AMELIORATING POTENTIAL OF CURCUMIN AND ASCORBIC ACID AGAINST HEPATOTOXICITY CAUSED BY SYNERGISTIC EFFECT OF HEAVY METALS IN MALE ALBINO RATS

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ABSTRACT

The ameliorative role of combination of curcumin and ascorbic acid (low and high doses) was determined against biochemical and histopathological changes in liver caused due to synergistic effects of heavy metals (As, Cd and Pb) present above permissible limits in drinking water for 60 and 90 days in 84 male albino rats. The histology of liver in rats treated with a mixture of low as well as high doses of heavy metals showed mild alternations after 60 days and intense damage after 90 days. The content of biochemical parameters like proteins and lipids were significantly decreased and cholesterol, phospholipids and fatty acids were significantly increased after 60 and 90 days of treatment. The rats treated with curcumin and ascorbic acid showed restoration of histological damage and content of biochemical components indicated amelioration of hepatotoxicity effectively up to 60/90 days depending upon the dose of heavy metals, curcumin and ascorbic acid.

Key words: Ascorbic acid, amelioration, curcumin, hepatotoxicity, arsenic, cadmium, lead, histopathology, proteins, lipids, liver

Heavy metal pollution is increasing day by day around the world and toxicity caused due to their non-biodegradable, biological accumulation and carcinogenic nature poses a significant threat to human health (Ahmadijokani et al., 2022). India is facing a major problem of heavy metals in the groundwater and heavy metal toxicity was reported in groundwater of various states of India such as Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal. The main source of Arsenic (As) exposure to 100 million people is through drinking water (Nordstrom et al., 2010). Cadmium (Cd) is a toxic heavy metal and its biological half-life is more than 20 years (Johri et al., 2010). Cd toxicity inhibits the liver metabolic enzyme systems containing sulphhydril groups and rupture of oxidative phosphorylation in mitochondria, which results in increased lipid peroxidation, hepatic congestion, ischemia, and hypoxia (Habeebu et al., 1998). Lead (Pb) poisoning is the most widely studied occupational and environmental hazards. The uptake of Pb decreases the intelligence level, destroys emotional stability and also causes hearing disabilities in children (Ibrahim et al., 2011). Hepatotoxicity refers to toxicity of the liver resulting from various chemicals and xenobiotics including heavy metals and their metabolites (Navarro et al., 2006). The effect of long-term exposure to a mixture of heavy metals like As, Cd and Pb at low doses produced hepatotoxic effects in albino rats (Bhattacharjee et al., 2016).

Curcumin has long been recognized for its pharmaceutical properties and efficacy as a food colorant (El-Desoky et al., 2021) and serves as anti-inflammatory and anti-tumorous agent. It ameliorates oxidative stress and inhibits the generation of Reactive Oxygen Species (ROS) both in vivo and in vitro (Eybl et al., 2006). It was used for the cure of oxidative stress and liver disease caused by Pb (Abdel-Moneim et al., 2015). Ascorbic acid, also known as vitamin C has a therapeutic role in the protection against heavy metals due to its antioxidant properties (Shaukat et al., 2018). The objective of the present study was to determine the changes in histopathology and biochemical components in the liver of male albino rats caused due to combination of heavy metals i.e. As, Cd, Pb at doses higher than the Maximum Permissible Limit of Bureau of Indian Standards (BIS) in drinking water for duration of 60 and 90 days and the possibility of combination of curcumin and ascorbic acid in different doses for protective effect against these changes.

MATERIALS AND METHODS

A total of 84 sexually mature male albino rats weighing 100-150 gm were procured from Disease
Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar after clearing the proposal through the Institutional Animal Ethics Committee (IAEC). The Institutional Animal Ethics Committee (IAEC) vide letter no IAEC/2019/104-123 dated 15/07/2019 approved this experimental protocol. The Institutional Animal Ethics Committee (IAEC) vide letter no IAEC/2019/104-123 dated 15/07/2019 approved this experimental protocol. The rats were given 5-fold and 10-fold concentrations of maximum permissible limit of As(0.05 mg/ l), Cd (0.003 mg/ l) and Pb (0.01 mg/ l) as recommended by the Bureau of Indian Standards (BIS) in a mixture through drinking water. The rats were provided with As in the form of sodium arsenite, Cd in the form of cadmium chloride, and Pb in the form of lead acetate. The curcumin and ascorbic acid were given to rats by oral gavage one hour before exposure to heavy metals. The rats were treated for a minimum of 60 days (42 rats- Group 1a to VIIa) and a maximum of 90 days (42 rats- Group Ib –Group VIIb). The grouping and treatment of rats were done as follows with six rats in each group:

