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Potential protective role of curcumin powder to regulate arsenic-induced hepatorenal toxicity and hyperlipidemic metabolic dysfunction in rat model

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Abstract: The present work was conceptualized to determine the potential protective effects of curcumin on arsenic-induced kidney damage in male albino rat model. Thirty six male albino rats were selected, weighed about 175±10g and classified into four groups (9 rats in each group) such as C group (control with basal diet), Cur group (curcumin 200mg/kg body weight), AI group (arsenic-induced 5mg/kg body weight) and AI + Cur group (arsenic 5mg/kg+curcumin 200mg/kg body weight), respectively. Arsenic and curcumin were offered through the gavage method once daily with basal diet. The different analyzed parameters showed that arsenic-induced elevation of aspartate amino transferase, alkaline phosphatase, bilirubin urea, alanine aminotransferase and creatinine significantly decreased with curcumin application in AI + Cur group. Similarly, the statistically significant decline of low-density lipoprotein (LDL), cholesterol, triglyceride and increased in high-density lipoprotein (HDL) was observed in rats of AI + Cur group with curcumin treatment as compared to the rats of AI group. The level of different enzymes of the liver as well as kidney was noted depleted on arsenic exposure whereas increased in level was observed with curcumin application in AI + Cur group. Moreover, pathological histology changes were also recorded. The outcomes suggest that curcumin has a potential effect against arsenic-induced toxicity in biological model.

Keywords: Curcumin, arsenic, efficacy, liver, kidney, rat model.

INTRODUCTION

Hepatorenal toxicity is a fatal disease that causes the destruction of liver as well as kidney tissues. Heavy metals are the major cause of such toxicity. These metals enhance the level of reactive oxygen species (ROS) that generates oxidative stress (OS) and cell damage in soft organs like liver and kidney. The environmental pollution due to industrial effluents are considered to be the main source for the exposure of heavy metals (Ziemacki *et al*., 1989). Experimental exposure of heavy metal in the animal model with nephrotoxicity induced by individual and mixture of heavy metals have been studied (Molina-Jijón *et al*., 2011). Such exposure of heavy metals can be cured using natural bioactive components present in the food. In this regard, the curcumin which is an active compound present in turmeric plant *(Curcuma longa* that belongs to family "*Zingiberaceae")* helps to decrease renal dysfunction and maintains antioxidant enzymes during heavy metal toxicity (He *et al*., 2006). It also regulates the liver functions by preventing lipid peroxidation and maintains the liver enzymes and their activities due to scavenging free radicals and protects the liver from CAT (catalase), antioxidants enzymes and SOD (superoxide dismutase).

Curcumin plays a potential role against hazardous environmental pollutants, also used as anticarcinoma, antifibrogenic and antischolastic agents. Curcumin elevates the mucin level, which protects from damaging the walls of the stomach from gastric juice. Studies performed on rats and also on cell lines of humans in *vitro* established that curcumin has a potential effect to inhibit cancer formation at three-stage, tumor growth, promotion of tumors and angiogenesis. Prostate and colon cancer, in *vitro* study, shows that curcumin inhibits tumor growth and cell proliferation which is due to direct antioxidant activity as well as free radicals scavenging potential and also indirectly enhance glutathione levels (Shao *et al*., 2002). Therefore, this study was designed to estimate the dosage level and influence of curcumin in the attenuation of hepatorenal toxicity.

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MATERIALS AND METHODS

Sample collection & study plan

Curcumin extracts 95% and arsenic was commercially obtained from Sigma (Missouri, USA). Thirty-six rats weighing between 175±10g were procured from the Department of Physiology, GC University Faisalabad, Pakistan. After 5-7 days of adjustment, rats were separated into four groups, 9 rats were allocated in each group. The diet plan of each group is presented in table 1 with different labeling. Arsenic and curcumin were offered through the gavage method once daily with a basal diet. This study was continued for 30 days followed by a week adjustment period. Blood samples were collected and spray-coated silica jell vacationers were used for serum separation. Serum samples were stored on -20^{o}C temperature until biochemical tests.

Biochemical Tests

Liver and Kidney Function Tests

The determination of Aspartate aminotransferase (AST), alanine aminotransferase (ALT) was performed using the methodology described by Anyanwu and coworkers (1971). Serum bilirubin was analyzed by the following protocol as mentioned by Garber (1981). On the other hand, the commercial kit was used for the determination of serum urea concentration (1971). The serum creatinine was evaluated through the protocol as described by Chromý and his colleagues (2008).

