

Tetrahydrocurcumin Potentially Attenuates Arsenic Induced Oxidative Hepatic Dysfunction in Rats

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Abstract

Arsenic (As) compounds are reported as environmental toxicants and human carcinogens. Exposure to arsenic imposes a big health issue worldwide. Tetrahydrocurcumin (THC) is an antioxidative substance, which is derived from curcumin, the component of turmeric. In view of this fact, the purpose of this study was to delineate the ameliorative role of THC against arsenic-induced hepatotoxicity in rats. In this context, we evaluated the mode of action of chronic oral exposure of sodium arsenite as the source of arsenic at 5 mg/kg/BW with THC (80 mg/kg/BW) for 28 days. Hepatotoxicity was evaluated by the increased activities of serum hepatospecific enzymes namely aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase and total bilirubin along with increased elevation of lipid peroxidative markers, thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content and conjugated dienes. The toxic effect of arsenic was also indicated by significantly decreased activities of membrane bound ATPases, enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and glucose-6-phosphate dehydrogenase along with non-enzymatic antioxidants like reduced glutathione, total sulfhydryl groups, vitamins C and E. Administration of THC exhibited a significant reversal of arsenic-induced toxicity in hepatic tissue. All these changes were supported by reduction of histopathological observations of the liver. These results suggest that THC has a potential protective effect over arsenic-induced hepatotoxicity in rat.

Keywords: Arsenic; Tetrahydrocurcumin; Liver; Oxidative stress; Rat

Introduction

Arsenic is a naturally occurring metalloid found in the environment, coming under one of the heavy metal and human carcinogen [1]. Bi-inorganic form of As in drinking water are trivalent (As^{+3}) such as arsenic trioxide and sodium arsenite and pentavalent (As^{+5}) such as arsenic pentoxide and the various arsenates. Recent studies have confirmed that the As^{+3} form of As highly toxic than As^{+5} and it has a higher affinity for thiol groups [2,3]. Mainly drinking water is a major source of Arsenic in the environment to public health [4]. Other vehicles of As are agricultural applications such as insecticides, herbicides, fungicides, algacides, sheep dips, wood preservatives and by-products of fossil fuels [5]. As compounds are also used in the manufacture of the products like glass, semiconductors, dyestuffs, smelters, cotton gins, cigarette and as an additive to metal alloys [6].

Furthermore, long term chronic arsenic exposure lead to skin lesions, hypertension, cardiovascular diseases, developmental abnormalities, diabetes, hearing loss, haematological disorders, neurological and reproductive problems, Blackfoot disease and cancer [7-11] and may affect a number of organs lung, skin, Bladder, kidney, liver, testis, uterus and prostate [12]. Even at low doses of arsenic might able to produce dermatological symptoms like raindrop pigmentation, hyperpigmentation, hyperkeratosis, squamous cell carcinoma and basal cell carcinoma [13,14]. Many other reports are available against arsenic induced hepatotoxicity but exact mechanism of Arsenic not fully understood. From earlier findings, arsenic exert its toxic effects through variety of mechanisms in which most noticeable one is the excess generation of reactive oxygen species (ROS).

As is more attractive for various Sulfhydryl group (SH) of cells and release its toxic effects via directly interact with SH groups [15]. Moreover, recent studies suggest that during the metabolism of arsenic in the cells lead to oxidative stress with generation of reactive oxygen species (ROS) like superoxide anion ($O_2^{\cdot-}$), hydroxyl radical

($\cdot OH$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), and peroxy radicals, which are responsible for various toxic effects [16] like oxidative damage to cellular macromolecules including DNA, proteins and lipids by disturbance of antioxidant defence system [17]. As is a potent hepatotoxic agent because liver is the primary target organ for arsenic methylation [2,18]. Islam et al. [2] have been reported that the oxidative stress may produce acquired tolerance to apoptosis, enhanced cell proliferation, altered DNA methylation, genomic instability, and aberrant estrogens signalling involved in the liver toxicity caused by arsenic. Recently, antioxidants are commonly used as medications to treat various forms of liver injury. Plant derived chelating agents are possess powerful antioxidant properties and neutralize the Arsenic induced reactive oxygen species activities, when compared with synthetic antidotes [19]. On the other hand, other reviews have suggested that antioxidants could reduce side effects [20,21].

Tetrahydrocurcumin (THC) possess strong antioxidant activity due to the presence of hydroxyl and methoxyphenyl groups in 4th and 3rd position [22] and shows identical β -diketone of 3rd and 5th substitution in heptane moiety [23]. THC (tetrahydrodiferuloylmethaneone) is a phenolic compound and major colourless metabolites of curcumin (diferuloylmethane) which is derived from the plant *Curcuma longa* L [24]. Holder et al. [25] have been identified that metabolite in intestinal

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Received July 07, 2013; Accepted September 13, 2013; Published October 11, 2013

Citation: Muthumani M (2013) Tetrahydrocurcumin Potentially Attenuates Arsenic Induced Oxidative Hepatic Dysfunction in Rats. J Clin Toxicol 3: 168. doi:10.4172/2161-0495.1000168

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and hepatic cytosol from humans and rats. THC and curcumin have similar structures but differs in that THC lacks the double bonds and is more stable, soluble than curcumin [26]. In addition, both invitro and invivo studies suggested that THC scavenge free radicals such as super oxide, hydroxyl radicals, ferric ions, and peroxy radical efficiently [26,27]. Sugiyama, [23] has been concluded that the beta-diketone moiety and phenolic hydroxyl groups of THC must exhibit antioxidant activity.

Previously, hepatoprotective effect of tetrahydrocurcumin has been demonstrated in chloroquine-induced toxicity in rats [28]. But still, the protective effect of THC against arsenic-induced liver damage hasn't been explored so far. Therefore, the present study was considered of interest to investigate the antioxidant activity of THC against arsenic induced hepatotoxicity in rats.

Materials and Methods

Chemicals

Sodium arsenite (NaAsO₂), 1,1',3,3'- tetramethoxy propane, bovine serum albumin, Hank's balanced salt solution, Ficol histopaque-1077, phosphate buffered saline and SYBR green-I were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and solvents were of certified analytical grade and purchased from S.D. Fine Chemicals, Mumbai or Himedia Laboratories Pvt. Ltd., Mumbai, India. Reagent kits were obtained from span Diagnostics, Mumbai, India.

