Aqueous bark extract of *Terminalia arjuna* protects against high fat diet aggravated arsenic-induced oxidative stress in rat heart and liver: involvement of antioxidant mechanisms

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**ABSTRACT**

**Back ground:** *Terminalia arjuna* (TA) Wight & Arn. (Combretaceae) is a tree having an extensive medicinal potential. The plant is used traditionally in the treatment of various ailments. Arsenic, a well-known groundwater contaminant causes various disorders via oxidative stress. Nutrition can affect arsenic toxicity by several plausible mechanisms. The present study is aimed at investigating the effect of aqueous bark extract of TA against high fat diet aggravated arsenic-induced oxidative damages in rat liver and heart tissues. **Methods:** For the present study, the average weight of rats remained within a range of 145g-170g. Finally, the animals were divided into ten groups. Each group of animals comprised of 6 rats. Control group of rats were fed with the standard laboratory diet that contained (for 100 g) 13.9 g protein (14.2% of total energy), 61.8 g carbohydrate (63.4%), 3.9 g fat (9%) and appropriate amounts of vitamins and minerals. The arsenic treated groups of rats as well as the rats of the positive control groups were also fed with standard laboratory diet with the above mentioned composition. The HFD contained (per 100 g) 11.1 g protein (11.4% of total energy), 32.8 g carbohydrate (33.6%) and fat 26.1 g (60%). The rats of the HFD groups were pair fed in respect to the rats of the control group. The rats of group VIII, IX and X were also fed with HFD. After acclimatization to laboratory environment, the rats of the treated groups (i.e., group V, VII, VIII, IX and X) were administered sodium arsenite at a dose of 0.75mg/kg body weight (5% of LD₅₀), i.p. every day for a period of 8 days. The rats of the group VIII, IX and X were co-treated also with aqueous TA bark extract at the dose of 20, 40 and 60mg/kg of bw, respectively. **Results:** We have determined heart weight to body weight ratio as well as analyzed various functional markers, oxidative stress bio-markers and also the activity of the antioxidant enzymes. Tissue morphological studies confirmed the biochemical investigations. **Conclusion:** The results raise the possibility of the aqueous bark extract of TA being considered for future use as a therapeutic antioxidant intervention.

**KEY WORDS:** Antioxidant, arsenic, heart, high fat diet, liver, oxidative stress, rat, *Terminalia arjuna*

**INTRODUCTION**

Arsenic pollution in the environment is becoming a major concern for environmental and occupational health, owing to its widespread toxic and multidimensional effects on humans and aquatic life and plants through polluted ground water and food chains. Groundwater contamination with arsenic in West Bengal, India, is reported to be the largest arsenic calamity in the world. Arsenic toxicity involves oxidative damage that plays a vital role for biochemical alteration. Arsenic possesses the ability to generate reactive radicals, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNA. These reactive radical species include a wide variety of oxygen-, carbon-, sulfur- and nitrogen-radicals, originating not only from superoxide radical, hydrogen peroxide, and lipid peroxides but also in chelates of amino-acids, peptides, and proteins complexes with the toxic metals. This metal generates reactive species, which in turn may cause neurotoxicity, hepatotoxicity and nephrotoxicity in humans and animals.
the remethylation step. This step seems to be most critical from a toxicological point of view. A second mode of arsenic-nutrition interaction involves epigenetic effects and fetal reprogramming via DNA methylation\textsuperscript{7}. Our previous study has established that high fat diet aggravates arsenic-induced oxidative damages in rat heart and liver\textsuperscript{8}. But, till date there is no information in the scientific literature regarding protective effect of aqueous bark extract of TA against high fat diet aggravated arsenic-induced oxidative damages in rat heart and liver. Aqueous extract of the bark of TA has earlier been demonstrated to have beneficial effect in various health situations. Herein, we provide evidences that the aqueous bark extract of TA has the capability to protect against high fat diet aggravated arsenic-induced oxidative stress in vivo in rat heart and liver tissues and antioxidant mechanism(s) may be responsible for such protections.

MATERIALS AND METHODS

Chemicals
Sodium arsenite was purchased from Sigma Aldrich, USA. All the other chemicals used including the solvents, were of analytical grade and obtained from Sisco Research Laboratories (SRL), Mumbai, India, Qualigens (India/Germany), SD fine chemicals (India), Merck Limited, Delhi, India.

Preparation of aqueous TA bark extract
Five gm of TA bark powder was dissolved in 25ml of double distilled water. After proper mixing, it was kept overnight with cotton plugging (for approximately 16 hours). Then, it was centrifuged twice at 1300g for 10 minutes. The supernatant, thus obtained, was collected, deep frozen and lyophilized. The yield of the aqueous TA bark extract, in our case was 10\textsuperscript{7}.

Animals
Male Wistar rats, weighing 140–200 g, were obtained from a CPCSEA registered animal supplier. The animals were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Government of India. The protocols using animals had the approval of the Institutional Anil Ethics Committee (IAEC), Department of Physiology, University of Calcutta.

Co-treatment of rats with high fat diet and arsenic and protection by aqueous TA bark extract
The rats were maintained in the animal room at a temperature of 25 ± 1 °C, humidity 50 ± 10% and a 12-h light/dark cycle. The rats were provided with a standard diet containing 18% protein (casein) and water ad libitum for 7 days (this is referred to as the quarantine period). The 18% protein diet was considered as an adequate dietary protein level, which was used on earlier occasions\textsuperscript{8}.

For the present study, the average weight of rats remained within a range of 145g-170g. Finally, the animals were divided into ten groups. Each group of animals comprised of 6 rats and the different groups are listed below:

- **Group I**: Control
- **Group II**: Rats treated only with aqueous TA bark extract at the dose of 20mg/kg of body weight (bw) (T20)
- **Group III**: Rats treated only with aqueous TA bark extract at the dose of 40mg/kg of bw (T40)
- **Group IV**: Rats treated only with aqueous TA bark extract at the dose of 60mg/kg of bw (T60)
- **Group V**: Rats treated with arsenic (As) only
- **Group VI**: Rats fed on high fat diet (HFD) only
- **Group VII**: Arsenic treated rats fed on high fat diet (As + HFD)
- **Group VIII**: Arsenic treated rats fed on high fat diet along with aqueous TA bark extract at the dose of 20mg/kg of bw (As+HFD+T20)
- **Group IX**: Arsenic treated rats fed on high fat diet along with aqueous TA bark extract at the dose of 40mg/kg of body weight (As+HFD+T40)
- **Group X**: Arsenic treated rats fed on high fat diet along with aqueous TA bark extract at the dose of 60mg/kg of bw (As+HFD+T60)

After acclimatization to laboratory environment, the rats of the treated groups (i.e., group V, VII, VIII, IX and X) were administered sodium arsenite at a dose of 0.75mg/kg body weight (5% of LD\textsubscript{50}), i.p. every day for a period of 8 days. The rats of the group VIII, IX and X were co-treated also with aqueous TA bark extract at the dose of 20, 40 and 60mg/kg of bw, respectively.