Lipid Profile Tests

Levels of triglycerides and serum lipid profile of rats like HDL (high-density lipoprotein), LDL (low-density lipoprotein) and serum cholesterol were observed according to the method of Kim *et al*. (2011).

Liver and kidney Antioxidant Status

Superoxide dismutase (SOD) status of kidney and liver was analyzed by the method described by Nishikimi *et al*. (1972). CAT of kidney and liver was found as stated by Aebi (1984). Glutathione peroxidase (GPX) of kidney and liver was assessed as mentionaed by Paglia and Valentine (1967). Glutathione Reductase (GR) of liver and kidney was noted by following the method of Factor *et al*. (1998).

Histopathological Examination

Histological studies were performed by the methodology stated by Goslin *et al*. (1990). Rat's liver (1 cm ± 1 cm ± 0.5 cm) was taken and fixed in 40 g/L methanol for 48 h and inserted in paraffin. The microtome was used to prepare the section of thickness (5µm), which were stained by using hematoxylin and eosin for histological study. Van-Gieson staining was studied for estimating the degree of fibrosis in the liver.

STATISTICAL ANALYSIS

Statistical analysis was done of various studies results. Analysis of Variance Technique (ANOVA) was given in Completely Randomized Design (CRD) used to analyze the results of all study groups to determine the level of significance illustrated by Steel *et al*. (1997).

RESULTS

Liver function ALP, ALT, AST (IU/L) and total Bilirubin

The level of liver function tests was significantly ($p<0.05$) different in rats belongs to C, AI and AI + Cur

Table 1: Diet plan for rat efficacy model

Group	Diet Plan
C	Basal diet AIN-93M
Cur	Basal diet + Curcumin (200mg/kg)
AI	Basal diet + arsenic (5 mg/kg)
AI + Cur	Basal diet + arsenic 5 mg/kg + Curcumin 200mg/kg

Table 2: Effect of curcumin on liver and kidney function in healthy and arsenic-induced rats

Parameters	Treatments			
	C	Cur	AI	AI + Cur
ALP (IU/L)	129.97±3.8^{d}	126.88±4.34^{e}	198.2±3.67^{a}	176.36±2.85^{b}
ALT (IU/L)	76.53±2.01^{J}	74.31±3.35^{J}	144.6±1.12^{c}	112.23±1.57^{g}
AST (IU/L)	79.77±1.57^{i}	75.66±2.01^{J}	121.2±2.68^{f}	99.15±2.12^{h}
Bilirubin mg/dL	2.66±0.34^{r}	2.63±0.25^{r}	4.53±0.43^{p}	3.01±0.18^{q}
Urea mg/dL	6.08±0.09^{n}	6.00±0.11^{n}	7.57±0.21^{k}	6.42±0.22^{m}
Creatinine mg/dL	0.53±0.01^{v}	0.50±0.02^{v}	0.72±0.01^{s}	0.60±0.02^{t}

Table 3: Effect of curcumin as an antioxidant in the liver and kidney of healthy and arsenic-induced rats

Organ	Parameters	Treatment Groups			
		C	Cur	AI	AI+Cur
Liver	SOD (U/mg)	1233.6 ± 52.6^{f}	1652.7 ± 48.7^{b}	1100.4 ± 27.4^{g}	1351.2 ± 47.0^{e}
	CAT (U/mg)	0.073 ± 0.01^{x}	0.094 ± 0.003^{w}	0.056 ± 0.002^{y}	0.081 ± 0.004^{x}
	GPX (U/mg)	0.78 ± 0.04^{r}	0.99 ± 0.06^{p}	0.65 ± 0.08^{t}	0.87 ± 0.11^{q}
	GR (µmol /mg)	7.01 ± 0.87^{k}	8.72 ± 0.67^{h}	5.81 ± 0.60^{m}	8.00 ± 0.44^{i}
Kidney	SOD (U/mg)	1540.3 ± 73.3^{d}	1713.0 ± 126.1^{a}	1349.3 ± 100.5^{e}	1611 ± 135.1^{c}
	CAT (U/mg)	0.076 ± 0.009^{x}	0.093 ± 0.002^{w}	0.060 ± 0.003^{y}	0.089 ± 0.009^{w}
	GPX (U/mg)	0.60 ± 0.07^{u}	0.71 ± 0.03^{s}	0.54 ± 0.06^{v}	0.67 ± 0.07^{t}
	GR (µmol /mg)	6.14 ± 0.79^{l}	7.99 ± 0.21^{i}	4.69 ± 0.71^{n}	7.24 ± 0.65^{j}