Drug

THC was a gift provided by Sabinsa Corporation (Piscataway, NJ, USA). Chemical structure of THC is shown in Figures 1 and 2.

Animals and diet

Healthy adult male albino rats of Wistar strain, bred and reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University were used for the experiment. Males were preferred in order to avoid complications of the oestrous cycle. Animals of equal weight (170-190 g) were selected and housed in polypropylene cages lined with husk and kept in a semi-natural light/dark condition (12 h light/12 h dark). The animals had free access to water and were supplied with standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India), constitution of protein (22.21%), fat (3.32%), fibre (3.11%), balanced with carbohydrates (> 67%), vitamins and minerals. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University (Registration Number: 853/2011/CPCSEA) and the animals were cared in accordance with the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals".

Experimental design

In the present study, NaAsO₂ was administered intragastrically at a dose of 5 mg/kg body weight/ day for 4 weeks, which was 1/8 of the oral LD50 values in rats [29]. Control group received the vehicles only, experimental rats were subdivided into two groups (2 and 3). Drug control group received the THC (dissolved in 0.5% of carboxy methyl cellulose, CMC) alone.

A pilot study was conducted with three different doses of THC (20, 40 and 80 mg/kg) to determine the dose dependent effect of THC

in Arsenic treated hepatotoxic rats. After 4 weeks of experiment, it was observed that THC treatment at the doses of 20, 40 and 80 mg/kg significantly ($p < 0.05$) lowered the levels of serum transaminases, thiobarbituric acid reactive substances and elevated the levels of reduced glutathione in the liver of Arsenic intoxicated rats (data have not shown). 80 mg/kg of THC showed higher significant effect than the lower doses 20 and 40 mg/kg. Hence, we have chosen the highest dose (80 mg/kg) of THC for our study.

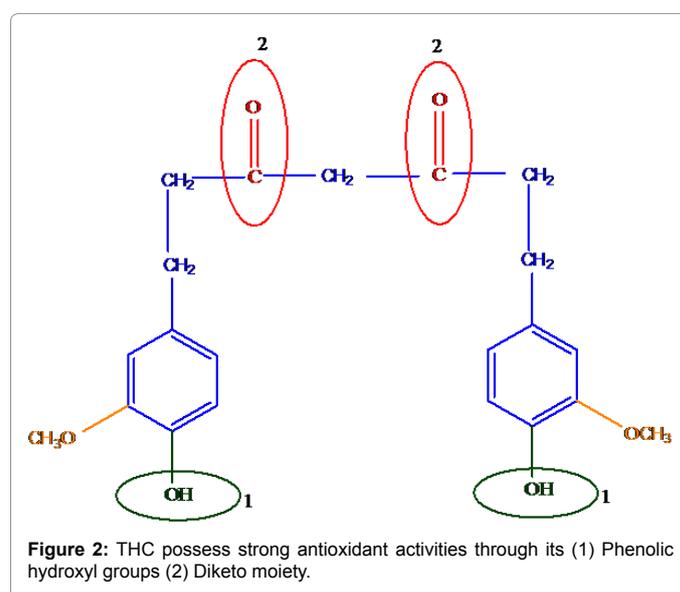
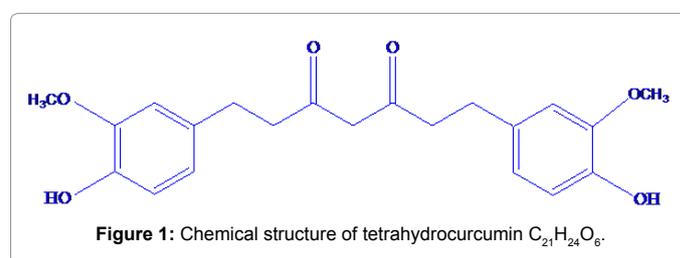
Group 1: In this group, rats received with normal saline and CMC solution (Vehicles) daily using an intragastric tube for 4 weeks and treated as control.

Group 2: In this group, rats received arsenic in the form of NaAsO₂ (5 mg/kg BW day) dissolved in normal saline daily using an intragastric tube for 4 weeks and treated as arsenic group.

Group 3: In this group, rats received THC dissolved in CMC (80 mg/kg BW day) along with arsenic in the form of NaAsO₂ (5 mg/kg BW day) dissolved in normal saline daily using an intragastric tube for 4 weeks and treated as As + THC group.

Group 4: In this group, rats received THC dissolved in CMC solution (80 mg/kg BW day) daily using an intragastric tube for 4 weeks and treated as THC group.

Food and water intake was recorded and rats were weighed every week. Fourty-eight hours after the administration of the last dose, the animals were anaesthetised with an intramuscular injection of ketamine hydrochloride (24 mg/kg) and sacrificed by decapitation. Blood was collected in heparinised and unheparinised tubes for the separation of plasma and serum. Liver tissue was surgically removed, washed with



cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers. Liver tissue (250 mg) was sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0°C in cold centrifuge. The supernatant was separated and used for the assay of various biochemical estimations. For comet assay, liver was cut into small pieces and washed with Hank's balanced salt solution. The individual cell suspensions were obtained by enzymic digestion with collagenase at 37°C. After filtration on 100 and 40 mm mesh successively, the resulting cell suspensions were centrifuged for 10 min at 3000 rpm. The cell pellet was then suspended in phosphate buffered saline and used for the estimation of DNA damage.

Biochemical assays

Activities of serum marker enzymes: The activities of serum aspartate aminotransferase (E.C. 2.6.1.1), alanine aminotransferase (E.C. 2.6.1.2), alkaline phosphatase (E.C.3.1.3.1), lactate dehydrogenase (E.C. 3.1.3.1) and total bilirubin were assayed using commercially available diagnostic kits (Sigma diagnostics (I) Pvt. Ltd., Baroda, India). Gamma glutamyl transferase (E.C. 2.3.2.2) activity was determined by the method of Rosalki, et al. [30] using α -glutamyl-p-nitroanilide as substrate.