Group Ia/ Group Ib: 0.25mg/ l of As+ 0.015mg/ l of Cd+0.05mg/ l of Pb (LDHM/ 5-fold)
Group Iia/ Group IIb: 0.50mg / l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb (HDHM/ 10-fold)
Group IIIa/Group IIIb: 0.25mg/ l of As+0.015mg/ l of Cd+0.05mg/ l of Pb + 200 mg of curcumin and 100 mg of ascorbic acid /kg of Body weight (LDHM+ high dose C +A)
Group IVA/Group IVb: 0.25mg/ l of As+0.015mg/ l of Cd+0.05mg/ l of Pb + 100mg of curcumin and 50 mg of ascorbic acid /kg of Body weight (LDHM +low dose C +A)
Group Vb/Group Vb: 0.50mg/ l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb +200 mg of curcumin and 100 mg of ascorbic acid /kg of Body weight (HDHM + high dose C +A)
Group VIa/VIIb: 0. 50mg/ l of As+0. 03 mg/ l of Cd+0.10 mg/ l of Pb +100 mg of curcumin and 50 mg of ascorbic acid /kg of Body weight (HDHM + low dose C +A)

Group VIIa/Group VIIb: Control

At the end of 60 and 90 days of treatment, the rats were dissected with cervical dislocation, the liver was taken and processed for histological studies by a method of Luna (1968). The photographs of histological slides were obtained under an optical light microscope (Magnus) under 100X and 400X magnifications with the help of Magvision software. The liver tissue was homogenized and the tissue homogenate was centrifuged at 5000 r.p.m. for about 20 minutes and the supernatant was collected and stored in a deep freezer for biochemical estimations. The different biochemical parameters like proteins (Lowry et al., 1951), lipids (Folch et al., 1957), phospholipids (Ames, 1966), cholesterol (Zlatkins et al., 1968), and fatty acids (Lowry et al., 1976) were analysed quantitatively in homogenates of liver tissues. The comparison for different parameters of between the treated rats and control rats was done using SPSS one way ANOVA. Comparisons were made for treated rats at 60 and 90 days by using Tukey’s method.