C (basal diet in healthy rats) Cur (basal diet+ curcumin 200mg/kg body weight in healthy rats)
AI (basal diet+5 mg/kg arsenic-induced rats)
AI+Cur (basal diet+ 5 mg/kg arsenic-induced rats + curcumin 200mg/kg body weight)
$^{a-y}$Means with different superscripts within a row differ significantly ($p<0.05$)

group diets (table 2). Furthermore, the results indicate that the non-significant (*p>0.05*) difference in ALP, ALT, AST (IU/L) and total bilirubin in healthy rats belongs to C and Cur groups. The arsenic-induced rats in AI and AI + Cur groups showed significant differences in ALP, ALT, AST (IU/L) and total bilirubin as compared to the C group. The ALP, ALT, AST (IU/L) and total bilirubin in AI + Cur group rats were 21.84, 32.37, 22.05 and 21.84 units reduced for rats of AI group. The reduction of ALP, ALT, AST (IU/L) and total bilirubin rats fed AI + Cur group showed that adding curcumin in the diet of rats has a positive impact to return these metabolites in a normal range.

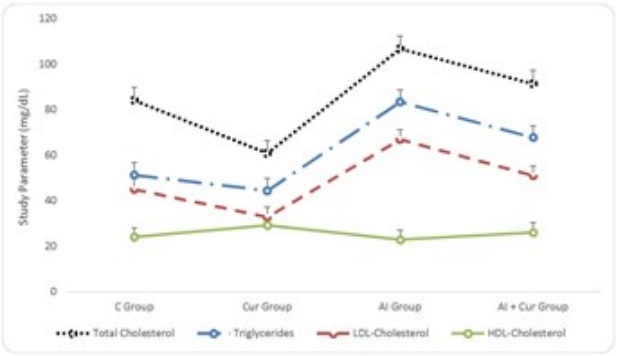

Fig. 1: Effect of curcumin on lipid profile in healthy and arsenic-induced rats.

Kidney function tests urea and creatinine (mg/dL)

Level of creatinine and urea (mg/dL) were significantly different (*p<0.05*) in C, Cur, AI and AI+Cur diet groups (table 2)**.** The level of urea and creatinine (mg/dL) remained unchanged in healthy rats belongs to C and Cur diet groups. However, the arsenic-induced rats in AI and AI + Cur groups showed a significant difference in creatinine and urea (mg/dL). The level of creatinine and urea (mg/dL) in AI + Cur group rats was 32.37 and 22.05 units reduced from rats belong to the AI group. The reduction of creatinine and urea (mg/dL) in rats fed AI+Cur group showed that adding curcumin in the diet of rats has a positive effect to return these metabolites in a normal range.

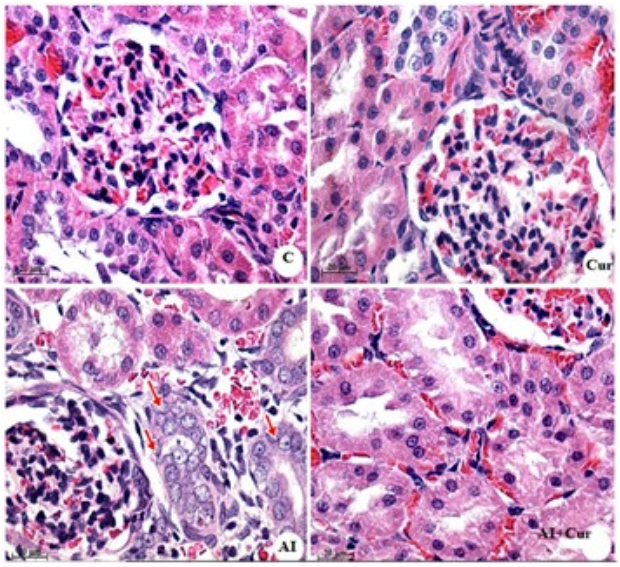

Fig. 2: Histopathological study of arsenic-induced hepatotoxicity: (C) Control; (Cur) curcumin-treated rats at a dose of 200mg/kg body weight; (AI) Arsenic induced rats at a dose of (5mg/kg) body weight; (AI + Cur) arsenic-induced rats fed on curcumin