Determination of blood α -aminolevulinic acid dehydratase (ALAD): The activity of blood α -aminolevulinic acid dehydratase (ALAD) was assayed according to the procedure of Berlin and Schaller [31]. Total volume of 0.2 mL of heparinized blood was mixed with 1.3 mL of distilled incubated for 10 min at 37°C for complete hemolysis. After adding 1 mL of standard ALA, the tubes were incubated for 60 min at 37°C. The reaction was stopped after 1 h by adding 1 mL of trichloroacetic acid (TCA). After centrifugation equal volume of Ehrlich reagent (0.59 g of dimethylamino benzaldehyde in 12.5 mL glacial acetic acid + 6 mL perchloric acid + 1 mL 2.5% mercuric chloride) was added and the absorbance was recorded at 555 nm after 5 min. The values are expressed as nmol/min/mL.

Determination of liver d-aminolevulinic acid dehydratase (ALAD): Liver α -aminolevulinic acid dehydratase (ALAD) was determined by following the method of Goering et al. [32]. 20% tissue homogenate (w/v) was prepared in 0.05 M Tris-HCl (pH7.4) and 0.25 M sucrose. The incubation medium (0.3 mL) contained 4 mg protein, 50 mM glycine, 50 mM-sodium citrate, 25 mM-sodium onophosphate, 10 mM-magnesium chloride, 5 mM-pyridoxal 5-phosphate and 10 mM disodium EDTA. The mixture was incubated for 45 min at 37°C in darkness. The aminoketones formed were converted into pyrroles by condensation with acetyl acetone. The activity of the enzyme was determined by adding Ehrlich reagent and the absorbance was recorded at 555 nm after 5 min. The values are expressed as nmol/min/mg protein.

Determination of lipid peroxidation and oxidative stress markers: Lipid peroxidation in liver was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances and lipid hydroperoxides by the method of Niehius and Samuelson [33], Jiang et al. [34] respectively. Protein carbonyl content was determined by the method of Levine et al. [35]. The levels of conjugated dienes were assessed by the method of Rao and Recknagel [36].

Determination of non-enzymatic and enzymatic antioxidants: Reduced glutathione was determined by the method of Ellman [37]. Total sulfhydryl groups were measured by the method of Ellman [37]. Vitamin C concentration was measured as previously reported

[38]. Vitamin E (a-tocopherol) was estimated by the method of Desai [39]. Superoxide dismutase activity was determined by the method of Kakkar et al. [40]. The activity of catalase was determined by the method of Sinha [41]. Glutathione peroxidase activity was estimated by the method of Rotruck et al. [42]. Glutathione S-transferase activity was determined by the method of Habig et al. [43]. Glutathione reductase was assayed by the method of Horn and Burns [44]. The estimation of glucose-6-phosphate dehydrogenase was carried out by the method of Beutler [45]. Total protein content of tissue homogenate was estimated as described previously [46].

Estimation of membrane-bound ATPase: The sediment after centrifugation was resuspended in ice-cold Tris-HCl buffer (0.1M) pH 7.4. This was used for the estimations of membrane-bound enzymes and protein content. The membrane bound enzymes such as Na⁺/K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase activity were assayed by estimating the amount of phosphorous liberated from the incubation mixture containing tissue homogenate, ATP and the respective chloride salt of the electrolytes [47-49].

Estimation of DNA damage by single-cell gel electrophoresis (Comet assay): DNA damage was estimated by alkaline single-cell gel electrophoresis (Comet assay) according to the method of Singh et al. [50]. In this method, the cells were first lysed to form nucleoids. During electrophoresis, DNA fragments (from damaged DNA) streamed towards anode while the undamaged DNA trapped within the nucleus. When they are stained with SYBR green-I, damaged DNA gave the appearance of a comet tail and undamaged DNA gave spherical appearance. Prefrosted slides were prepared by pouring 3.0–5.0 mL of 1% normal agarose over clean glass slides. It was allowed to dry at room temperature and placed in hot-air oven at 70°C–80°C for 30 min. A freshly prepared suspension of 100 μ L of hepatocytes in 1% low-melting point agarose (LMPA; 1:3 ratio) was cast on to prefrosted microscopic slides, immediately covered with cover slip and kept for 10 min in a refrigerator to solidify. Then the cover slip was removed and a top layer of 100 μ L of LMPA was added and the slides were again cooled for 10 min. The cells were then lysed by immersing the slides in the lysis solution for 1 h at 4°C. After lysis, slides were placed in a horizontal electrophoresis tank. The unit was filled with electrophoresis buffer to a level of 0.25 cm above the slides. The cells were exposed to the alkaline electrophoresis solution for 20 min to allow DNA unwinding. Electrophoresis was conducted in a cold condition for 20 min at 25 V and 300 mA. After electrophoresis, the slides were placed horizontally and neutralized with Tris-HCl buffer. Finally, 50 μ L of SYBR green-I (1:10,000) dilution) was added to each slide and analyzed using a fluorescence microscope. To prevent additional DNA damage, all steps were conducted under dimmed light or in the dark. Twenty five images were randomly selected from each sample and were examined at 200 magnification in a fluorescence microscope connected to a personal computer-based image analysis system, Komet v. 5.0 (Kinetics Imaging Ltd., Liverpool, UK). The relative amount of DNA appearing in the tail of the comet (percent tail DNA), tail length and tail moment (% tail DNA \times length) were linearly related to DNA break frequency.

Histopathological studies

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10%formalin-saline and dehydrated by passing successfully in different mixture of ethyl alcohol, water, cleaned in xylene and embedded in paraffin. Sections of the tissues (5–6 μ m thick) were prepared by using a rotary microtone and stained with haematoxylin and eosin dye, which was mounted in a neutral deparaffined xylene

medium for microscopical observations. Six rats from each group were sacrificed for analyzing the hepatic histological examinations.

Statistical analyses

Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package (SPSS[®] for Windows, V. 17.0, Chicago, USA). Results were presented as mean \pm SD. Differences were considered significant if $P < 0.05$.

Results

Table 1 depicts the effects of Arsenic on body weight gain, food and water intake and relative liver weight in control and experimental rats. In As treated rats, water and pellet diet consumption significantly ($p < 0.05$) decreased with a decrease in body weight. A significant ($p < 0.05$) decrease in relative liver weight was recorded in Arsenic treated rats when compared with control rats. Pre-treatment with THC effectively attenuated the As-induced alterations in food and water intake and body weight when compared with Arsenic treated rats. Administration of THC alone to rats did not show any alterations in these parameters and did not differ significantly from that of the normal control group.