Collection of blood and tissue samples
At the end of the treatment period, the rats were kept fasting overnight and sacrificed through cervical dislocation after subjecting them to mild ether anesthesia. The chest cavity was surgically opened through vertical incision and blood was carefully collected through cardiac puncture for the preparation of serum and the heart surgically removed. The liver was also removed surgically after carefully opening the abdominal cavity. The collected heart and liver tissues were rinsed well in cold saline, soaked properly with a piece of blotting paper and stored in sterile plastic vials at -20°C for further biochemical
analyses. For tissue morphological studies, a suitable amount of the cardiac and the hepatic tissues were placed immediately in appropriate fixatives. Each set of experiment was repeated at least two times.

**Measurement of body weight and determination of heart weight to body weight ratio**

The body weights of rats of all the groups before and after the treatment were noted. Heart weight of each of the rats was measured following sacrifice and heart weight to body weight ratio was determined using the body weight of each of the rats on the day of sacrifice.

**Preparation of tissue homogenate**

A 10% tissue homogenate of each of heart and liver was prepared in cold in two separate buffer solutions, viz., ice cold 0.1M phosphate buffer (pH 7.4) and 1mM EDTA buffer, respectively, using a Potter Elvenjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA) for 30 sec. The homogenates were kept in cold and processed for biochemical analyses within 30 minutes of preparation.

**Isolation of mitochondria from heart and liver tissues**

The mitochondria from heart and liver tissues were isolated according to the procedure of Dutta et al., (2013). A portion of the heart and liver tissues were cleaned and cut into small pieces. Five hundred mg of both the tissues were placed separately in 10 ml of sucrose buffer [0.25(M) sucrose, 0.001(M) EDTA, 0.05(M) Tris-HCl (pH 7.8)] at 25°C for 5 min. The tissues were then homogenized separately in cold for 1 minute at low speed by using a Potter Elvenjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA). The homogenates were centrifuged at 1500 rpm for 10 minutes at 4°C. The supernatant was poured through several layers of cheesecloth and kept in ice. This filtered supernatant was centrifuged at 4000 rpm for 5 minutes at 4°C. The supernatant, thus obtained, was further centrifuged at 14000 rpm for 20 minutes at 4°C. The final supernatant was discarded and the pellet was re-suspended in sucrose buffer and stored at -20°C until further analysis. However, most of the enzymatic assays were carried out with freshly prepared mitochondria.

**Measurement of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) activities**

Serum AST and serum ALT activities were measured by standard methods. Non-hemolyzed serum was mixed with glutamate pyruvate transaminase substrate and incubated for 30 min at 37°C. Then, 2, 4-dinitrophenyl hydrazine (DNPH) solution was added, mixed and kept for 20 min at room temperature. Thereafter, 0.4(N) NaOH was added, mixed and kept at room temperature for 10 min. The intensity of the developed colour was noted at 540 nm after setting the UV/VIS spectrophotometer to zero with water. (Bio-Rad, Hercules, CA, USA). The serum enzyme activities were expressed as IU/L.

**Measurement of serum total lactate dehydrogenase (LDH), lactate dehydrogenase 1 (LDH1) and lactate dehydrogenase 5 (LDH5) activities**

The serum total LDH activity was obtained by measuring the oxidation of NADH (0.1 mM) to NAD+ at 340 nm using 1.0 mM sodium pyruvate as substrate, after incubating the serum samples at 37°C for 30 min according to the method of Strittmatter (1965) with some modifications. The enzyme activity was expressed as IU/L.

The serum LDH1 activity was obtained by measuring the oxidation of NADH (0.1 mM) to NAD+ at 340 nm using 1.0 mM sodium pyruvate as substrate, after incubating the serum samples at 65°C which destroys all isozymes except LDH1 for 30 min according to the method of Strittmatter (1965) with some modifications. The enzyme activity was expressed as IU/L.

The serum LDH5 activity was obtained by measuring the oxidation of NADH (0.1 mM) to NAD+ at 340 nm using 1.0 mM sodium pyruvate as substrate, after incubating the serum samples at 55°C which destroys all isozymes except LDH5 for 30 min according to the method of Strittmatter (1965) with some modifications. The enzyme activity was expressed as IU/L.

**Measurement of cardiac and hepatic tissue lipid peroxidation (LPO) level, reduced glutathione (GSH) and protein carbonyl (PCO) contents**

The lipid peroxides in the cardiac and hepatic tissue homogenates were determined separately as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust and some modifications as adopted by Bandyopadhyay et al., (2004). Briefly, the homogenates were mixed with thiobarbituric acid–trichloroacetic acid (TBA–TCA) reagent with thorough shaking and heated for 20 min at 80°C. The samples were then cooled to room temperature. The absorbance of the pink chromogen present in the clear supernatant after centrifugation at 12,000g for 10 min at room temperature was measured at 532 nm using a UV–VIS spectrophotometer (Bio-Rad, Hercules, CA, USA). The values were expressed as nmoles of TBARS/mg of protein.

The GSH content (as acid soluble sulfhydryl) of both the cardiac and hepatic tissues was estimated separately by its reaction with DTNB (Ellman’s reagent) following the method of Sedlak et al., (1968) with some modifications by Bandyopadhyay et al., (2004). Incubated sample was mixed with Tris–HCl buffer, pH 9.0, followed by DTNB for color development. The absorbance was measured at 412 nm using a UV–VIS spectrophotometer to determine the GSH content. The values were expressed as nmoles of GSH/mg of protein.
The PCO content was estimated by DNPH assay\(^{16}\). One fourth of a milliliter of the homogenates of cardiac and hepatic tissues were separately taken in respective tubes and 0.5 ml DNPH in 2.0 M HCl was added to the tubes. The tubes were vortexed every 10 min in the dark for 1 h. Proteins were then precipitated with 30% TCA and centrifuged at 4000g for 10 min at 4°C. The pellet was washed three times with 1.0 ml of ethanol: ethyl acetate (1:1, v/v). The final pellet was dissolved in 1.0 ml of 6.0 M guanidine HCl in 20 mM potassium dihydrogen phosphate (pH 2.3) solution. The absorbance was determined spectrophotometrically at 370 nm. The PCO content was calculated using a molar absorption coefficient of 2.2X 10\(^{-4}\) M\(^{-1}\) cm\(^{-1}\). The values were expressed as nmoles /mg of protein.

