameliorates hepatic inflammation and preserves liver architecture. Therefore, its use as a hepatoprotective agent should be encouraged.

Keywords: *Emblica officinalis*, Copper, Inflammation, Liver fibrosis, Hepatoprotective.

INTRODUCTION

Recently liver disease has become major cause of morbidity and mortality worldwide. WHO survey claims liver cirrhosis to be the fifteenth major cause of death in adults worldwide with a prevalence of 1.5billion cases worldwide. In South-East Asian region, liver cirrhosis stands as tenth major cause of death[1].There are a variety of factors leading to liver toxicity and cirrhosis. These include hereditary factors, environmental toxins and micro-organisms. Exposure to oxidant species such as Copper (Cu) is one such factor. Increased Serum Cu levels have been reported in chronic cases of viral hepatitis, Wilson's and Non-Wilson's copper toxicosis in multiple national and international studies[2].

Considered to be the first metal used by man, today Cu is one of the most commonly used commercial metals[3]and has found extensive application in agriculture, cosmetics, architecture, plumbing, electric and telecommunication industries[4]. These advances in commercial and industrial usage of copper have led to contamination of water sources with copper. Increased amount of copper is found in drinking water coming from copper pipes or from water treated with algaecides[5]. Discharge of industrial effluents into water streams also increases copper in water[6].

Multiple studies have revealed increased copper content in vegetables grown on sewerage water and in those where copper-based pesticides and insecticides have been used. The highest concentration is found in green leafy vegetables and liver meat[7]. Copper bioaccumulation has been reported in several fish species as well[8].

Several international studies have found that copper concentration in multivitamins exceeds the permissible limit with few samples containing as much as 10 times the allowed limit of copper[9]. It is also a documented contaminant of herbal medicines and medicinal plants and has been found in slimming products as well[10].

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[11]. Excess copper leads to damage of membrane lipids by formation

of per-oxy radicals and also causes peroxidation of the hepatic lysosomal membranes[12].It decreases GSH levels and diminishes the activity of cytochrome C oxidase and catalase and impairs liver mitochondrial respiration[13].

Emblica officinalis is commonly known as Amla or Indian gooseberry. It is grown in tropical countries of the Asian continent including China, India, Indonesia, Sri Lanka and Pakistan. It is claimed to be the best source of vitamin C and contains 30 times the amount of ascorbic acid found in oranges. It has found extensive use in Ayurveda, Unani, Arabic and Tibetan medicine and is used since ancient times as a liver, skin and hair tonic[14]. It is also used locally in the form of powder, pickles, chutneys and murabba (jams).

Emblica officinalis is a rich source of many anti-oxidants. The tannins (Emblicanin A and Emblicanin B) and ascorbic acid present in it act as potent anti-oxidants and prevent tissue damage by inhibiting lipid peroxidation. They also augment antioxidant system and scavenge free radicals, ROS and NO in a dose dependent manner[15]. Amla extract increases naturally occurring anti-oxidant enzymes including superoxide dismutase, GSH, catalase, GSH transferase and GSH reductase[16].The ascorbic acid, phenols and flavonoids present in *Emblica officinalis* also act as metal chelators. The protective effects of *Emblica officinalis* against metals like lead, cadmium and mercury have been reported in literature[17].

Emblica officinalis is claimed to restrict inflammation by preventing immune cells infiltration at the site of inflammation and by reducing the formation of several chemokines and NO. It also promotes the formation of immuno-protective cytokines to speed the repair process [18]. A study conducted on murine model showed that concurrent administration of *Emblica officinalis* extract with arsenic decreased the levels of TNF-α, IL-1β and IL-6 in serum with recovery in B and T lymphocyte populations. There was also reduced arsenic burden in lymphoid organs like spleen and thymus which can be attributed to metal chelation properties of *Emblica officinalis*[19]. It is also reported to decrease acute and chronic inflammation by decreasing edema and limiting the formation of granulomas in mice models[20].