Table 2 illustrates the effects of Arsenic on hepatic marker enzymes in control and experimental rats. In arsenic treated rats, the activities of serum hepatospecific enzymes, were significantly ($P < 0.05$) increased, when compared with control rats. But, oral administration of THC (80 mg/kg bw/day) to normal rats did not show any significant ($P < 0.05$) effect on hepatic markers. Pre-treatment of THC (80 mg/kg bw/day) with arsenic significantly ($P < 0.05$) decreased the levels of serum hepatic markers and bilirubin.

Figure 3 exhibits the levels of blood and hepatic ALAD in control and experimental animals. As intoxicated animals showed a significant ($p < 0.05$) decrease in the levels of blood and hepatic ALAD when compared with control rats. Pre-administered with THC significantly ($p < 0.05$) restored the altered levels of blood and hepatic ALAD by Arsenic to near normalcy levels in rats when compared with As-treated rats.

Table 3 represents the effect of Arsenic on TBARS, LOOH, PC and

CD in control and experimental animals. The rats treated with Arsenic the levels of TBARS, LOOH, PC and CD were significantly ($p < 0.05$) increased when compared with control rats. Pre-administration of THC in arsenic intoxicated rats significantly ($p < 0.05$) decreased the level of these oxidative stress markers in liver when compared with arsenic treated rats.

Table 4 shows the effect of As on GSH, TSH, vitamins C and E in control and experimental animals. The levels of GSH, TSH, vitamins C and E were significantly ($p < 0.05$) decreased in the liver tissues of arsenic intoxicated rats when compared to control rats. Pre-administration of THC in arsenic treated rats significantly ($p < 0.05$) protected the depleted levels of GSH, TSH, vitamins C and E contents in liver when compared with arsenic treated rats. THC alone treated rats did not show any alterations in these parameters when compared with control group.

Table 5 illustrates the effect of As on SOD, CAT, GPx, GR, GST and G6PD in control and experimental animals. A significant ($p < 0.05$) decrease in the activities of SOD, CAT, GPx, GR, GST and G6PD were observed in arsenic intoxicated rats when compared with control rats. Pre-treatment with THC in arsenic intoxicated rats showed a significant ($p < 0.05$) increase in the activities of antioxidant enzymes in the liver when compared with arsenic intoxicated rats.

Figure 4 shows the effect of Arsenic on the activities membrane bound ATPase enzymes (Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase) in control and experimental animals. A significant ($p < 0.05$) decrease in the activities of membrane bound ATPase enzymes were observed in arsenic intoxicated rats as compared with control rats. Pre-administration with THC in arsenic treated rats significantly ($p < 0.05$) elevated the activities of ATPase enzymes in liver when compared with arsenic alone treated rats.

Figure 5 and 6 shows the alterations in the comet assay parameters of control and experimental rats. A significant ($p < 0.05$) increase in different comet assay parameter such as % DNA in tail, tail length and tail movement was observed in rats treated with Arsenic when compared with control rats. Pre-treatment THC along with Arsenic significantly ($p < 0.05$) reduced the % DNA in tail, tail length and tail movement in hepatocytes. Control and THC alone treated rats showed no DNA migration.

Variables	Control	Arsenic	Arsenic + THC	THC	
Body weight	Initial (g)	184 \pm 2.62	189 \pm 6.21	191 \pm 5.46	187 \pm 7.02
	Final (g)	216 \pm 9.88	162 \pm 7.45	195 \pm 10.24	218 \pm 8.46
Body weight gain (%)	15.78	5.95	12.46	16.07	
Food intake (g/100 g bw/day)	22.15 \pm 2.06 ^a	17.34 \pm 0.72 ^b	19.56 \pm 1.18 ^c	25.02 \pm 0.78 ^a	
Water intake (mL/rat/day)	17.13 \pm 1.22 ^a	11.04 \pm 1.78 ^b	14.47 \pm 1.14 ^c	18.43 \pm 0.68 ^a	
Organ-body weight ratio (%) liver	2.41 \pm 0.64 ^a	3.52 \pm 0.58 ^b	3.07 \pm 1.42 ^c	3.76 \pm 0.59 ^a	

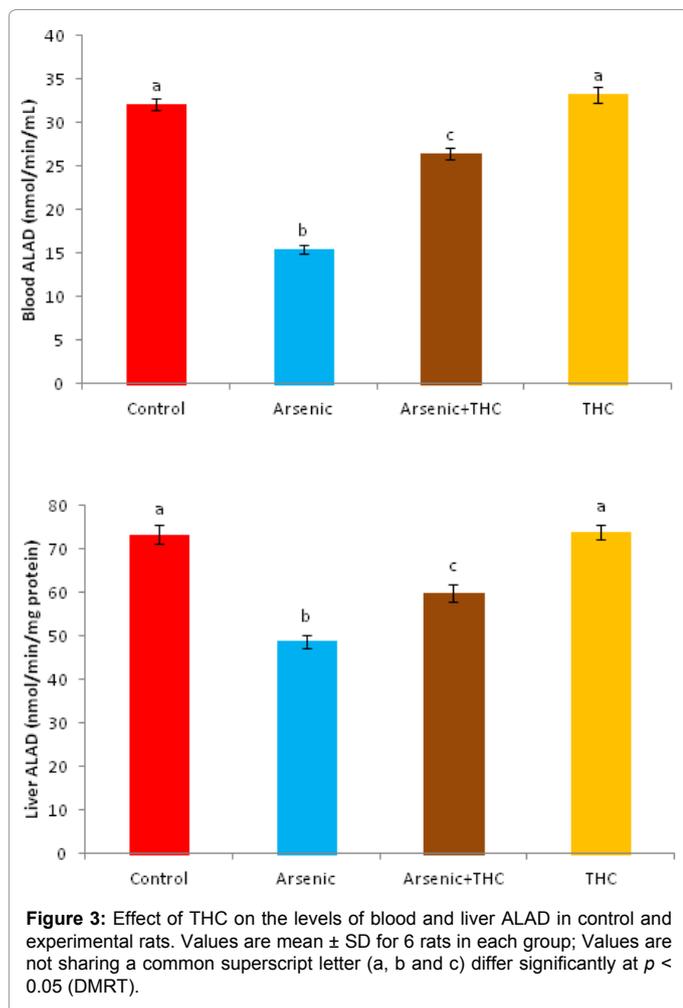
Values are expressed mean \pm SD for six rats in each group; ^{a, b and c}Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

Table 1: Changes in body weight, body weight gain, food intake, water intake and organ-body weight ratio in control and experimental rats.

