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Hepatoprotective efficacy of curcumin against arsenic trioxide toxicity

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ABSTRACT

Objective: To evaluate the efficacy of curcumin in combating arsenic induced hepatic oxidative stress, histopathological changes and the hepatic arsenic accumulation in rat model. **Methods:** Oxidative stress was induced by oral administration 4 mg/kg b.wt of arsenic trioxide (As2O3,) for 45 days in experimental rats. The level of liver arsenic concentration, lipid peroxidation, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) were determined in adult male Wistar rats. Hepatotoxicity was assessed by quantifying the aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phophatase (ALP). Hepatoprotective efficacy of curcumin (15 mg/kg b.wt) was evaluated by combination treatment with As_2O_3. **Results:** As_2O_3 administration leads to the generation of reactive oxygen species (ROS), arsenic accumulation, serum marker enzymes release and decrease in antioxidant enzymes in liver. Retention of arsenic in liver caused increased level of lipid peroxidation with a concomitant decline in the glutathione dependant antioxidant enzymes and antiperoxidative enzymes. Curcumin treatment protected the liver from arsenic induced deterioration of antioxidant levels as well as oxidative stress. And also a significant decrease in hepatic arsenic accumulation and serum marker enzymes was observed. Histopathological examination revealed a curative improvement in liver tissue. **Conclusions:** These findings lead to the conclusion that curcumin may have the potential to protect the liver from arsenic-induced toxic effects.

1. Introduction

Arsenic is one of the most important global environmental toxicants and its exposure in humans comes mainly from consumption of drinking water contaminated with inorganic arsenic [1]. In clinical trials it is considered as a first choice cancer chemotherapeutic against certain leukemia and has potential against a variety of other cancers, including solid tumors [2]. Specifically, arsenic trioxide (As_2O_3,) is used in the treatment of acute promyelocytic leukemia and it greatly improves the clinical outcome even in refractory or multiple relapsed cases [3]. But, toxic side effects of arsenicals are often a major concern, including the potential for fatal hepatotoxicity [4].

The liver is a major target organ for both arsenic metabolism and toxicity. Arsenic induced hepatic injury is known to be exerted through excess production of reactive oxygen species, namely superoxide (O2 $^{\bullet}$ $^{-}$), hydroxyl ($^{\bullet}$ OH), and peroxy (ROO $^{\bullet}$) radicals and hydrogen peroxide (H_2O_2) [5]. The harmful expressions of arsenic are primarily due to an imbalance between pro-oxidant and antioxidant homeostasis in physiological system and also due to its fascination to bind sulfhydryl groups of proteins and thiols of glutathione (GSH) [6]. Thus, an agent able to reduce the toxic potential of arsenic in liver cells would clearly be a useful compound for arsenical chemotherapy.

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione (diferuloylmethane)] is a naturally occurring polyphenolic compound derived from the root of Curcuma longa Linn. a perennial herb belonging to ginger family Zingiberaceae [7]. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities [8]. Curcumin could effectively be considered a good candidate for cancer prevention when used alone and for cancer treatment in combination with other conventional therapies as it is able to target multiple signaling pathways implicated in this disease [9]. Thus, in the present study, curcumin was selected as the most potential natural compound to prevail over arsenic induced hepatotoxicity.

Arsenic exposure leads to the incidence of hepatotoxicity as manifested by increase in the levels of total bilirubin, alanine aminotransferase, aspartate aminotransferase,

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and malondialdehyde [10]. Curcumin is well known as a multifunctional drug with anti-oxidative, anti-cancerous, and anti-inflammatory activities [11]. More and more preclinical studies support the idea that curcumin, a plant-derived natural polyphenol, could be a promising anticancer drug[12]. Hepatoprotective, antioxidant, antimutagenic and anticarcinogenic effects of curcumin have been shown by previous workers [13]. This study was designed to evaluate the protective role of curcumin upon As_2O_3 induced hepatotoxicity.

2. Materials and methods

2.1. Chemicals and reagents

Arsenic trioxide, curcumin, sodium pyruvate, thiobarbituric acid and triton X-100, phenazine methosulphate, nitroblue tetrazolium (NBT) and bovine serum albumin were obtained from Sigma-Aldrich, Bangalore, India. L-aspartate, α -oxoglutarate, 2,4-dinitro phenyl hydrazine, nicotinamide adenine dinucleotide (Reduced), thiobarbituric acid, 5,5′-dithiobis-nitro benzoic acid, nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione, 1-chloro-2,4 dinitro benzene (CDNB) were purchased from Merck Specialties Pvt. Ltd., Mumbai, India. All other chemicals were purchased from Sisco Research Laboratories (SRL), India.