Emblica officinalis is reported to protect human liver from different environmental toxins such as heavy metals and alcohol by antagonizing apoptosis and lipid peroxidation[21]. It was found to be effective against ethanol induced liver damage and non-alcoholic steatohepatitis (NASH) in murine models[22]. Its fruit extract when given in a dose of 200mg/ kg/ day for 1 week to rats was found to be protective against carbon tetrachloride induced liver damage. It prevented necrosis, vacuolization of hepatocytes and fatty changes in liver. It also attenuated derangement in levels of ALT, AST, total protein and albumin-globulin ratio[23]. Similar hepatoprotective effects of *Emblica officinalis* were reported in Sulphur dioxide[24], Sodium arsenite[25] and Iron induced hepatic injury in rats[26].Since there is lack of hepatoprotective drugs, therefore, this study is designed to establish role of *Emblica officinalis* as a hepatoprotective agent both in copper induced liver toxicity and in general toxic insults to liver. To determine the role of *Emblica officinalis* fruit in reducing Cu induced inflammation and distortion of hepatic lobules of adult albino rats.

MATERIAL AND METHODS

This study was a randomized animal trial done in Department of Anatomy, Shaikh Zayed Medical College vide IRB F-39 /185/Acad/1417 dated 9-8-2016. Initial sample size of 4 rats in each group was estimated by using 95% confidence level and 95% power using Power & Precision 3.0 software. The sample size was increased to 12 rats per group so that the parameters under observation could be compared with good power of study. 36 adult male albino rats (2-4 months of age) with average weight of 250-300g were marked and divided in three groups at random after 1 week of acclimatization. They were kept in three separate cages labelled A, B and C. Rats showing signs of any ailment (lethargy, decrease in appetite and sleep time, sneezing, nasal or eye discharge, breathing problems and unexplained bleeding) were excluded from the study. Extract from fresh fruits of *Emblica officinalis* was made in PCSIR laboratory, Lahore. 20g of this extract was dissolved in 500 ml of normal saline to make extract solution.

Group A (Control):

The rats of this group were fed 1.5ml saline once daily by orogastric tube for 4 weeks.

Group B (Cu treated):

Animals of this group were fed 1.5ml of Copper sulphate stock solution once daily by orogastric tube for 4 weeks.

Group C (Cu and *Emblica officinalis* treated):

Rats of this group were fed by orogastric tube with 1.5ml of Copper sulphate solution once daily. After 8 hours a single dose of 1.5 ml *Emblica officinalis* solution was given via orogastric tube.The animals were fed with Cu and *Emblica officinalis* extract for 4 weeks. 24-hours after the last dose, they were euthanized. A midline incision was made from chin to groin. This incision was extended laterally to

expose the liver. Liver was taken out by dissecting the falciform ligament and hepatic vessels. Livers were washed with saline after isolation and were placed in labelled jars containing 10% Formaldehyde solution for fixation. After tissue processing, paraffin blocks of liver were made. Each block was tagged with the name of respective animal. 5 µm thick sections were cut by a rotatory microtome and stained with H & E stain for detailed histological evaluation. Presence of multiple mononuclear cells like neutrophils and lymphocytes within the liver parenchyma and portal triad was labelled as inflammation.

Statistical Analysis:

SPSS 22 software was used to analyze the obtained data. The data for qualitative parameter such as shape of hepatic lobule and presence or absence of inflammation is stated by using frequency and percentages in each group. Comparison of diameter of hepatic lobules among groups was made by using one way ANOVA and post-hoc Tukey test. P-value of ≤0.05 was considered significant.