Groups	Control	Arsenic	Arsenic + THC	THC
AST (min/mg protein)	120.48 \pm 7.84 ^a	168.02 \pm 9.12 ^b	143.09 \pm 6.77 ^c	118.41 \pm 4.78 ^d
ALT (min/mg protein)	46.68 \pm 4.52 ^a	78.37 \pm 3.14 ^b	59.46 \pm 5.23 ^c	38.92 \pm 5.18 ^d
ALP (min/mg protein)	89.64 \pm 6.75 ^a	118.58 \pm 8.04 ^b	109.07 \pm 7.47 ^c	82.96 \pm 6.05 ^d
LDH (min/mg protein)	149.78 \pm 8.64 ^a	186.52 \pm 7.58 ^b	169.57 \pm 9.02 ^c	140.66 \pm 6.17 ^d
GGT (μ g/mg protein)	0.54 \pm 0.05 ^a	0.98 \pm 0.06 ^b	0.76 \pm 0.07 ^c	0.46 \pm 0.02 ^d
Bilirubin (μ g/mg protein)	0.18 \pm 0.04 ^a	0.54 \pm 0.07 ^b	0.34 \pm 0.06 ^c	0.09 \pm 0.05 ^d

Values are mean \pm SD for 6 rats in each group; ^{a, b, c and d}Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT).

Table 2: Effect of THC on the levels of AST, ALT, ALP, LDH, GGT and Bilirubin in the liver of control and experimental rat



Groups	Control	Arsenic	Arsenic + THC	THC
ROS (pmol/mg protein)	12.83 \pm 3.05 ^a	34.04 \pm 3.98 ^b	23.76 \pm 1.67 ^c	11.96 \pm 2.99 ^a
TBARS (nmol/100g wet tissue)	4.27 \pm 0.65 ^a	9.02 \pm 0.85 ^b	6.91 \pm 1.02 ^c	3.24 \pm 0.71 ^d
LOOH (nmol/100g wet tissue)	0.95 \pm 0.12 ^a	2.92 \pm 0.34 ^b	1.64 \pm 0.25 ^c	0.83 \pm 0.07 ^d
CD (nmol/100g wet tissue)	29.49 \pm 4.62 ^a	52.94 \pm 7.34 ^b	38.02 \pm 5.21 ^c	24.36 \pm 2.42 ^d
PC (nmol/mg protein)	8.24 \pm 0.57 ^a	17.53 \pm 0.68 ^b	13.46 \pm 0.62 ^c	5.96 \pm 0.76 ^d

Values are mean \pm SD for 6 rats in each group; a, b, c and d Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT). The levels of TBARS were expressed as nmol/100g wet tissue. The levels of LOOH and CD were expressed as nmol/100g wet tissue. The levels of PC were expressed as nmol/mg protein.

Table 3: Effect of THC on the levels of TBARS, LOOH, CD and PC in the liver of control and experimental rats.

Histopathological studies show that arsenic administration induces various pathological changes in the liver (Figure 7). Control rats and THC treated rats showed a normal architecture of the liver. Exposure of arsenic resulted in changes in liver architecture as indicated by portal triad with mild inflammation, cell infiltration, focal necrosis and giant cell formation. Arsenic along with THC supplementation showed near normal hepatocytes with mild portal inflammation.

Discussion

The present study was designed to evaluate the protective and ameliorative efficacy of THC on Arsenic induced oxidative hepatic dysfunction in rats. From the earlier findings, reduction of body weight is used as a key indicator for the deterioration of rat general health status [51] and an excessive Arsenic exposure has changed body weight, absolute and relative liver weights, leading to significant decrease in animal growth and alterations in organ-body weight ratio in the present study. Similarly, these observations were related to our previous publication by Muthumani and Milton prabu [52] who reported that high Arsenic exposure have significantly induced disturbances of the intake of water and food, total body weight, absolute and relative liver weights of rats. Accordingly, all the morphological changes observed in arsenic intoxicated rats were ameliorated by administered with THC.

Exposure of arsenic leads to liver injury indicating that the cellular leakage of serum hepatic marker enzymes into blood stream and loss of functional integrity of hepatic membrane architecture [53]. Our results were representing a significant increase in the levels of AST, ALT, ALP and LDH from hepatocyte membrane damage due to Arsenic intoxication. GGT is known as an important enzyme for liver function and recently it has been used as an oxidative stress marker through generation of free radicals via Fenton reaction in the presence of iron [54]. Previous report of Muthumani and Milton prabu [52] showed that the increase level of total bilirubin concentration was associated with free radical production in rats treated with arsenic. Pre-

Groups	Control	Arsenic	Arsenic + THC	THC
GSH (μ g/mg protein)	64.45 \pm 3.53 ^a	38.54 \pm 2.91 ^b	52.32 \pm 3.21 ^c	66.93 \pm 4.02 ^d
TSH (μ g/mg protein)	18.04 \pm 0.25 ^a	9.73 \pm 0.26 ^b	13.29 \pm 0.45 ^c	22.79 \pm 0.39 ^d
Vitamin C (μ mol/mg tissue)	2.94 \pm 0.09 ^a	1.28 \pm 0.06 ^b	2.03 \pm 0.04 ^c	3.48 \pm 0.08 ^d
Vitamin E (μ mol/mg tissue)	0.32 \pm 0.04 ^a	0.17 \pm 0.03 ^b	0.26 \pm 0.04 ^c	0.38 \pm 0.06 ^d

Values are mean \pm SD for 6 rats in each group; a, b, c and d Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT). The levels of GSH and TSH were expressed as μ g/mg protein. The levels of vitamins C and E were expressed as μ mol/mg tissue.

Table 4: Effect of THC on the levels of GSH, TSH, vitamins C and E in the liver of control and experimental rats.