2.2. Animals

Twenty four male Wistar rats weighing 180-200 g were purchased from the Small Animal Breeding Station (SABS) of Government Veterinary College, Mannuthy, Thrissur, Kerala, India and acclimatized for six days. All the animals were maintained under standard laboratory conditions of temperature (25 ℃) and 12 hour light and dark cycles throughout the experimental period. The rats were provided with laboratory chow (Hindustan Lever Ltd., India) and tap water adlibitum. Experiments were conducted as per the guidelines of Institutional Animal Ethical Committee, School of Biosciences, Mahatma Gandhi University.

2.3. Experimental protocol

The rats were divided into four groups of six rats each, a normal control group, a curcumin control which received 15 mg/kg b.wt of curcumin, one As_2O_3 (4 mg/kg b.wt) administered group and a combination group treated with 4 mg/kg b.wt of As_2O_3 and 15 mg/kg b.wt of curcumin. 0.2% DMSO solution was used as vehicle for curcumin administration. Experimental groups received this via oral intubation daily for a period of 45 days.

At the end of the experimental period animals were decapitated, blood was collected and centrifuged at 3 000 rpm for 20 minutes; the clear serum obtained was used for the determination of marker enzymes. Liver was removed immediately, washed in ice cold 0.15M NaCl and blotted on a filter paper. Then the tissue was weighed and homogenized by using Teflon glass homogenizer (1/10th weight/volume) in ice cold tris-HCl buffer (0.2M, pH 7.4). The homogenate was centrifuged at 10 000g for 20 min at 4 ℃ and the supernatant was used for the estimation of lipid peroxidation and various enzymatic and non enzymatic assays.

2.4. Serum enzyme analysis

The activities of lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were detected by kinetic method using the kit of Agappe Diagnostic Ltd., India. Activities of these serum enzymes were measured by using semi auto analyzer (RMS, India).

2.5. Detection of arsenic accumulation in liver tissue

Liver tissue of experimental rats were digested by thermal acid microwave digestion and diluted with double distilled water. Total arsenic deposition in liver tissue was analyzed by standard inductively coupled plasma-optical emission spectroscopy (Optima 2000 DV ICP-OES, Perkin Elmer, Inc. USA).

2.6. Tissue analysis

Tissue catalase (CAT) activity was determined from the rate of decomposition of H_2O_2 [14]. Glutathione peroxidase (GPx) activity was determined by measuring the decrease in glutathione (GSH) content after incubating the sample in the presence of H_2O_2 and sodium nitrite [15]. Glutathione reductase (GR) activity was assayed by the method of Goldberg and Spooner [16]. The amount of NADPH consumed during GSSG (glutathione oxidised) reduction was used as the index of enzyme activity. Superoxide dismutase (SOD) activity was measured by the method of Kakkar *et al*. [17]. One unit was taken as the amount of enzyme that gave 50% inhibition of NBT reduction/mg protein. Glutathione-S-transferase (GST) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB [18]. Reduced GSH was determined according to the method of Ellman [19] based on the formation of a yellow coloured complex with DTNB. Lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1′1′3′3′ tetramethoxypropane as standard [20]. Protein content in the tissue was determined [21] using bovine serum albumin (BSA) as the standard.

2.7. Histopathology

Small sections of liver, fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of 5-6 μm were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam-1000 camera at original magnification of 100X.

2.8. Statistical analysis

The results were analyzed using statistical programme SPSS/PC+, version 10 (SPSS Inc. Chicago, IL, USA). One way

ANOVA was employed for comparison among the three groups. LSD post hoc multiple comparison test was used to determine significant difference among groups. $P<0.05$ was considered significant.

3. Results

3.1. Effect of curcumin on arsenic trioxide induced variation in the liver arsenic accumulation

Figure 1A shows that treatment with As_2O_3 caused a significant ($P<0.05$) increase in the concentration of arsenic in the liver tissue. Continuous administration of As_2O_3 for a period of 45 days at a dose of 4 mg/kg body weight caused arsenic deposition in liver. Co-treatment with curcumin significantly ($P<0.05$) decreased the accumulation of arsenic.