**Measurement of the activities of Cu-Zn superoxide dismutase (Cu-Zn SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) of rat cardiac and hepatic tissues**

Copper–zinc superoxide dismutase (SOD1) activity was measured by hematoxylin autooxidation method of Martin et al., (1987) with some modifications as adopted by Dutta et al., (2014)\(^{17,18}\). Briefly, the weighed amounts of cardiac and liver tissues were homogenized (10%) separately in ice-cold 50 mM phosphate buffer containing 0.1 mM EDTA, pH 7.4. The homogenates were centrifuged at 12,000g for 15 min at 4°C and the supernatant was collected. Inhibition of hematoxylin auto-oxidation by the cell free supernatant was measured at 560 nm using a UV–VIS spectrophotometer (Bio-Rad Smart spec Plus). The enzyme activity was expressed as units/ mg of tissue protein.

The CAT activity was assayed by the method of Beers et al., (1952) with some modifications as adopted by Chattopadhyay et al., (2003)\(^{19,20}\). A weighed amount of cardiac and liver tissue was homogenized (5%) separately in ice-cold 50 mM phosphate buffer, pH 7.0. The homogenate was centrifuged in cold at 12,000g for 12 min at 4°C. The supernatants, thus obtained, were then collected and incubated with 0.01 ml of absolute ethanol at 4°C for 30 min, after which 10% Triton X-100 was added so as to have a final concentration of 1%. The samples, thus obtained, were used to determine CAT activity by measuring the breakdown of H\(_2\)O\(_2\) spectrophotometrically at 240 nm. The enzyme activity was expressed as µmoles of H\(_2\)O\(_2\) consumed/ mg tissue protein.

The GR activity was measured according to the method of Krohne-Ehrich et al., (1977)\(^{21}\). The assay mixture in a final volume of 3 ml contained 50 mM phosphate buffer, 200 mM KCl, 1 mM EDTA and water. The blank was set with this mixture. Then, 0.1 mM NADPH was added together with suitable amount of cardiac or liver tissue homogenate into the cuvette as the source of enzyme. The reaction was initiated with 1 mM oxidized glutathione (GSSG). The decrease in NADPH absorption was monitored spectrophotometrically at 340 nm. The specific activity of the enzyme was calculated as units/ mg tissue protein.

The GPx activity was measured according to the method of Paglia and Valentine with some modifications as adopted by Dutta et al., (2014)\(^{22,23}\). A weighed amount of cardiac and hepatic tissues were separately homogenized (10%) in ice cold 50 mM phosphate buffer containing 2 mM EDTA, pH 7.0. The assay system contained, in a final volume of 1 ml, 0.05 M phosphate buffer with 2 mM EDTA, pH 7.0, 0.025 mM sodium azide, 0.15 mM glutathione, and 0.25 mM NADPH. The reaction was started by the addition of 0.36 mM H\(_2\)O\(_2\). The linear decrease of absorbance at 340 nm was recorded using a UV/VIS spectrophotometer. The specific activity was expressed as nmols of NADPH produced/ mg tissue protein.

**Measurement of the activities of pyruvate dehydrogenase (PDH) and some of the Kreb’s cycle enzymes**

The PDH activity of rat cardiac and hepatic tissues were measured spectrophotometrically according to the method of Chretien et al., (1995) with some modifications by following the reduction of NAD\(^+\) to NADH at 340 nm using 50 mM phosphate buffer, pH 7.4, 0.5 mM sodium pyruvate as the substrate and 0.5 mM NAD\(^+\) in addition to the enzyme\(^{24}\). The enzyme activity was expressed as units/ mg tissue protein.

Isocitrate dehydrogenase (ICDH) activity of rat cardiac and hepatic tissues were measured according to the method of Duncan et al., (1979) by measuring the reduction of NAD\(^+\) to NADH at 340 nm with the help of a UV–VIS spectrophotometer\(^{25}\). One ml assay volume contained 50 mM phosphate buffer, pH 7.4, 0.5 mM isocitrate, 0.1 mM MnSO\(_4\), 0.1 mM NAD\(^+\) and the suitable amount of mitochondria as the source of enzyme. The enzyme activity was expressed as units/ mg tissue protein.

Alpha-Ketoglutarate dehydrogenase (a-KGDH) activity of rat cardiac and hepatic tissues were measured spectrophotometrically according to the method of Duncan et al., (1979) by measuring the reduction of 0.35 mM NAD\(^+\) to NADH at 340 nm using 50 mM phosphate buffer, pH 7.4 as the assay buffer. 0.1 mM a-ketoglutarate as the substrate and the suitable amount of mitochondria as the source of enzyme\(^{25}\). The enzyme activity was expressed as units/ mg tissue protein.
Likewise, succinate dehydrogenase (SDH) activity of rat cardiac and hepatic tissues were measured spectrophotometrically by following the reduction of potassium ferricyanide \([K_Fe(CN)_6]\) at 420 nm according to the method of Veeger et al., (1969) with some modifications\(^2\). One ml assay mixture contained 50 mM phosphate buffer, pH 7.4, 2% (w/v) BSA, 4 mM succinate, 2.5 mM K\(_2\)Fe(CN)\(_6\) and a suitable amount of mitochondria as the source of enzyme. The enzyme activity was expressed as units/ mg tissue protein.

**Measurement of the activities of some of the mitochondrial respiratory chain enzymes**

NADH-Cytochrome c oxidoreductase activity was measured spectrophotometrically by following the reduction of oxidized cytochrome c at 565 nm according to the method of Goyal et al., (1995)\(^7\). One ml of assay mixture contained in addition to the enzyme, 50 mM phosphate buffer, 0.1 mg BSA, 20 mM oxidized cytochrome c and 0.5 (M) NADH. The activity of the enzyme was expressed as units/ mg tissue protein.

Cytochrome c oxidase activity was also determined spectrophotometrically by following the oxidation of reduced cytochrome c at 550 nm according to the method of Goyal et al., (1995)\(^7\). One ml of assay mixture contained 50 mM phosphate buffer, pH 7.4, 40 mM reduced cytochrome c and a suitable aliquot of the enzyme. The enzyme activity was expressed as units /mg tissue protein.