RESULTS

1. Shape of Hepatic Lobule:

Shape of hepatic lobules of all rats in group A (Control) was normal. The lobules were polygonal in shape with a central centrilobular vein. 5 to 6 Portal triads were visible in periphery of each lobule. In group B (Cu treated), all rats had hypertrophy and distortion of hepatic lobules whereas in group C (Cu+EO treated), 10 (83.3%) rats had normal and 2 (16.7%) had enlarged and distorted hepatic lobules (Fig-1,Fig-2,Fig-3,Fig-5, Fig-6). Hypertrophy of lobules was assessed by calculating number of hepatic lobules/ central veins in one HPF and was later confirmed by micrometry. Fisher's exact test showed that there was an association between shape of hepatic lobule and groups.(Table-1)

	Shape of hepatic lobule		Total
	Normal	Hypertrophy	
Group A	12 (100.0%)	0 (0.0%)	12 (100.0%)
Group B	0 (0.0%)	12 (100.0%)	12 (100.0%)
Group C	10 (83.3%)	2 (16.7%)	12 (100.0%)
p-value	**< 0.001***		

Table-1: Distribution of Shape of Hepatic Lobule in Control and Experimental Groups.

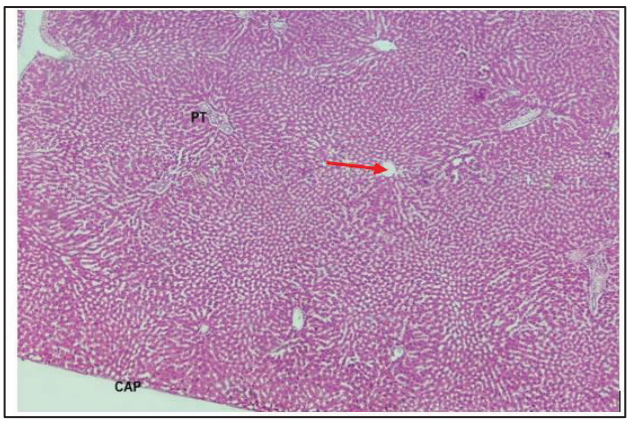

Fig-1: Photomicrograph of the liver of adult albino rat from Control group showing normal polygonal hepatic lobule. Capsule (Cap), portal triad (PT), central vein (red arrow) are also visible (H & E stain, 40X).

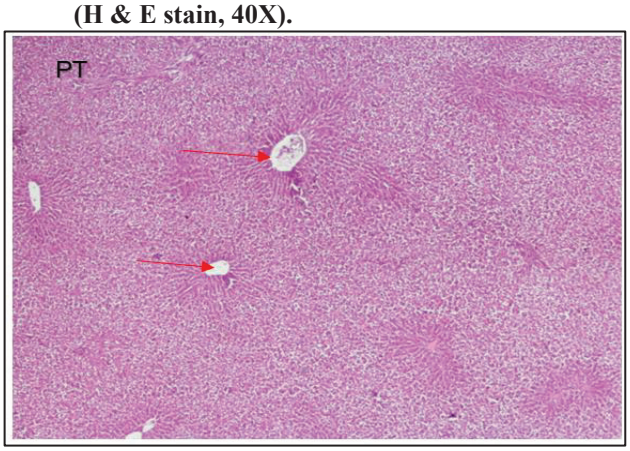

Fig-2: Photomicrograph of liver of adult albino rat of group B (Cu treated) showing disrupted margins of hepatic lobule, dilated central vein (red arrow) and portal triad (PT). (H&E stain, 40X).

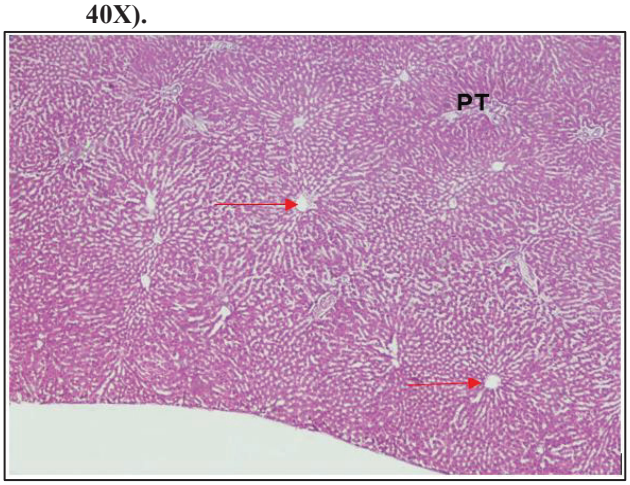

Fig-3: Photomicrograph of liver of adult albino rat of group C (Cu+EO treated) showing normal polygonal lobule architecture. Portal triad (PT) and central vein (red arrow) are also visible. (H&E stain, 40X).