3.2. Effect of curcumin on arsenic trioxide induced changes in the liver marker enzymes in serum

Liver markers were monitored by estimating AST, ALT and ALP in the serum of rats. The diagnostic marker enzyme activities were significantly increased ($P<0.05$) in arsenic treated rats, indicating hepatic dysfunction. Curcumin co-treatment exerted a reduction in the activity of AST (Figure 1B) ALT (Figure 1C) and ALP (Figure 1D).

3.3. Effect of curcumin on arsenic trioxide induced changes in the liver lipid peroxidation and antioxidant enzyme activity

The effect of curcumin on lipid peroxidation and the activity of antioxidant enzymes were outlined in the Table 1. Thiobarbituric acid assay was used to measure the extent of lipid peroxidation induced by As_2O_3 in liver of rats. Results indicated that TBARS level was significantly ($P<0.05$)

Table1.
Effect of curcumin on arsenic trioxide induced changes in lipid peroxidation and antioxidant status.

Parameters	Normalcontrol	Curcumin control	Arsenic	Arsenic + Curcumin
TBARS(nM/mg protein)	3.18±0.18	2.14±0.13	5.47±0.27a	3.98±0.15b
SOD(U/mg protein)	9.11±0.11	9.73±0.14	7.63±0.15a	8.78±0.13b
GST(μM of CDNB- GSH conjugate formed/min/mg protein)	1.23±0.08	1.41±0.09	0.51±0.09a	1.05±0.10b
GPx(μg of GSH consumed/min/mg protein)	11.22±0.31	11.63±0.41	8.17±0.34a	10.84±0.46b
CAT(μ moles of H_2O_2 consumed /min /mg protein)	32.99±2.34	32.56±4.25	23.01±2.15a	29.93±2.14b
GSH(μM/g tissue)	53.78±7.50	59.37±5.61	34.88±5.80a	47.31±4.71b
GR(nmol of NADPH oxidised/min/mg protein)	0.68±0.03	0.75±0.02	0.43±0.02a	0.59±0.02b

Normal control, Curcumin control (Curcumin- 15 mg/kg b.wt), Arsenic (As_2O_3-4 mg/kg b.wt), Arsenic (As2O3 4 mg/kg b.wt) + Curcumin (Curcumin-15 mg/kg b.wt). Data represented as mean ±SD, n=6. a$P<0.05$ versus normal control, b$P<0.05$ versus As_2O_3 treated groups.