Groups	Control	Arsenic	Arsenic + THC	THC
SOD (min/mg protein)	8.54 \pm 0.32 ^a	5.08 \pm 0.18 ^b	6.59 \pm 0.24 ^c	9.96 \pm 0.58 ^d
CAT (min/mg protein)	122.48 \pm 8.24 ^a	86.65 \pm 6.08 ^b	102.49 \pm 7.81 ^c	126.39 \pm 6.93 ^d
GPx (min/mg protein)	6.02 \pm 0.48 ^a	3.91 \pm 0.53 ^b	5.02 \pm 0.61 ^c	7.78 \pm 0.74 ^d
GST (min/mg protein)	0.64 \pm 0.12 ^a	0.31 \pm 0.14 ^b	0.52 \pm 0.17 ^c	0.85 \pm 0.21 ^d
GR (μ g/mg protein)	0.32 \pm 0.04 ^a	0.19 \pm 0.05 ^b	0.25 \pm 0.04 ^c	0.38 \pm 0.06 ^d
G6PD (μ g/mg protein)	3.07 \pm 0.72 ^a	1.24 \pm 0.49 ^b	2.02 \pm 0.53 ^c	4.05 \pm 0.41 ^d

Values are mean \pm SD for 6 rats in each group; a, b, c and d Values are not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (DMRT). SOD – One unit of enzyme activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute/mg protein. CAT – μ mol of H₂O₂ utilized/min/mg protein. GPx – μ g of GSH consumed/min/mg protein. GST – μ mol of CDNB-GSH conjugate formed/min/mg protein. The activities of GR were expressed as nmol of NADPH oxidized/min/mg protein. G6PD were expressed as nmol of NADPH formed/min/mg protein.

Table 5: Effect of THC on the levels of SOD, CAT, GPx, GST, GR and G6PD in the liver of control and experimental rats

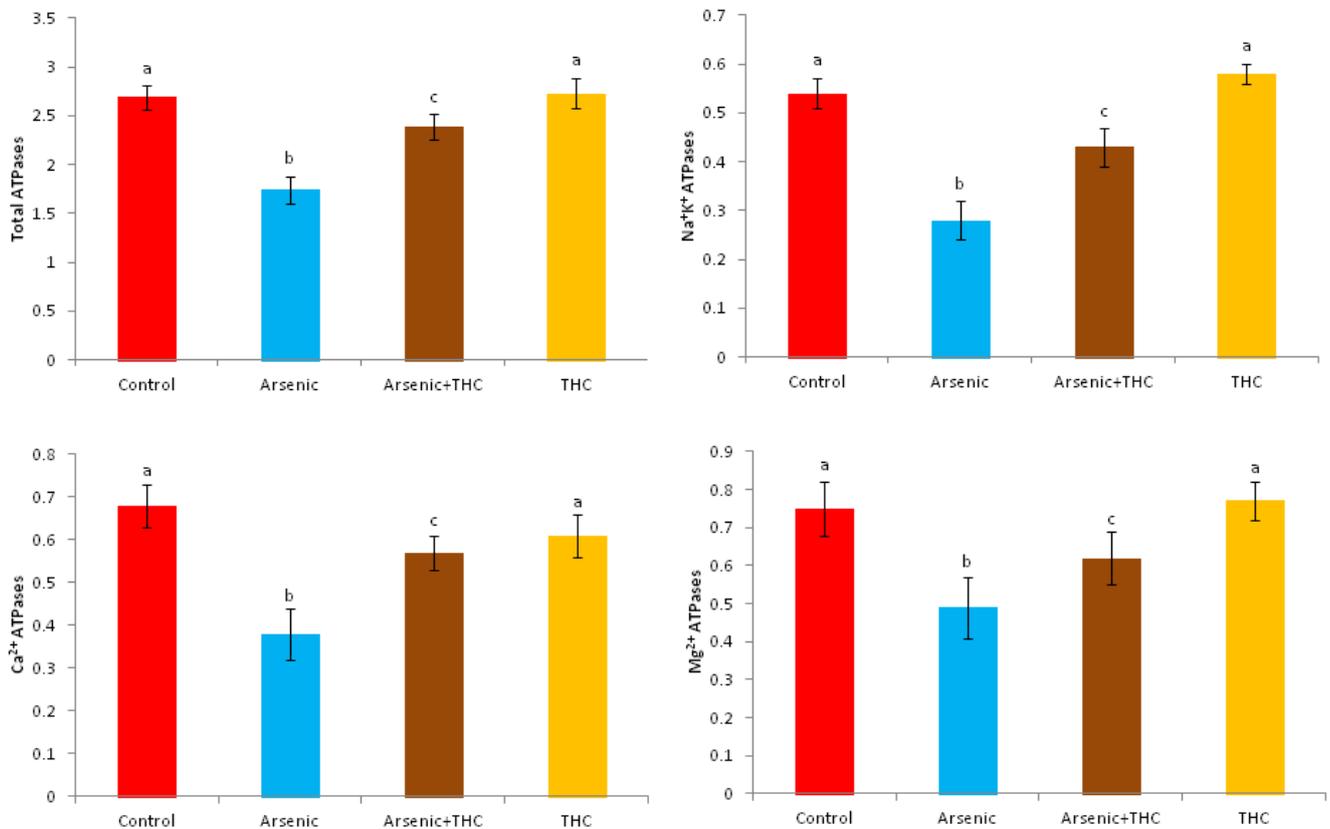


Figure 4: Effect of THC on the activities of membrane bound adenosine triphosphatases (ATPase) enzymes in the liver of control and experimental rats. Values are mean \pm SD for six rats in each group; Values are not sharing a common superscript letter (a, b, and c) differ significantly at $p < 0.05$ (DMRT); Units for ATPases $\mu\text{g pi liberated/min/mg protein}$.