Influence of Curcumin on antioxidant enzymes status in the liver

Table 3 presents the significantly different (*p<0.05*) level of GR (µmol/mg), CAT, SOD and GPX (U/mg) in the liver after the different diets. Level of GR (µmol/mg) CAT, SOD and GPX (U/mg) was significantly (*p<0.05*) higher in healthy rats fed belong to Cur group. On the other hand, the arsenic-induced rats in AI and AI + Cur groups revealed a significant difference in SOD, CAT, GPX (U/mg) and GR (µmol/mg). The levels of GR (µmol/mg), CAT, SOD and GPX (U/mg) in AI + Cur group rats were 2.82, 0.025, 250.8 and 0.22 units increased for rats of AI group, respectively.

Impact of Curcumin on Antioxidant enzymes in kidney

Levels of GR (µmol/mg), CAT, SOD and GPX (U/mg) in kidneys were significantly ($p<0.05$) different in rats fed C, Cur, AI and AI + Cur diets (table 3). Level of GR (µmol/mg), CAT, SOD and GPX (U/mg) were significantly higher ($p<0.05$) in healthy rats fed diet Cur. However, the arsenic-induced rats in AI and AI + Cur groups displayed a significant difference in GR (µmol/mg), CAT, SOD and GPX (U/mg). The levels of GR (µmol/mg), CAT, SOD and GPX (U/mg) in AI + Cur group rats were 2.55, 0.025, 250.8 and 0.22 units increased for rats of AI group, respectively. The reduction of CAT, SOD, GPX (U/mg) and GR (µmol/mg) in rats fed AI+Cur group showed that adding curcumin in the diet of rats has a positive impact.

Effect of curcumin on lipid profile cholesterol, triglyceride, LDL and HDL

Level of cholesterol, triglyceride, HDL and LDL (mg/dL) were significantly ($p<0.05$) different in rats fed C, Cur, AI and AI + Cur diets (fig. 1). Level of cholesterol, triglyceride and LDL (mg/dL) were significantly decreased in curcumin fed rats of Cur group and elevated levels of these parameters in arsenic-induced AI + Cur group return to normal as compared to AI group, respectively. But the level of HDL was increased in curcumin fed healthy rats and arsenic-induced rats of Cur and AI+Cur groups instead of AI group, respectively. However, the concentrations of cholesterol, LDL and triglyceride (mg/dL) in AI + Cur group rats were 15.39, 15.89 and 15.63 units reduced and HDL has increased 3.22 units for rats of AI group, respectively. The reduction of cholesterol, triglyceride, LDL and increase of HDL (mg/dL) in rats AI + Cur group showed that adding curcumin in the diet of rats has a positive impact to return these metabolites in a normal range.

DISCUSSION

In arsenic-induced rats, the concentration of total bilirubin, ALP, AST and ALT (IU/L) was significantly ($p<0.05$) higher when compared by the curcumin-based diet group. Similar results were found when 200mg/kg curcumin was offered to the rats twice in a week with duration of six weeks (Gao *et al*., 2013) but the lower doses (50mg/kg) showed no beneficial impact against As-induced elevation of ALT activity (2010) However, the present study offered higher amount (200mg/kg) of curcumin, which provides better results. In addition to this, the elevation in liver enzymes ALP, ALT, and AST (IU/L) has been reported by Sheikh *et al*. (2013) also stated that arsenic causes hepatotoxicity. However, curcumin can play its role to reduce the increased level of ALP, AST, ALT, and bilirubin in the present study showing the possible hepato-toxicity in arsenic exposed rats.

The significantly ($p<0.05$) higher levels of serum urea and creatinine (mg/dL) were found in arsenic-induced rats as compared to the control group. This elevation of can be significantly ($p<0.05$) reduced due to the supplementation of curcumin as evident in the present study. Similar results were found by Karim *et al*. (2010) when treatment of rats with 50mg/kg body weight of turmeric (per day) and 10 mg/kg body weight of sodium arsenate (III) (per day) was given for ten weeks. In addition to this, Mohamad *et al*. (2009) reported that pre-treatment with berberine leads to significantly decrease in the concentration of serum urea and creatinine exposed to mercury(II) chloride ($HgCl_2$; 0.4mg/kg, BN (100mg/kg body weight) for 7 days showing the dramatic improvement in degenerative changes in renal corpuscles and kidney tubules that supports the finding of present research work.