2. Inflammation in portal triad:

Presence of inflammation in portal triad was confirmed by presence of mononuclear infiltrate. Fig-4 shows the percentage of presence or absence of inflammation in group A, B and C.

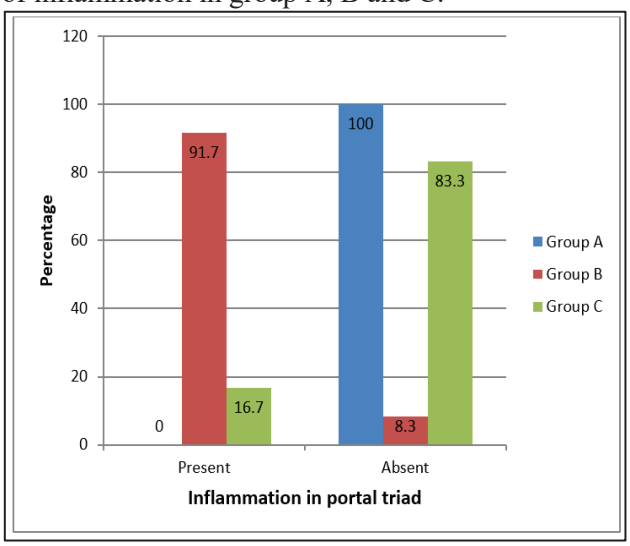

Fig-4: Bar chart showing inflammation in portal triad among groups.

Fisher's exact test showed that there was an association between inflammation in portal triadand groups with p-value <0.001 (Table-2).

	Inflammation in Portal Triad		Total
	Present	Absent	
Group A	0 (0.0%)	12 (100.0%)	12 (100.0%)
Group B	11 (91.7%)	1 (8.3%)	12 (100.0%)
Group C	2 (16.7 %)	10 (83.3%)	12 (100.0%)
p-value	< 0.001*		

Table-2: Inflammation in portal triad in control and experimental groups.

*Statistically significant difference (p-value < 0.05)

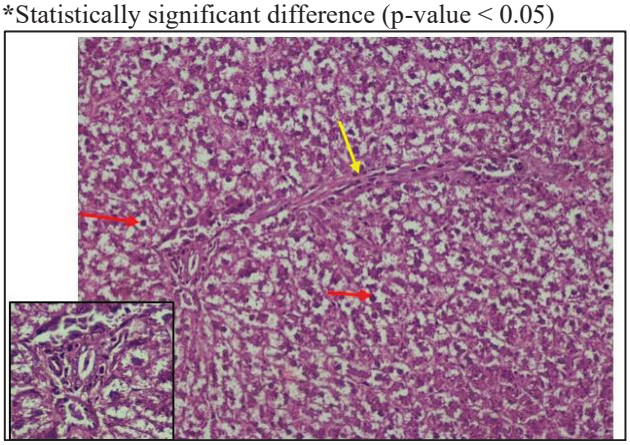

Fig-5: Photomicrograph of adult albino rat of group B showing lymphocytes (red arrow) and fibrosis (yellow arrow) in portal triad. Inset shows lymphocytic infiltration in portal triad (H&E stain, 400X).