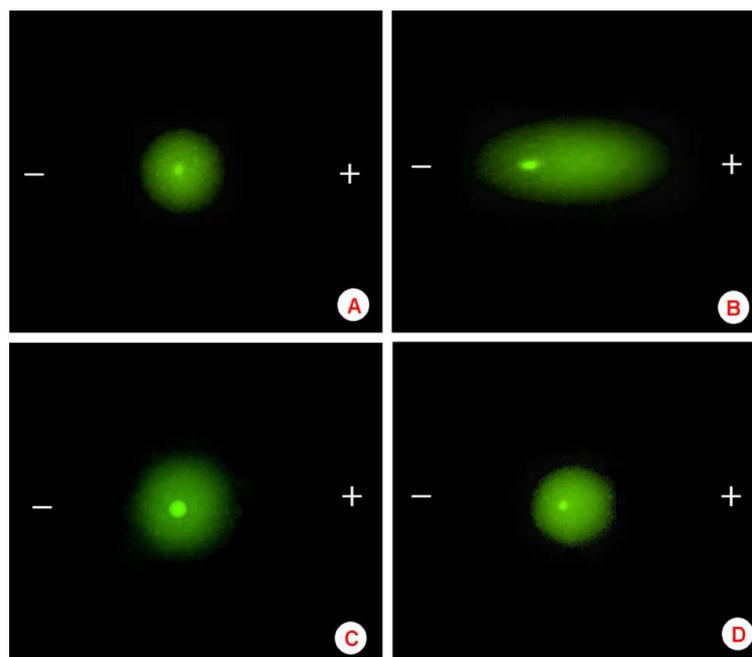


Figure 5: Representative photographs of comets stained with SYBR green-I at a magnification of 200X showing the DNA migration pattern in hepatocytes. (A) Control group shows no DNA migration. (B) Arsenic treated group shows extensive DNA migration. (C) THC administered arsenic intoxicated group shows the minimal DNA migration. (D) THC administered group shows no DNA migration. The symbols - and + represents cathode and anode, respectively, during electrophoresis.

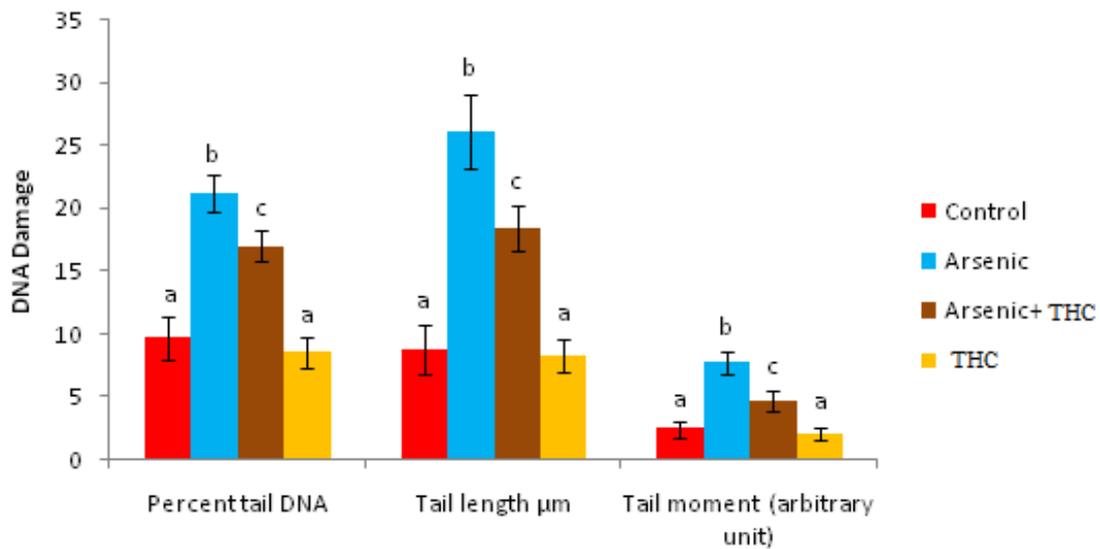


Figure 6: Effect of THC on DNA damage in the hepatocytes of control and experimental rats. Arbitrary unit = Percentage tail DNA \times tail length; Values are mean \pm SD for six rats in each group; Values are not sharing a common superscript letter (a, b and c) differ significantly at $p < 0.05$ (DMRT).

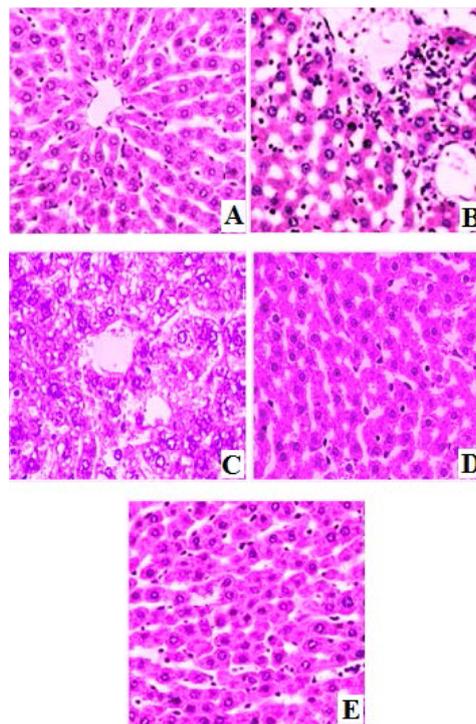


Figure 7: Representative photographs from the liver of control and experimental rats: Histologic features (A-E) of liver sections stained with H&E treatment. Typical images were chosen from each experimental group. (Original magnifications 200X). (A) Control group showing normal hepatic architecture. (B&C) Arsenic treated group showing the severe hepatocellular necrosis, inflammatory cell infiltration and apoptotic changes in liver tissue. (D) THC+ Arsenic treated group showing almost normal histological features of liver. (E) THC alone treated rat showing the normal histological features of liver tissue.

administration of THC decreased the levels of AST, ALT, ALP, LDH, GGT and bilirubin, thus offering protection against arsenic induced toxicity by stabilizing the cell membrane of hepatocytes [55].

δ -Aminolevulinic acid dehydratase (ALAD) is a sulfhydryl containing enzyme that catalyzes the asymmetric condensation of two molecules of aminolevulinic acid (ALA-substrate) to prophobilinogen

during heme synthesis pathway. Administration of Arsenic exerted a significant depletion in the levels of blood and liver ALAD. Since arsenic has a high affinity for sulfhydryl, this may lead to possible inhibition of ALAD activity. An inhibition in the activity of blood and liver ALAD may lead to a significant accumulation of ALA. Therefore, there was a possibility of increased ALA might generate more reactive oxygen

intermediates (ROIs) and thus oxidative stress [56]. Pre-administration of THC significantly restored these blood and liver ALAD activities due to the direct scavenging effects of reactive oxygen species and its ability to inhibit thiol group oxidation [27].