**Indirect assessment of the generation of superoxide anion free radical** \((O_2^-)\) by xanthine oxidase and xanthine dehydrogenase

Xanthine oxidase (XO) activity of rat cardiac and hepatic tissues were assayed by measuring the conversion of xanthine to uric acid following the method of Greenlee et al., (1964)\(^8\). Briefly, the weighed amounts of cardiac and hepatic tissues were separately homogenized in cold (10%) in 50 mM phosphate buffer, pH 7.8. The homogenates were centrifuged at 500g for 10 min at 4°C. The resulting supernatants were further centrifuged at 12,000g for 20 min in cold. The supernatant, thus obtained, was collected and used for spectrophotometric assay of the enzyme at 295 nm using 0.1 mM xanthine in 50 mM phosphate buffer, pH 7.8, as the substrate. The enzyme activity was expressed as milli Units/ mg tissue protein.

Xanthine dehydrogenase (XDH) activity was measured by following the reduction of NAD\(^+\) to NADH according to the method of Strittmatter (1965) with some modifications\(^9\). In brief, the weighed amounts of rat cardiac and hepatic tissues were homogenized separately in cold (10%) in 50 mM phosphate buffer with 1 mM EDTA, pH 7.2. The homogenates were centrifuged in cold at 500g for 10 min at 4°C. The supernatants, thus obtained, were further centrifuged in cold at 12,000g for 20 min. The final supernatant was used as the source of the enzyme, and the activity of the enzyme was measured spectrophotometrically at 340 nm with 0.3 mM xanthine as the substrate (in 50 mM phosphate buffer, pH 7.5) and 0.7 mM NAD\(^+\) as an electron donor. The enzyme activity was expressed as milli units/ mg tissue protein.

**Measurement of nitric oxide (NO\(^-\)) concentration in mitochondria**

The NO\(^-\) concentration in mitochondria of both the cardiac and hepatic tissues were measured separately spectrophotometrically at 548 nm according to the method of Fiddler by using Griess reagent\(^29,30\). The reaction mixture in a spectrophotometer cuvette (1 cm path length) contained 100 µL of Griess Reagent, 700 µL of the sample and 700 µL of distilled water. The NO\(^-\) concentration was expressed as µM/mg of protein.

**Estimation of tissue protein**

The protein content of the different samples was determined by the method of Lowry et al., (1951)\(^31\).

**Tissue morphological and histochemical studies**

(a) **Staining of tissue sections using hematoxylin-eosin, and periodic acid Schiff (PAS) stains**

A portion of the extirpated rat heart and liver were fixed immediately in 10% formalin and embedded in paraffin following routine procedure as used earlier by Dutta et al., (2014)\(^4\). Sections of heart and liver tissues (5 µm thick) were prepared. The cardiac tissue sections were stained with hematoxylin-eosin stain and the sections obtained from the liver tissue were stained with hematoxylin-eosin stain and periodic acid Schiff (PAS) stain. The stained tissue sections were examined under Leica microscope and the images were captured with a digital camera attached to it.

(b) **Determination of lipid content in cardiac and hepatic tissues by using Sudan stain**

The rat cardiac and hepatic tissue sections were stained with Sudan stain by following the method of Canson\(^12\). The tissue sections were examined under Leica microscope and the images were captured with a digital camera attached to it. The digitized images were then analyzed using image analysis system (ImageJ, NIH Software, Bethesda, MD) and the total lipid content as represented in each image was measured and expressed as the % lipid volume (% area).

(c) **Quantification of fibrosis by confocal microscopy**

The rat heart and liver tissue sections (5 µm thick) were stained with
Sirius red (Direct Red 80; Sigma Chemical Co., St. Louis, MO, USA) according to the method of Dutta et al., (2014) and imaged with a laser scanning confocal system (Zeiss LSM 510 META, Germany) and the stacked images through multiple slices were captured. The digitized images were then analyzed using image analysis system (ImageJ, NIH Software, Bethesda, MI) and the total collagen area fraction of each image was measured and expressed as the % collagen volume.

Scanning electron microscopy (SEM)
Small pieces of heart and liver tissues were fixed overnight with 2.5% glutaraldehyde. After washing three times with PBS, the pieces were dehydrated for 10 min at each concentration of a graded ethanol series (50, 70, 80, 90, 95 and 100%). The dehydrated pieces were immersed in pure tert-butyl alcohol and were then placed into a 4°C refrigerator until the tert-butyl alcohol solidified. The frozen tissue pieces were dried by placing them into a vacuum bottle. The cardiac and liver tissue surface morphology was evaluated by scanning electron microscopy (SEM; Zeiss Evo 18 model EDS 8100).

Statistical evaluation
Each experiment was repeated at least two times with different rats. Data are presented as means ± S.E. Significance of mean values of different parameters between the treatments groups were analyzed using one way post hoc tests (Tukey’s HSD test) of analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatments. Pairwise comparisons were done by calculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS

Changes in body weight and heart weight to body weight ratio of animals of different groups
The pre-experimental body weights of all the groups of rats remained within the range of 145g-170g. In case of As-treated group, post-experiment body weight was found to be decreased by 4.29% compared to pre-experiment body weight. Similarly the post-experiment body weight of animals of HFD group gradually was found to be increased by 19.40% (P<0.001) compared to pre-experiment body weight. However, in case of animals co-treated with arsenic and HFD, the post-experiment body weight was also found to be decreased during the period of experiment by 1.41% compared to pre-experiment. In As + HFD + T20, As + HFD + T40 and As + HFD+ T60 groups, body weight was found to be increased by 1.41%, 1.39% and 0.69% (P<0.001) respectively than pre-experimental weight (Fig. 1A). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on body weight.

The heart weight/body weight ratio was also found to be increased in As, HFD, As + HFD co-treated groups than control group by 50%, 20% and 16% (P<0.001). Only 3.45%, 10.34% and 13.79% decrease (P<0.001) were observed in TA protected groups compared to As+HFD co-treated group (Fig. 1B). Furthermore, the heart weight to body weight ratio of animals of HFD group was also found to be increased significantly than the control group (P<0.001). Aqueous TA bark extract, alone, at increasing concentrations did not show any effect on heart weight to body weight ratio.