The level of cholesterol, LDL triglycerides in rats of all groups were significantly ($p<0.05$) decreased whereas HDL increased by administration of curcumin as compared to control group. It is evident from the previous studies that the administration of curcumin helps to improve the lipid profile by reducing the cholesterol and triglycerides and enhancing the HDL levels that ultimately reduce the risk of heart failure (Karabourniotis *et al*., 2020; 2010). Similarly, curcumin was offered to the rabbits and found a significant decrease in cholesterol as compared to the control group which described hypocholesterolemic effect of curcumin (Zorofchian Moghadamtousi *et al*., 2014). In addition to this, the impact of L3 (an analog of curcumin) has shown its potential against hyperlipidemia that supports the findings of the present study (Farkhondeh & Samarghandian, 2016).

In this study, it was noticed that, the level of antioxidants enzymes (SOD, CAT, GPX and GR) significantly decreases in arsenic-induced rats when compared with normal rats, which showed the arsenic-induced OS that generates ROS and considered as a first defensive pathway as a result of free radical injury (Sumathi *et al*., 2012). The down regulation of GPX and SOD in lead-induced toxicity was observed with the same pattern of activity of an enzyme. The level of GR (µmol/mg) and CAT, SOD, GPX (U/mg) and in AI + Cur group rats were 2.55, 0.025, 250.8 and 0.22 units increased for rats of AI group, respectively. GR (µmol/mg) and CAT, SOD, GPX (U/mg) were significantly ($p<0.05$) increased in liver tissues of AI+Cur group rats as compared to control group 0.99, 0.0008, 117.6, and 0.09 units, respectively which specified that arsenic affected OS. Sumathi *et al*. (2012) reported a similar effect which was observed from present outcomes. Another modification in reaction to As-toxicity was noticed in genes expression that encode antioxidant enzymes (Tarasub *et al*., 2012). The

hydrophobicity of curcumin makes its entry into the cell very easily and results in the inhibition of ROS generation, which activate the pathway of OS caused by metal toxicity. Curcumin binds metal with low affinity (near 1mM) compared with storage proteins and biological transport. One curcumin molecule may undergo many cycles of binding metals, releasing it to different conditions (Moghaddam *et al*., 2019).

The same histopathological as well as biochemical changes in arsenic-induced nephrotoxicity was found as reported by Tarasub *et al*. (2012). Histological approach was used to study the level of injury in kidney induced by arsenic (fig. 2). The hypertrophy of proximal tubular cells and hydropic swelling resulted in the renal damage (panel AI), as compared with control (panel C). Treatment of arsenic group with curcumin (panel AI + Cur) resulted in the improvement of proximal tubular cell damage and observation was done with arsenic alone while compared with panel AI + Cur and panel AI (arsenic-induced), the toxicity of arsenic may be ascribed that some of the arsenic metallothionein's are degraded to release the arsenic is toxic. Curcumin illustrates the ability to reduce the changes associated with the toxicity of metal. This is possibly due to the combination of some stem cells and to regenerate such lost cell masses. The hepato-renal protective impact may attribute to the metals (low dose) in the intestine with increased intestinal metabolism. The pro-oxidant curcumin can prompt the response of GSH-antioxidant and thus can deliver hepatic protection (Avila-Rojas *et al*., 2019).

CONCLUSION

In conclusion, this study revealed that exposure to arsenic induces adversative effects and profound changes in the hepatic and kidney functions related to OS and reduced antioxidant activities of enzymes. Curcumin displayed ameliorating properties against arsenic-induced hepatic toxicity and nephrotoxicity. Development in the biochemical and antioxidant enzymes (GSH, CAT and SOD) was identified with curcumin-treated groups. The post-treatment of curcumin was more effective than the co-treatment of curcumin. Consequently, the use of curcumin is mentioned as a prophylactic measure against As-induced hepatic renal toxicity. Curcumin showed a positive impact on the function of liver and kidney in the prevention of toxicity caused by arsenic. Antioxidant status has been improved to prevent free radicals. The lipid profile showed normal values by curcumin administration.

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