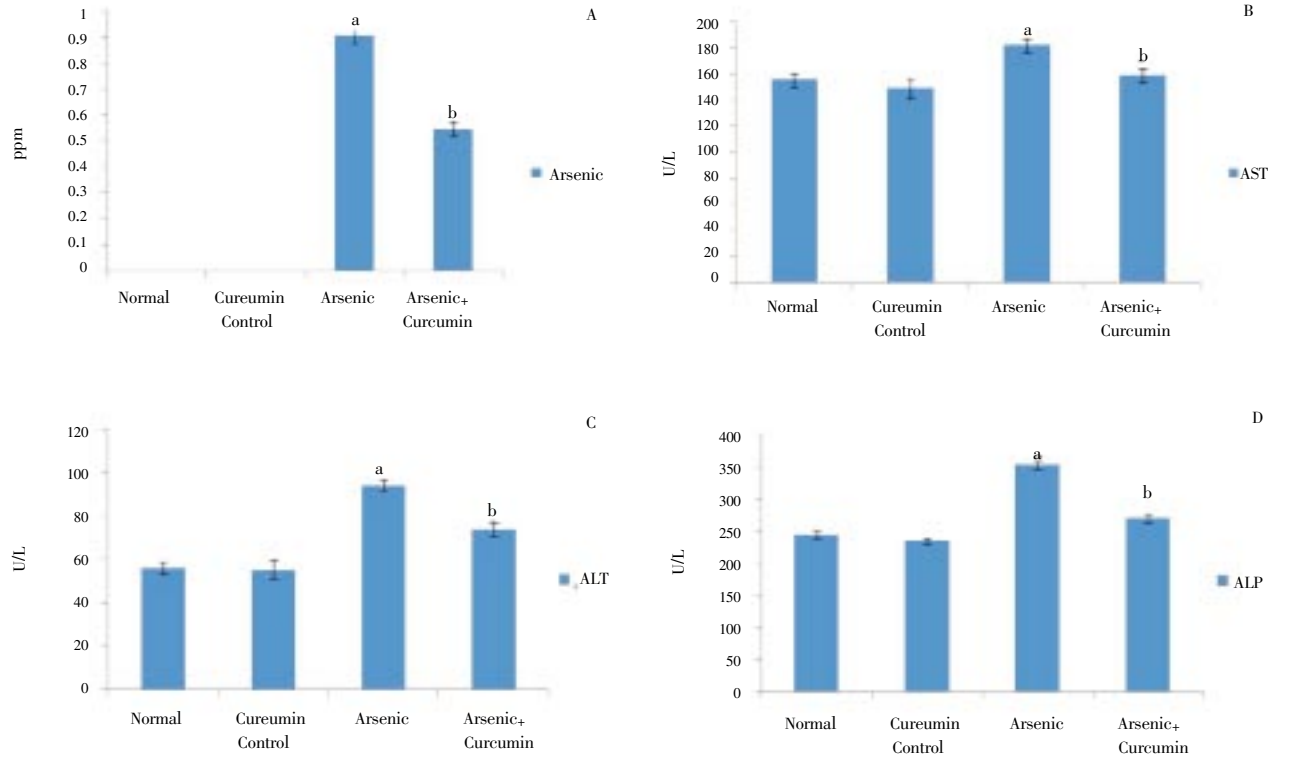

Figure 1. Effect of curcumin on arsenic trioxide induced variations in arsenic accumulation and the liver marker enzymes in serum.
(A)-Liver arsenic concentration, (B)-Aspartate aminotransferase, (C)-Alanine aminotransferase and (D)-Alkaline phosphatase; Data represented as mean ±SD, n=6. a$P<0.05$ versus normal control, b$P<0.05$ versus As_2O_3 treated groups.

increased in liver of rats treated with As_2O_3. Co-treatment with curcumin caused significant ($P<0.05$) decrease in liver TBARS compared to the As_2O_3-treated rats.

A significant ($P<0.05$) decline in the level of GSH was noticed in liver tissue of arsenic treated rats as compared to controls. As_2O_3 administered rats showed significant decrease ($P<0.05$) in the activity of SOD, CAT, GPx, GST and GR in hepatic tissue. Co-treatment with curcumin exhibited significant increase in the GSH levels compared to As_2O_3 rats. The curcumin treatment showed significant ($P<0.05$) recovery in the antioxidant enzymes activity (Table 1).

3.4. Effect of curcumin on arsenic trioxide induced changes in the liver histopathology.

Haematoxylin and eosin staining of the hepatic tissue showed significant structural abnormalities i.e. moderate sinusoidal dilation, hemorrhage, focal necrosis and cholangiofibrosis in arsenic treated rats (Figure 2C). Structural abnormalities in As_2O_3 treated liver was significantly prevented by co-treatment with curcumin. Curcumin co-treated rats showed only mild sinusoidal dilation; no other significant pathological changes were observed (Figure 2D). Liver tissue of normal control (Figure 2A) and curcumin control (Figure 2B) showed normal hepatocellular morphology.