RESULTS AND DISCUSSION

The liver of control rats at both 60 and 90 days of treatment showed radially arranged hepatic cords with hepatocytes organized into cords separated by sinusoids (Figs. 1A, 2A). The histological structure of the liver showed various alternations in heavy metal treated groups. LDHM was found to cause dilation of the central vein after 60 days (Fig. 1B) and dilation of central vein, degeneration of hepatocytes, and vacuolisation after 90 days (Fig. 2B). HDHM treated group showed an irregular arrangement of hepatic cords after 60 days along with dilation of central vein and infiltration of leukocytes in central vein after 90 days (Figs. 1C, 2C). LDHM + low dose curcumin and ascorbic acid treated group showed degeneration of hepatocytes, dilation of central vein and dilation of sinusoidal space after 60 days (Fig. 1D) and infiltration of leukocytes in central vein and necrosis of hepatocytes after 90 days (Fig. 2D). LDHM + high dose curcumin and ascorbic acid showed restored central vein and hepatocytes after 60 days (Fig. 1E) along with reduced sinusoidal space restored hepatic cords after 90 days (Fig. 2E). HDHM+low dose curcumin and ascorbic acid showed degeneration of hepatocytes in hepatic cords as well as infiltration by the large mass of leucocytic inflammatory cells after 60 days of treatment (Fig. 1F) and irregular arrangement of hepatocytes and necrosis after 90 days of treatment (Fig. 2F). HDHM+low dose curcumin and ascorbic acid showed infiltration of leukocytes in central vein after 60 days of treatment (Fig. 1G) and irregular arrangement of hepatocytes and necrosis after 90 days of treatment (Fig. 2G). The histological damage was observed in the liver of rats treated with a mixture of low and high doses of heavy metals and this damage was more prominent in 90 days as compared to 60 days. These histological changes were restored in rats treated with a mixture of heavy metals and a combination of curcumin and ascorbic acid in a dose and time-dependent manner. Histopathological changes occurred in liver due to
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Fig. 1. T.S. of Liver (X100) of rats after 60 days of treatment (A) Control showing Hepatocytes (HC) in radially arranged hepatic cords (RHC) separated by sinusoids (S) (B) LDHM showing dilation of central vein (DCV) after 60 days of treatment. (C) HDHM showing irregular arrangement of hepatic cords (IHC) after 60 days of treatment. (D) LDHM+ low dose C+A showing dilation of central vein (DCV), degeneration of hepatocytes (DHC) and dilation of sinusoid spaces (DS) (E) LDHM+ high dose C+A showing restored central vein (RCV) (F) HDHM+ low dose C+A showing degeneration of hepatocytes (DHC) in hepatic cords and infiltration by large mass of leucocytic inflammatory cells (LIC) (G) HDHM+ high dose C+A showing infiltration of leukocytes in central vein (ILC).
Fig. 2. T.S. of Liver (X100) of rats after 90 days of treatment (A) control rat showing Hepatocytes (HC) in radially arranged hepatic cords (RHC) separated by sinusoids (S) (B) LDHM showing dilation of central vein (DCV), degeneration of hepatocytes (DHC) and vacuolization (V) (C) HDHM showing infiltration of leukocytes in central vein (ILC) and necrosis of hepatocytes (N) (D) LDHM+ low dose C+A showing restored central vein (RCV), reduced sinusoidal space (RS) and restored hepatic cords (RHC) (E) LDHM+ high dose C+A showing irregular arrangement of hepatic cords (IHC), dilation of central vein (DCV) and infiltration of leukocytes in central vein (ILC) (F) HDHM+ low dose C+A showing irregular arrangement of hepatocytes (IHC) and necrosis (N) (G) HDHM+ high dose C+A showing restored central vein (RCV), restored hepatocytes (RHC) in hepatic cords and reduced the dilation of sinusoids (RSD)
As toxicity i.e. moderate sinusoidal dilatation, focal necrosis, and these abnormalities in rat liver were cured by co-treatment with curcumin (Mathews et al., 2012). Pb toxicity caused the necrosis of hepatocytes and leucocytic infiltration of hepatic cells in liver of rats (Mehana et al., 2012). The chronic low dose exposure to a mixture of heavy metals i.e. As, Cd, and Pb caused histopathological changes in rat liver i.e. vacuolation in some hepatocytes, fatty degeneration in other hepatocytes, and congestion within central veins and some sinusoids (Bhattacharjee et al., 2016). Liu et al. (2023) also reported histopathological and cytotoxicity caused by different heavy metals (cadmium (Cd), arsenic (As), lead (Pb), and nickel (Ni) contributed to the detoxification ability of curcumin against heavy metals. The content of protein (60 days) and lipid (60 and 90 days) in liver of rats was observed to be significantly low in LDHM, HDHM, HDHM+ high dose C+A and HDHM + low dose C+A as compared to control rats (Table 1). After 90 days of treatment, the protein content in liver of rats was observed to be significantly less in LDHM, HDHM, LDHM + low dose C+A, HDHM+ high dose C+A, and HDHM + low dose C+A as compared to control rats. The non-significant variation in protein and lipid content of liver of LDHM + high dose C+A and LDHM + low dose C+A as compared to control rats indicated the amelioration of hepatotoxicity caused due to low dose of heavy metals at 60 days. However, at 90 days only LDHM + high dose C+A caused amelioration of heavy metals toxicity in protein content. The significantly less content of protein in all the treated groups and that of lipid in LDHM+ low dose C+A, LDHM + high dose C+A treated rats was observed in 90 days as compared to 60 days of treatment. The combination of heavy metals (As, Cd and Pb) leads to overproduction of free radicals which caused damage to protein and lipids by causing oxidation of proteins and peroxidation of membrane lipids (Martemucci et al., 2022). The free radical scavenging properties of antioxidants like curcumin and ascorbic acid prevent the overproduction of free radicals thus protecting the proteins and lipids (Sadighara et al., 2023). The mixture of As, Cd and Pb significantly lowered levels of protein and albumin in liver as compared to control rats (Bhattacharjee et al., 2016). Pb-treated rats showed the suppression of total protein content in liver and curcumin retorted it to normal level as compared to control (Abdelhamid et al., 2020). The liver plays a central role in lipid metabolism, serving as the center for lipoprotein uptake, formation and export to circulation. Alterations in hepatic lipid