Changes in activities of AST and ALT
In As treated group, the activities of AST and ALT were found to be increased by 31.25% and 44% (P<0.001) respectively, compared to the control group. Increase of 12.50% and 12% (P<0.001) in the activities of AST and ALT from control group in high fat diet fed group were also observed. In case of animals co-treated with As and HFD, the activities of AST and ALT were found to be increased by 66.88% and 72% (P<0.001) respectively, compared to the control group. Followed by treatment with increasing doses of TA bark extract, the activities of AST and ALT were found to be protected from As+HFD co-treated group. In As+HFD+T20, the activities of AST and ALT were found to be protected by 21.35% and 11.63% (P<0.001) from As+HFD co-treated group respectively. Similarly, the AST and ALT activities were found to be protected by 40.07% and 37.21% (P<0.001) in As+HFD+T40 group and by 51.31% and 62.80% (P<0.001) in As+HFD+T60 group respectively, from the As+HFD co-treated group (Fig. 1C). Aqueous TA bark extract, alone, at increasing concentrations did not show any effect.

Changes in serum total LDH, LDH1 and LDH5 activities
Total LDH, LDH1 and LDH5 activities in serum were found to be increased significantly by 57.14%, 142.86% and 159.26% (P<0.001) respectively, in As treated group compared to control group. In HFD fed group, the total LDH, LDH1 and LDH5 activities in serum were found to be increased by 27.14%, 78.57% and 48.15% (P<0.001) respectively than control group. In As+HFD co-treated group, an increase of 157.14%, 321.43% and 225.93% (P<0.001) were found in total LDH, LDH1 and LDH5 activity compared to control animals. In As+HFD+T20 group, the LDH, LDH1, LDH5 activities were found to be protected by 16.67%, 23.73% and 67.80% (P<0.001) compared to As+HFD co-treated group. Similarly, the LDH, LDH1, LDH5 activities were also found to be protected in case of As+HFD+T40 and As+HFD+T60 by 45.56%, 50.85%, 43.18% and 60%, 67.80%, 65.91% (P<0.001) respectively, compared to As+HFD co-treated group. Aqueous TA bark extract, alone, at increasing concentrations did not show any effect on the activities of these enzymes. (Fig. 1D).
Changes in LPO level, GSH and protein carbonyl content in rat heart and liver tissues

The LPO level in heart tissue was found to be increased by 28.13%, 18.75% and 81.25% (P<0.001) in As, HFD and As+HFD co-treated group respectively, in comparison with the control group. The elevated level of LPO was found to be protected by 3.45%, 17.24% and 39.66% (P<0.001) in heart tissue of As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups compared to As+HFD co-treated group. The LPO level in liver tissue was found to be protected by 31.82%, 25% and 47.73% (P<0.001) in As, HFD and As+HFD co-treated group respectively, in comparison with control group. The elevated level of LPO was found to be protected by 3.08%, 20% and 26.15% (P<0.001) in liver tissue of As+HFD+T20, As+HFD+T40 and As+HFD+T60 group compared to As+HFD co-treated group.Aquous TA bark extract, alone, at increasing concentrations did not show any effect on LPO level (Fig. 2A).
The level of GSH in heart and liver tissues in As treated group were found to be decreased by 18.18% and 22.22% (P<0.001) respectively, compared to control group. In HFD and As+HFD co-treated group, the decrease in GSH content in heart and liver tissues were found by 12.73%, 13.89% and 30.91%, 31.94% (P<0.001) respectively, in comparison with control group. The GSH content of As+HFD+T60 group was found to be protected significantly from As+HFD co-treated group in heart and liver tissues by 42.11% and 51.02% respectively (P<0.001). TA aqueous bark extract, alone, at increasing concentrations did not show any effect (Fig. 2B).

The PCO level in heart and liver tissues of As treated group were significantly found to be increased by 65.71% and 35.42% (P<0.001) respectively, compared to control animals. In HFD fed group, the PCO level was found to be increased by 37.14% and 16.67% (P<0.001) in heart and liver, respectively. A significant increase of PCO level by 111.43% and 95.83% (P<0.001) were found in heart and liver tissues of As+HFD co-treated group compared to control group. The PCO levels of As+HFD+T60 group were found to be protected significantly from As+HFD co-treated group in heart and liver tissues by 59.46% and 38.30% respectively, (P<0.001) (Fig. 2C). Aqueous TA bark extract, alone, at increasing concentrations did not show any effect.

![Graphs](image-url)

Figure 2: Protective effect of aqueous TA bark extract on changes of (A) lipid peroxidation (LPO) level, (B) reduced glutathione (GSH) content, (C) protein carbonylation (PCO) level of heart and liver tissues of rats of various groups during the period of experiment. As: Arsenic treated group, HFD: High fat diet treated group, As+HFD: Arsenic and high fat diet co-treated group, T20= group treated with aqueous TA bark extract at the dose of 20mg/ml, T40= group treated with aqueous TA bark extract at the dose of 40mg/ml, T60= group treated with aqueous TA bark extract at the dose of 60mg/ml, As+HFD+T20= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 20mg/ml, As+HFD+T40= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 40mg/ml, As+HFD+T60= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 60mg/ml. The values are expressed as Mean ± S.E.; *P < 0.001 compared to control values using ANOVA, ^P < 0.001 compared to only arsenic treated values using ANOVA. \(^P < 0.001\) compared to arsenic and high fat diet co-treated values using ANOVA.
Changes in antioxidant enzyme activities of rat heart and liver tissues

In As treated group, Cu-Zn SOD activity was found to be decreased by 50% and 27.27% (P<0.001) in heart and liver tissues respectively, from the control groups. In high fat diet fed only group, Cu-Zn SOD activity was found to be decreased by 40% and 13.64% (P<0.001) respectively, in heart as well as in liver tissues compared to control. In heart and liver tissues of As+HFD co-treated group, a decrease of 60% and 63.64% (P<0.001) were found respectively, in Cu-Zn SOD activity compared to control group. The Cu-Zn SOD activities of As+HFD+T60 group were found to be protected significantly compared to As+HFD co-treated group in heart and liver tissues by 150% and 162.5%, respectively (P<0.001). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on the enzyme activity (Fig. 3A).

Glutathione reductase (GR) activity in As treated group was found to be decreased from control group by 25.93% and 22.58% (P<0.001) in heart and liver, respectively. In HFD group GR activity was also found to be decreased by 11.11% in heart and by 14.52% (P<0.001) in liver which was significantly different compared to control. GR activity in heart and liver tissues of As+HFD co-treated group were also found to be decreased significantly by 44.44% and 38.71% (P<0.001) compared to what has been observed in control rats. The GR activities of As+HFD+T60 group were found to be protected significantly from As+HFD co-treated group in heart and liver tissues by 86.67% and 52.63%, respectively (P<0.001) (Fig. 3A). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on GR activity of both the tissues.