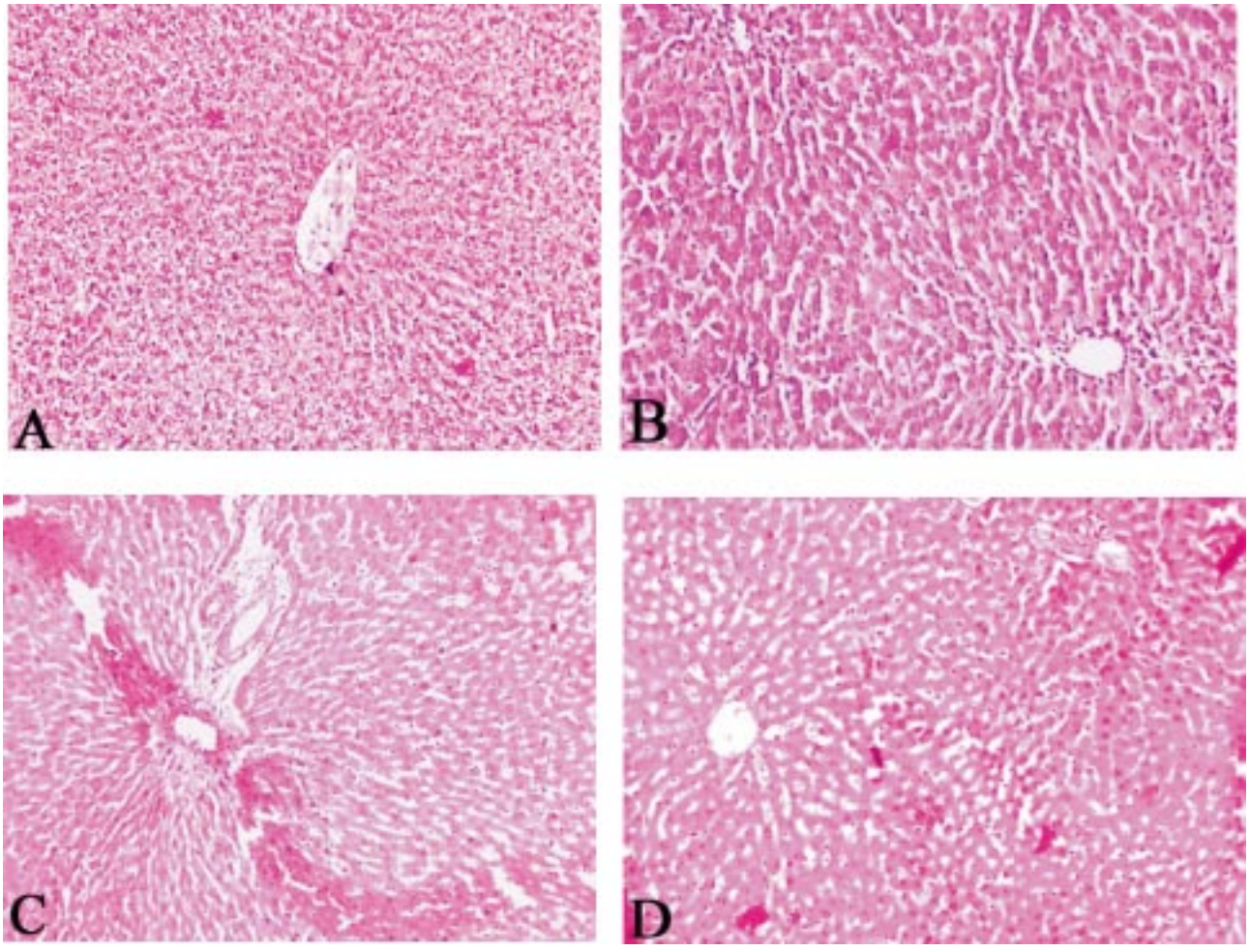

Figure 2. Histopathology of liver tissue: Histopathological changes occurred in rat liver after As_2O_3 administration and prevention by the treatment with curcumin (hematoxylin and eosin, 100 X). (A) Normal control, (B) Curcumin control (15 mg/kg b.wt), (C) As_2O_3 (4 mg/kg b.wt), (D) As_2O_3 (4 mg/kg b.wt) + Curcumin (15 mg/kg b.wt).

4. Discussion

Curcumin or diferuloylmethane is a polyphenolic molecule derived from the rhizome of the plant Curcuma longa [22]. The present study demonstrates the curative potential of curcumin by reversing As_2O_3 induced hepatic damage. Arsenic has been showing stunning efficacy in the treatment of acute promyelocytic leukemia [2]. However, inorganic arsenic can have profound toxic effects even following short-term use. For instance, potentially fatal hepatic toxicity has been reported in a subset of patients receiving arsenic chemotherapy [4]. The dose of curcumin (15 mg/kg body weight) was selected from a recent study [23]. The dose of As_2O_3 was 4 mg/kg body weight daily, which has been shown to produce plasma concentration of arsenic within the near range of those presented in arsenic treated APL patients [24].

As_2O_3 treatment in this experiment resulted in a significant increase in the level of TBARS in hepatic tissue. This elevation might be because of the lower level of SH-groups and antioxidant enzymes also observed in this study. Generation of large amount of reactive oxygen species

(ROS) due to arsenic toxicity can overwhelm the antioxidant defense mechanism and damage cellular ingredients such as lipids, proteins and DNA; this in turn can impair cellular structure and function. Curcumin a known scavenger of free radicals, when administered in the current study, efficiently lowered the peroxidation levels thus protecting tissues from oxidative stress. In agreement with Kalpana *et al* [25], data obtained in the present study also showed that curcumin significantly decreased the levels of TBARS.