Further, lipids are also participating in function of membrane integrity, while TBARS, LOOH and CD were measured as the index of lipid peroxidation [57]. Elevated level of protein carbonyl content also formed from oxidation of protein due to Arsenic exposure was observed in the present investigation. Results from the present study, there was a significant decreased levels of TBARS, LOOH, PC and CD in the liver. Decreased levels of oxidative stress markers expressed in arsenic treated rats pre-administered with THC, due to its radical scavenging activity leads to prevention of lipid peroxidation [27].

GSH is a tripeptide non-enzymatic antioxidant and protects cells against arsenic induced reactive oxygen species [58]. Reduced GSH is required for vitamins C and E to regenerate its active forms. Further, GSH prevent the oxidation of sulfhydryl groups [59]. In our study, GSH level decreased in the liver of As-treated rats due to oxidation of GSH by free radicals [60] or sulfhydryl group of cysteine moiety in GSH has a high affinity for Arsenic forming As-GSH complex [15] or its electron donor ability [61] and finally these complexes excreted via the bile [60]. Vitamin E is an important lipid soluble antioxidant present in cells and play a major role in scavenging of free radicals including 1O_2 , $HO\cdot$, $\bullet O_2$, Peroxyl, and alkoxyl radicals in lipid peroxidation [62]. Moreover, Vitamin C reduces the vitamin E derived tocopheroxyl radical $\alpha\text{-TO}\cdot$ [63]. Pre-administration of THC lack the free radicals complex with GSH by the radical scavenging activity lead to increases the levels of total sulfhydryl group (TSH), vitamins C and E in the presence of GSH [27].

Several enzymatic antioxidants such as SOD, CAT, GPx, GST, GR and G6PDH are the first line of defence against oxidative stress induced by As. SOD and CAT are the two basic subcellular defence of antioxidant system that counteracts free radicals produced during As exposure. SOD dismutase the superoxide anion to H_2O_2 , CAT converts these hydroxyl radical into water molecule. A decreased activity of SOD and CAT in arsenic-exposed rats reflects elevated production of superoxide radical anions [64] and insufficient requirement of NADPH, which is required for the activation of CAT from its inactivated form [65]. Furthermore, glutathione-related enzymes, such as GPx, GR and GST act as antioxidants either directly or indirectly against Arsenic induced hepato toxicity. GST is an isoenzyme involved in the process of forming S-substitute in GSH during reacts with toxicants [66]. Due to the presence of selenium in GPx lead to inhibition of GPx activity through the arsenic-selenium complex [67]. Both GST and GPx reduce the organic hydro peroxides within membranes and lipoproteins in the presence of GSH. In addition decreased levels of GR and G6PD in arsenic treated rats showed the inhibition of GSH, which are required for the reduction of GSSG to GSH. Accordingly oral pre-administration of THC in the liver of arsenic-treated rats elevated the levels of enzymatic antioxidants which could be due to the orientation of functional groups in 3rd, 4th position of methoxy, hydroxyl group in benzene ring and positions of 3rd and 5th identical β -diketone in heptane moiety.

Membrane bound ATPases are the sulfhydryl group containing enzymes and lipid dependant [68]. Reduction in the activity of these enzymes might be due to enhanced lipid peroxidation by free radicals. Arsenic enhances the production of free radicals in the liver of rat and interferes with the antioxidant defence system leads to the alteration of structural integrity of membrane lipids and secondarily affects the

membrane bound enzymes. In the present study, reduced activity of Mg^{2+} ATPase and Na^+/K^+ ATPase are responsible for ionic imbalance caused by As which damages the membranous lipids. Moreover, Arsenic decreased the activity of Ca^{2+} ATPase observed in the result due to high affinity for SH groups. This could be due to the ability of THC to protect the sulfhydryl group from oxidative damage through the inhibition of lipid peroxidation by scavenging of free radicals [27]. Interestingly, the restoration of membrane bound enzymes supported by the restoration of serum marker enzymes.

Mostly, the assay of comet is a rapid, perceptive and versatile method for the quantification of DNA damage in the individual cells [69]. The increased levels of % DNA in tail, tail length, and tail movement were observed in the present study. Earlier studies demonstrated that the lipid peroxidation products of polyunsaturated fatty acids such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) play a major role in genotoxicity of the cell [70]. Results of the present studies are reliable with the previous reports, which show that arsenic produces DNA single strand breaks [71]. The levels of DNA damage significantly decreased in the hepatocytes of THC treated rats. THC is a well-known free-radical scavenger and thereby it reduces the ROS mediated lipid peroxidation and oxidative DNA damage by arsenic. Prabhu et al. [27] demonstrated that THC acts as an effective scavenger of reactive oxygen radicals in perfused hepatocytes and thereby reduced the oxidative stress.

Various pathological alterations were observed in the liver of arsenic intoxicated rats such as portal triad with mild inflammation, cell infiltration, focal necrosis, and giant cell formation. Pre-administration of THC recovered the histological alterations induced by arsenic in the liver.

From the present investigation, the chemical moieties which are attributed to the antioxidant property of THC are hydroxyl and methoxyphenyl groups in 4, 4' and 3, 3' position in benzene ring and shows identical β -diketone of 3rd and 5th substitution in heptane moiety. THC showed the highest potency, implying that hydrogenation of curcuminoids increased their antioxidant activity. Moreover THC is a phenolic chain-breaking antioxidant, which donates H atoms from the phenolic OH groups and the H atom donation takes place at the active methylene groups in the diketone moiety. Phenolic groups in THC are essential for activity, and are more effective at the *para* position than at the *ortho* position. In addition, an electron donating group at the *ortho* position relative to the phenolic group is also required for activity. This theoretical approach favors the necessity of a phenolic OH group for the antioxidant activity of THC. It concludes that arsenic induced hepato toxicity can be significantly prevented by the antioxidant efficacy of THC.

Conclusion

From the observations, we conclude that Arsenic administration results in pronounced oxidative stress and cellular damage to that liver tissue. Pre-administration of THC was found to be effective in ameliorating the biochemical abnormalities caused by Arsenic. The histopathological findings also confirmed the cytoprotection rendered by THC.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgement

Authors extend their thanks to the Professor and Head, Department of Zoology and UGC-SAP for their generous support in this study.

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