Glutathione peroxidase (GPx) activity in heart and liver tissues of As, HFD, As+HFD groups were found to be decreased by 51.35%, 43.24% and 59.46% (heart) and also by 26.32%, 15.79% and 44.74% (liver) (P<0.001), respectively, compared to control group of rats. In heart tissues of As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups, GPx activities were found to be protected by 26.67%, 80% and 123.33% (P<0.001) compared to As+HFD group. In liver tissues of As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups, GPx activities were found to be protected by 28.57%, 61.90% and 85.71% (P<0.001) compared to As+HFD group (Fig. 3A). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on GPx activity in both the tissues studied.

Changes in pyruvate dehydrogenase (PDH) activity and some of the Kreb’s cycle enzymes in rat heart and liver tissues

The activity of PDH was found to be decreased significantly by 13.33%, 8.33% and 18.33% (P<0.001) in heart tissues of As, HFD, As+HFD groups of compared to control group. In liver tissue, PDH activity of As, HFD, As+HFD groups was found be decreased by 15.38%, 7.70% and 23.08% (P<0.001) than control. In heart tissues of As+HFD+T20, As+HFD+T40 and As+HFD+T60 group, the PDH activity was found to be protected by 2.04%, 12.24% and 22.45% (P<0.001) compared to As+HFD group. The PDH activity in liver tissue was found to be protected by 10%, 20% and 40% (P<0.001) in As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups, respectively, compared to As+HFD group. Aqueous TA bark extract, alone, at increasing concentration did not show any effect on PDH activity (Fig. 3B).

Isocitrate Dehydrogenase (ICDH) activity was found to be decreased significantly by 31.25%, 25% and 37.5% (P<0.001) in heart tissue of As, HFD, As+HFD treated groups of rats compared to control group of animals. In liver tissue, ICDH activity of As, HFD, As+HFD treated groups were found to be decreased by 20%, 6% and 40% (P<0.001) compared to control group of rats. In heart tissues of As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups of rats, the ICDH activity was found to be protected by 6%, 16% and 80% (P<0.001) compared to As+HFD group. The ICDH activity in liver tissues were found to be protected by 50%, 60% and 70% (P<0.001) in As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups of rats, respectively, compared to As+HFD group. Aqueous TA bark extract, alone, at increasing concentration did not show any effect on ICDH activity (Fig. 3B).

Alpha-Ketoglutarate Dehydrogenase (a-KGDH) activity was found to be decreased significantly by 12.73%, 7.27% and 30.91% (P<0.001) in heart tissues of As, HFD, As+HFD groups of rats compared to control group. In liver tissue, a-KGDH activity of As, HFD, As+HFD groups of rats were found to be decreased by 23.08%, 12.82% and 48.72% (P<0.001) when compared to the activity of the rats of control group. In heart tissues of As+HFD+T20, As+HFD+T40 and As+HFD+T60 group, the a-KGDH activity was found to be protected by 18.42%, 31.58% and 44.74% (P<0.001) compared to As+HFD fed group of rats. The a-KGDH activity in liver tissue was found to be protected by 40% and 85% (P<0.001) in As+HFD+T40 and As+HFD+T60 groups of rats, respectively when compared As+HFD fed group of rats (Fig. 3B). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on this enzyme.

Succinate dehydrogenase (SDH) activity was found to be decreased significantly by 31.25%, 25% and 37.5% (P<0.001) in heart tissues of As, HFD, As+HFD groups of rats compared to control group of rats. In liver tissue, SDH activity of As, HFD, As+HFD fed groups was found to be decreased by 20%, 36% and 40% (P<0.001) compared to control. In heart tissues of As+HFD+T20, As+HFD+T40 and
As+HFD+T60 groups of rats, the SDH activity was found to be protected by 6%, 16% and 80% (P<0.001) compared to As+HFD group of rats. SDH activity in liver tissues was found to be protected by 50%, 60% and 70% (P<0.001) in As+HFD+T20, As+HFD+T40 and As+HFD+T60 groups of rats, respectively compared to As+HFD group of rats (Fig. 3B). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on SDH activity.

Figure 3: Protective effect of aqueous TA bark extract on changes of (A) Cu-Zn superoxide dismutase (Cu-Zn SOD) activity, glutathione reductase (GR) activity and glutathione peroxidase (GPx) activity, (B) pyruvate dehydrogenase (PDH) activity, isocitrate dehydrogenase activity (ICDH), a-ketoglutarate dehydrogenase (a-KGDH) activity and succinate dehydrogenase (SDH) activity of heart and liver tissues of rats of various groups during the period of experiment. As: Arsenic treated group, HFD: High fat diet treated group, As+HFD: Arsenic and high fat diet co-treated group, T20= group treated with aqueous TA bark extract at the dose of 20mg/ml, T40= group treated with aqueous TA bark extract at the dose of 40mg/ml, T60= group treated with TA at the dose of 60mg/ml, As+HFD+T20= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 20mg/ml, As+HFD+T40= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 40mg/ml, As+HFD+T60= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 60mg/ml. The values are expressed as Mean ± S.E.; *P < 0.001 compared to control values using ANOVA. ^ P < 0.001 compared to only arsenic treated values using ANOVA. # P < 0.001 compared to arsenic and high fat diet co-treated values using ANOVA.