As_2O_3 treatment caused arsenic accumulation in liver cells. The increased concentration of arsenic in liver may be due to an insult in the detoxification mechanism. The liver is also the major site of arsenic methylation, which is catalyzed by arsenic methyl-transferase using S-adenosyl methionine (SAM) as the substrate [26]. This enzyme catalyzes the transfer of a methyl group from SAM to trivalent arsenic producing methylated and dimethylated arsenic compounds. Arsenic is methylated to monomethyl arsenic acid (MMA) and finally to dimethyl arsenic acid (DMA) [27]. Our study also showed that the exposure to As_2O_3 significantly increased ROS production and enhanced oxidative stress in hepatocytes. One of the most important arsenic detoxification mechanisms is the glutathione (GSH) system. Oxidative stress-mediated hepatotoxic effect of arsenic is mainly due to the depletion of GSH in liver. Depletion of hepatic GSH facilitates accumulation of arsenic in the liver and thus causes oxidative stress [28]. Therefore, curcumin-induced increased GSH level during exposure to toxic electrophiles generated by arsenic as well as its rapid elimination/excretion from the body play an important role in decreasing oxidative stress. Hepatocellular arsenic deposition may be the prime reason for liver toxicity. Curcumin treatment significantly reduced the arsenic accumulation and structural abnormalities in hepatic tissue.

In the present study, treatment with As_2O_3 caused significant increase in the activities of hepatic marker enzymes in serum; this may be due to the leakage of the enzymes to the blood stream. The increase in the activities of these enzymes in plasma indicates liver damage and alteration in liver function. Treatment with curcumin significantly decreased the activities of these enzymes in serum suggesting that it offers protection by preserving the structural integrity of the cell. Administration of curcumin preserved the integrity of the hepatocellular membrane. Our results were in agreement with Yu *et al* [29] who demonstrated that curcumin prominently reduced tissue injury as well as ALT and AST activity. The leakage of enzymes because of liver injury was prevented by the liver cell membrane stabilizing action of curumin.

A significant reduction in the activities of glutathione dependant antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) with a concomitant decline in the level of GSH level was observed in the liver tissue of As_2O_3 treated rats. GST plays a key role in cellular detoxification by catalyzing the reaction of glutathione with toxicants to form an S substituted glutathione [30]. SOD is the first antioxidant enzyme to deal with oxy-radicals by accelerating the dismutation of superoxide to hydrogen peroxide, while CAT is a peroxisomal heme protein that catalyses the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT acts mutually supportive anti oxidative enzymes, which provide protective defense against reactive oxygen species [31]. Reduced activity of CAT after exposure to As_2O_3 in the present finding could be correlated to increased generation of H_2O_2. Inorganic arsenic exposure results in the generation of ROS in various cellular systems, and its production has been proposed as one of the early biological events on arsenic-related carcinogenic process [32].

Curcumin has two o-methoxy phenolic OH groups attached to the β-diketone moiety having methylene CH2 group. It is believed that the H abstractions from these groups are responsible for the remarkable antioxidant activity of curcumin. The free radical scavenging activity of curcumin can arise by the resonance stabilization of its radicals from two phenolic OH groups (mainly) or from the CH2 group of the β-diketone moiety [33]. Therefore, curcumin not only is a phenolic antioxidant that mostly donates H atoms from the phenolic groups, but also is a β-diketone radical chain-breaking substance that can give H atom from methylene CH2. Treatment with curcumin significantly reduced intracellular ROS production by increasing the activity of GSH generating enzyme GR and ROS scavenging enzymes (GST, GPx, SOD and CAT) in hepatic tissue.

Curcumin is known as antioxidant and anti-inflammatory properties. It is the free radical scavenger and inhibited lipid peroxidation products [34]. The protective mechanism of curcumin may due to the strong antioxidant property i.e. it helped for the healing of hepatic parenchyma and regeneration of hepatocytes. Histopathology was also consistent with As_2O_3 induced damage in the concerned organ. Treatment by curcumin restored normal hepatic histopathology as well as various diagnostic biochemical variables towards normal indicating reversal of As2O3 induced hepatotoxicity and confirming the free radical scavenging property of curcumin.

In conclusion, the present study shows that curcumin treatment mitigates arsenic intoxication-induced oxidative damage, which could be due its antioxidant nature that combines free radical scavenging and metal chelating properties. As a single agent arsenic caused toxic effects in hepatic tissue but along with curcumin the side effects were significantly reduced. It can be summarized that curcumin could be a potential antioxidant against the oxidative stress generated by As_2O_3. However, it is suggested that curcumin might be a useful hepatoprotective agent in cancer therapy along with standard chemotherapeutic drug As_2O_3.

Conflict of interest statement

We declare that we have no conflict of interest

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