### Table 1: Biochemical contents in liver of rats treated with heavy metals, curcumin and ascorbic acid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (µg/g)</th>
<th>Lipids (µg/g)</th>
<th>Cholesterol (mg/g)</th>
<th>Phospholipids (µg/ml)</th>
<th>Fatty acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 Days</td>
<td>60 Days</td>
<td>90 Days</td>
<td>60 Days</td>
<td>90 Days</td>
<td>60 Days</td>
</tr>
<tr>
<td>LDHM</td>
<td>103.05±</td>
<td>18.73±</td>
<td>57.19±</td>
<td>18.05±</td>
<td>57.19±</td>
</tr>
<tr>
<td>HDHM</td>
<td>18.05±</td>
<td>18.05±</td>
<td>18.05±</td>
<td>18.05±</td>
<td>18.05±</td>
</tr>
<tr>
<td>LDHM + high dose C+A</td>
<td>139.57±</td>
<td>21.12±</td>
<td>142.26±</td>
<td>23.43±</td>
<td>106.41±</td>
</tr>
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<td>142.26±</td>
<td>23.43±</td>
<td>106.41±</td>
</tr>
</tbody>
</table>

Values Mean ± SE (n=3 from pooled samples of 6 rats); *Significant at p≤0.05 as compared to control; Values with different superscripts (a,b) in a column represents significant difference for 60 and 90 days (p≤0.05).
metabolism could contribute to the development of chronic liver disease (Waghe et al., 2017). The total lipids were elevated in As treated rats as compared to control rats and after the treatment of curcumin with As showed the normal level of total lipids as that of control rats (Mann et al., 2005).

After 60 days of treatment, cholesterol in liver of rats was observed to be significantly high in LDHM, HDHM as compared to control rats. The non-significant variation in cholesterol of liver of LDHM + high dose C+A, LDHM + low dose C+A, HDHM + low dose C+A, HDHM+ high dose C+A treated rats as compared to control rats indicated the ameliorative potential of curcumin and ascorbic acid for hepatotoxicity caused due to low dose of heavy metals at 60 days. However, at 90 days no amelioration of heavy metals toxicity was observed. Cholesterol level in liver was significantly increased in LDHM, HDHM, LDHM + high dose C+A, HDHM + low dose C+A, HDHM + high dose C+A in 90 days of treatment as compared to 60 days of treatment. One study reported high cholesterol levels in liver of male Wistar albino rats treated with heavy metal mixture (20mg/kg Pb, 1.61mg/kg Cd, 0.40mg/kg HgCl₂) (Ayanwu et al., 2020). The cholesterol level was high in male rats treated with a sub-acute heavy metal mixture of Pb (60mg/kg) and Cd(2mg/kg) (Abdulidha et al., 2020). This effect may be due to the increased disturbance of lipid metabolism after the accumulation of heavy metals in the liver. Pb increased the total cholesterol level in liver by inhibiting the activity of hepatic lipoprotein lipase enzyme and the treatment with curcumin + Pb reduced the total cholesterol level in liver tissue to reach the normal range of control rats (Abdelhamid et al., 2020). Buhari et al. (2020) reported that an increase in total cholesterol was observed in Pb, Hg and Cd toxicity in blood of US population. Significant increase in total cholesterol, triglycerides and LDH concentrations upon treatment with buprofezin (BPFN) dose and were restored to normal concentrations after treatment with Vitamin C and curcumin suplementation (Sadia and Qureshi, 2022). After 60 days and 90 days of treatment, the content of phospholipids was found to be significantly increased in liver of all the treated rats and fatty acids content was found to be significantly high in liver of LDHM, HDHM, LDHM +low dose C+A, HDHM + high dose C+A treated rats as compared to control rats. There was no amelioration by curcumin and ascorbic acid in the phospholipid content of liver after 60 and 90 days of heavy metal treatment whereas non-significant variation observed in LDHM + high dose C+A as compared to control indicated the amelioration by curcumin and ascorbic acid of fatty acid content in hepatotoxicity for 60 and 90 days of treatment. Phospholipids significantly increased in liver of LDHM, HDHM, HDHM + high dose C+A an HDHM +low dose C+A treated rats in 90 days as compared to 60 days of treatment. However, there was non-significant variation in the fatty acid content of liver in 90 days as compared to 60 days of treatment A significant increase in phospholipids is in accordance with various earlier toxicity studies caused by different heavy metals, 100 ppm of Cd exposure to rats significantly increased the phospholipid content in liver as compared to control rats (Afolabi et al., 2012). The triglycerides could be taken by the cells in the form of free fatty acids there was an increase in the level of free fatty acids in the liver due to liver problems (Arain et al., 2017). The free fatty acid content was increased with As treatment in drinking water in male albino rats as compared to control rats (Afolabi et al., 2015). The triglycerides/ free fatty acids could be managed by curcumin or ascorbic acid supplementation (Abdel-Moneim et al., 2015).

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AUTHOR CONTRIBUTION STATEMENT

NK designed research. RK conducted experiments. RK, NK and PS wrote the manuscript. NK and PS read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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