Changes in mitochondrial respiratory chain enzymes in heart and liver tissues
The activity of cytochrome C oxidoreductase was found to be decreased by 36.84%, 21.05% and 60.53% (P<0.001) in the heart tissues and by 50%, 30% and 64% (P<0.001) in liver tissues of As, HFD, As+HFD group of rats, respectively compared to control group of rats. In As+HFD+T20, As+HFD+T40 and As+HFD+T60 treated groups of rats the activity of cytochrome C oxidoreductase was found to be protected by 73.33%, 100%, 140% (P<0.001) in heart tissues and by 94.44%, 166.67%, 216.67% (P<0.001) in liver tissues in comparison to As+HFD treated group of rats. Aqueous TA bark ex-
The activity of cytochrome C oxidase was found to be decreased by 45.45%, 27.07% and 68.18% (P<0.001) in the heart tissues and by 37.5%, 18.75% and 53.13% (P<0.001) in liver tissues of As, HFD, As+HFD treated groups of rats, respectively, compared to the activity observed in the control group of rats. In As+HFD+T20, As+HFD+T40 and As+HFD+T60 treated groups of rats, the cytochrome C oxidase activity was found to be protected by 14.29%, 185.71%, 300% (P<0.001) in heart tissues and by 4%, 60%, 140% (P<0.001) in liver tissues in comparison to As+HFD treated group of rats. Aqueous TA bark extract, alone, at increasing concentration did not show any effect on the activity of this enzyme. Changes in NO• concentration in mitochondria of heart and liver tissues of rat
The NO• concentration in As treated group of rats was found to be increased by 50% and 45.45% (P<0.001) in heart and liver tissues, respectively, compared to control group of rats. In case of rats, co-treated with As and HFD, the NO concentration were found to be significantly increased than that observed in control rats, by 78.13% and 100% (P<0.001) in heart and liver tissue, respectively. In As+HFD+T20, As+HFD+T40 and As+HFD+T60 treated groups of rats, the NO concentrations were found to be protected by 14.04%, 22.81% and 43.86% in (P<0.001) heart tissue and by 20.45%, 36.36% and 43.18% (P<0.001) in liver tissue compared to As+HFD group of rats. Aqueous TA bark extract, alone, at increasing concentration did not show any effect on NO• concentration in both the tissues studied. Indirect assessment of superoxide radical generation (O2•−) in rat heart and liver tissues
We also examined whether arsenic and high fat diet administration to rats induced the generation of ROS. The results presented in Fig. 5A–E clearly indicate that there was an increase in the generation of (O2•−) in vivo following treatment of rats with arsenic and high fat diet. The activities of xanthine oxidase (XO), xanthine dehydrogenase (XDH), total enzyme activity, i.e., XO plus XDH, XO - XDH ratio and XO/XO + XDH ratio all increased significantly following arsenic and high fat diet treatment of rats. All these parameters were found to be increased when the rats were co-treated with arsenic and high fat diet. In the As+HFD +T60 group the XO, XDH, XO+XDH activities were found to be protected significantly by 33.33%, 41.67%, 34.48% (P<0.001) in heart tissues and by 26.09%, 33.90%, 31.03% (P<0.001) in liver tissues than that observed in the As+HFD treated group of rats. On treatment with aqueous TA bark extract, XO/ (XO+XDH) activity was shown to be protected further in both tissues, in comparison with As+HFD group of animals significantly. Aqueous TA bark extract, alone, at increasing concentration did not show any effect on the generation of this ROS.
Figure 5: Protective effect of aqueous TA bark extract on changes in the activity of (A) xanthine oxidase (XO), (B) xanthine dehydrogenase (XDH), (C) XO+XDH, (D) XO-XDH ratio, (E) XO/XO+XDH of heart and liver tissues of rats of various groups. As: Arsenic treated group, HFD: High fat diet treated group, As+HFD: Arsenic and high fat diet co-treated group, T20= group treated with aqueous TA bark extract at the dose of 20mg/ml, T40= group treated with aqueous TA bark extract at the dose of 40mg/ml, T60= group treated with aqueous TA bark extract at the dose of 60mg/ml, As+HFD+T20= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 20mg/ml, As+HFD+T40= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 40mg/ml, As+HFD+T60= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 60mg/ml. The values are expressed as Mean ± S.E.; *P < 0.001 compared to control values using ANOVA.

Tissue morphological and histochemical studies

Lipid content in rat heart and liver tissues

Lipid contents were found to be decreased in heart as well as increased significantly in liver tissues, respectively, in case of arsenic treated group (P<0.001). But, in case of HFD treated group of rats, the lipid content was found to be increased compared to control group. In the rats co-treated with As+HFD, the lipid content was found to be protected from being altered both the in cardiac and hepatic tissues significantly (P<0.001). In both tissues, aqueous TA bark extract decreased the lipid content in a dose-dependent manner significantly.
(P<0.001) (Fig. 6A-D). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on the lipid content of both the tissues studied.

**Studies using tissue sections stained with hematoxylin – eosin as well as PAS stains**

Tissue morphological studies revealed that arsenic (As) mediate damage to cyto-architecture of both cardiac and liver tissues. Degenerative changes in cardiac tissue and necrosis in liver tissue can be clearly demonstrated from the HE stained cardiac and liver tissue and PAS stained liver. Mild dilatation of central vein, mild inflammatory cell infiltration in portal tract and mild congestion of sinusoids and glycogen depletion were observed. In case of high fat diet treated

![Figure 6](image_url)

**Figure 6**: Protective effect of aqueous TA bark extract on changes of lipid contents in rat cardiac (A) and liver (B) tissues and the graphical representation of the changes of lipid contents in heart (C) and liver (D) tissues in various groups and changes of rat cardiac tissue morphology: (E) H and E stained (40X magnification), (H) acid Sirius stained (40X magnification) and changes of rat liver tissue morphology: (F) H and E stained (40X magnification) (G) PAS stained (40X magnification) (I) acid Sirius stained (40X magnification), The images captured by confocal laser scanning microscope for quantification of fibrosis of heart (J) and liver (K) tissues, the graphical representation of the changes of collagen content of heart (L) and liver (M) tissues; As: Arsenic treated group, HFD: High fat diet treated group, As+HFD: Arsenic and high fat diet co-treated group, T20= group treated with aqueous TA bark extract at the dose of 20mg/ml, T40= group treated with aqueous TA bark extract at the dose of 40mg/ml, T60= group treated with aqueous TA bark extract at the dose of 60mg/ml, As+HFD+T20= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 20mg/ml, As+HFD+T40= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 40mg/ml, As+HFD+T60= group co-treated with arsenic, high fat diet and aqueous TA bark extract at the dose of 60mg/ml. The values are expressed as Mean ± S.E.; *P < 0.001 compared to control values using ANOVA. ^ P < 0.001 compared to only arsenic treated values using ANOVA.
Studies on collagen content by Sirius red stain
Under confocal microscopy, with the study of Sirius red stained tissues, it had been revealed that, collagen content in As, HFD and As+HFD groups decreased in heart tissue and increased in liver tissue significantly (P<0.001). In heart tissues of the As+HFD+TA co-treated groups of rats the collagen contents were found to be increased and in case of hepatic tissues the collagen contents were found to be decreased (P<0.001). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on tissue collagen (Fig.6H-M).