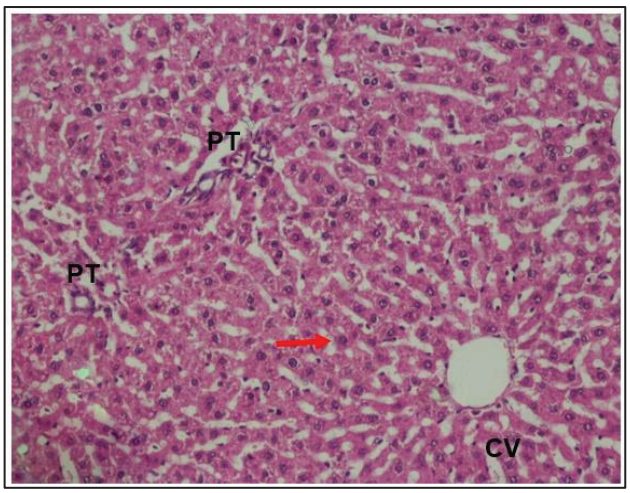

Fig-6: Photomicrograph of adult albino rat of group C showing normal portal triad (PT). Hepatocytes with rounded central nuclei (red arrow), hepatic sinusoids (blue arrow) and centrilobular vein (CV) are also visible. (H&E stain, 400X).

3. Diameter of hepatic lobule (µm):

The diameter of hepatic lobule in all groups was measured using ocular micrometer. The mean diameter of hepatic lobule in each group are given in Table-3

Group	Diameter of hepatic lobule (µm)			
	Mean	± SD	Minimum	Maximum
A	688.8	± 22.80	662	726
B	957.5	± 26.71	918	1012
C	691.5	± 9.568	676	710

Table-3: Diameter of hepatic lobule (µm) in control and experimental groups.

One-way ANOVA test was used to compare the mean diameter of hepatic lobule among groups which shows significant p value of <0.001. For multiple comparisons, post hoc Tukey test was used which showed that mean diameter of hepatic lobule in group B was significantly higher as compared to group A and C. However, no significant difference was found in the mean diameter of hepatic lobule between groups A and C (Table-4).

Multiple Comparison						
	S. No.	Groups	Groups	Mean Difference (I-J)	Std. Error	p-value
Diameter of hepatic lobule (µm)	1	A	B	-268.8	8.580	< 0.001*
			C	-2.750	8.580	0.945+
	2	B	C	266.1	8.580	< 0.001*

Table-4: Pair wise comparison of diameter of hepatic lobule (µm) among groups.

+ Statistically insignificant difference (p value > 0.05)

*Statistically significant difference (p value < 0.05)

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[27] and *Emblica officinalis*. In previous studies the protective role of *Emblica officinalis* on various hepatotoxic agents including heavy metals, alcohol and insecticides has been studied, but no research has yet been done on its protective role against Cu toxicity.

The hepatic lobules of rats treated with Cu (group B) appeared grossly enlarged and their normal polygonal structure was distorted. This can be attributed to hyperemia due to inflammation[12] as congestion was noted in hepatic sinusoids, central veins and in portal triads of rats in group B. Lymphocytic infiltration was also observed in portal triads of Cu treated rats (Fig-5). El-hak et al reported similar finding due to Cu toxicity which they attributed to Cu induced oxidative stress that leads to disturbance of normal liver architecture, congestion in vessels and hepatic sinusoids as well as inflammation and ultimately fibrosis in hepatic lobules[27].These findings were absent in animals of Control group and Cu+EO treated group (group A and group C). Amelioration of Cu toxicity by *Emblica officinalis* can be explained by the fact that it prevents oxidative stress. Restoration and protection of liver parenchyma by *Emblica officinalis* against carbon tetrachloride, ethanol and iron induced hepatotoxicity has been reported[25,26].

Micrometric studies of the hepatic lobules of rats showed statistically significant difference among groups (p-value <0.001, Table-4). This gross difference in diameter between groups can be explained by hypertrophy of hepatocytes coupled with congestion in sinusoids and mononuclear infiltration in Cu treated group as was reported by Ghinomiet al[28].

Limitations: This study is based on the murine model and may not be wholly applied to humans. The study could be of longer duration to ascertain chronic aspects of Cu toxicity.

CONCLUSION

The current research establishes the ameliorative role of *Emblica officinalis* against copper induced hepatotoxicity in rats. Its use may also be recommended in patients suffering from liver inflammation as it is cheap, easily available and is a safe dietary supplement.

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