Studies on rat cardiac and hepatic tissue surface morphology by Scanning Electron Microscopy (SEM)
It was observed that in both heart and liver tissues of As treated group, there was presence of furrow and a fuzzy appearance was noted. In HFD group of rats, in case of heart, there was mild furrow formation and in liver tissue, accumulation of large amount of fat had been observed. In As+HFD co-treated group of rats, the fat deposition was found to be increased and more prominent furrows were observed. However, in aqueous TA bark extract protected group of rats, the fuzziness and furrows were found to be disappeared with restoration of normalcy (Fig. 7). Aqueous TA bark extract, alone, at increasing concentration did not show any effect on surface morphology of both the tissues studied.
DISCUSSION

Oxidative stress is generally defined as an imbalance that favors the production of ROS over antioxidant defenses; however, the precise mechanisms by which ROS cause cellular injury remain elusive. The current study has shown that the aqueous bark extract of *Terminalia arjuna* can protect high fat diet-induced aggravation of arsenic-induced cardiac and liver injuries in rats. The central hypothesis is that high fat diet could enhance arsenic-induced hepatofibrogenesis as well as obesity and hypertension which are also the two major risk factors that lead to increased incidence of cardiac diseases including coronary artery disease, heart failure and cardiomyopathy. Concerns over the safety of synthetic antioxidants have shifted the global interests towards exploration of antioxidant compounds from natural sources. A plethora of phenolics extracted from several plant species have been reported to possess strong antioxidant activities. Phenolics are ubiquitously present in plants, and when plants are consumed as foods, these phytochemicals contribute to the intake of natural antioxidants in the diets of human as well as animals. Out of all the phenolics, the flavonoids belong to a large family of compounds with different degrees of hydroxylation, oxidation and substitution. Our previous study showed that the aqueous extract of TA contains tannins, cumarins and terpenoids and this extract revealed a higher amount of polyphenol which may be responsible for antioxidant activity of this extract. Arsenic itself reduced body weight by interfering with various metabolic pathways. When high fat diet is administered along with arsenic treatment, body weight also gradually reduced further. Arsenic toxicity has been reported to cause cardiac hypertrophy and reduction of body weight. Activities of both AST and ALT were significantly higher in arsenic treated rats indicating liver dysfunctions and increased activity of AST indicated cardiac disorders. Increment of the activities of AST and ALT in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which gives an indication on the hepatotoxic effect of arsenic. High fat diet also elicited the activities of AST and ALT.

LDH oxidizes lactic acid to pyruvic acid, using NAD+ as the coenzymes. It can also catalyze the reverse reaction. Of the five isozymes, LDH 1 predominates in the cardiac muscle and LDH 5 in the liver. Any significant increase in serum LDH 1 activity specifically indicates myocardial damage and serum LDH 5 increases during liver diseases such as hepatic dysfunction due to oxidative stress.

The arsenic-induced cardiac and liver tissue damages, in our experimental situation, is due to generation of oxidative stress as is evident from elevated levels of tissue LPO and protein carbonyl content and a decreased tissue level of reduced GSH, the well known bio-markers of oxidative stress. Arsenic may induce oxidative stress by enhancing tissue LPO and by altering the antioxidant system in the organs. Increased oxidative stress due to accumulated fat leads to deregulated production of adipocytokines and selective increase in ROS production.

A decrease in the activity of Cu–Zn SOD can be owed to an enhanced superoxide production during arsenic metabolism. Exposure to arsenic decreased the catalase activity. Wang and Huang, showed that arsenic induced increase of micronuclei by overproduction of H₂O₂. Catalase catalyzes the removal of H₂O₂ formed during the reaction catalyzed by SOD.

The depletion of reduced glutathione level in heart and liver of rats after exposure to arsenic may be accounted for by the decrease in GR activity. Arsenic can inhibit the activity of GR. The inhibition may be due to the interaction of trivalent arsenic with critical thiol groups in GR molecules. GPx reduces lipid hydroperoxide into lipid alcohols; this enzyme is coupled with GR. GSH serves as a substrate for GPx. Xanthine oxidoreductase, under normal conditions, exists in dehydrogenase form and uses NAD⁺ and there is no or very little production of superoxide anion. Under ischemic conditions, there is depletion of ATP and subsequent loss of membrane Ca²⁺ gradient. Increased Ca²⁺ levels activates Ca²⁺ dependent proteases which cause selective proteolysis of the dehydrogenase to convert it into xanthine oxidase (XO) which acts both on hypoxanthine and xanthine at the expense of molecular oxygen to produce superoxide anion free radical. Thus, XO in oxidative stress conditions may play an important role in contributing free radical mediated damage. The generation of ROS in heart and liver tissues was aggravated after the co-treatment of rats with arsenic and high fat diet by the endogenous formation of superoxide anion free radical and OH radical as evident from our present studies.

Mitochondria are the major source of ROS production in cells. In our study, we found that the activities of pyruvate dehydrogenase and the Kreb’s cycle enzymes like isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase and succinate dehydrogenase were decreased after treatment of rats with arsenic.

The impairment of electron transfer through NADH:ubiquinone oxidoreductase (complex I) and ubiquinol:cytochrome c oxidoreductase (complex III) may induce superoxide formation. Mitochondrial production of ROS is thought to play an adverse role in many pathologic disorders. In our present study, copper-ascorbate induced oxidative stress inhibits NADH cytochrome c oxidoreductase and cytochrome c oxidase enzymes of ETC. The activities of these enzymes were
found to be protected when the mitochondria were co-treated with aqueous TA bark extract. This strongly indicates that the extract possesses either some chelating property or is simply able to protect mitochondria from getting damaged due to ROS production, by itself being a quencher of reactive oxygen species.

In our present study, it was established that the oxidative stress-induced damages due to arsenic and high fat diet co-treatment were found to be protected when the rats were co-treated with aqueous TA bark extract. This is also clear from our tissue morphological as well as histochemical studies of both the cardiac and hepatic tissues, using light, confocal and scanning electron microscopy. This, further, strongly indicates that the extract possesses either some chelating property or is simply able to protect heart and liver tissues of rat from getting damaged due to ROS production, by itself being a quencher of reactive oxygen species or by various antioxidant mechanism(s) (Fig. 8).

**CONCLUSION**

From the findings of our present study it can be said that co-treatment of rats with aqueous extract of bark of *Terminalia arjuna* provides protective effects against arsenic-induced aggravation of high fat diet-induced oxidative stress mediated damages in cardiac and liver tissues in a dose-dependent manner and such protection may be exerted via various antioxidative mechanism(s).

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