Aqueous bark extract of *Terminalia arjuna* protects against adrenaline-induced hepatic damage in male albino rats through antioxidant mechanism(s): a dose response study

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**ABSTRACT**

**Background:** The present study aimed to evaluate the hepatoprotective effects of different doses of aqueous bark extract of *Terminalia arjuna* (TA) on adrenaline-induced hepatotoxicity in male albino rats. **Methods:** A total number of 48 adult healthy male albino rats were divided into eight groups comprising of 6 animals each. Group-I served as normal control group. All other groups (group-II and group-VI to VIII) were administered adrenaline sub-cutaneously at a dose of 0.3mg/kg body weight. Group- III to V was treated with different doses of aqueous bark extract of TA at doses of 10mg/kg bw/administered orally (For Group-III), 20mg/kg bw/administered orally (For Group-IV), 40mg/kg bw/administered orally (For Group-V). Group-VI, group-VII and group-VIII were also co-treated with different doses of aqueous bark extract of TA at doses of 10, 20 and 40 mg/kg bw/administered orally, respectively. **Results:** Treatment of rats with adrenaline induced alterations in the activities of serum lactate dehydrogenase total (LDH T), lactate dehydrogenase-5(LDH 5), serum glutamate pyruvate transaminase (SGPT), caused elevation in the level of lipid peroxidation and protein carbonylation, a decrease in glutathione content as well as altered the activities of antioxidant enzymes and the enzymes of Kreb’s cycle and respiratory chain. Tissue histomorphological studies also showed considerable damage following adrenaline treatment. Pre-treatment of rats with aqueous bark extract of TA significantly protected against these hepatic damage. **Conclusion:** The present studies suggest that the aqueous bark extract of TA may be beneficial in ameliorating adrenaline-induced oxidative stress mediated damages in the rat liver.

**KEYWORDS:** Adrenaline, antioxidant, hepatotoxicity, oxidative stress, rats, *Terminalia arjuna*.

**INTRODUCTION**

Reactive oxygen species (ROS), reactive nitrogen species (RNS) and other free radicals causes hepatic tissue injury by causing lipid peroxidation, depletion of cellular antioxidants and through oxidation of critical cellular proteins as well as by bringing alterations in the activities of the antioxidant enzymes\(^1\). Adrenaline, a catecholamine, is generally considered as a hormone involved in “fight or flight” mechanism\(^2\). However, its role in the genesis of oxidative stress in humans is being increasingly recognized and is considered more dangerous in bringing about hepatic disorders. Adrenaline acts by binding to a variety of adrenergic receptors present in our system. Epinephrine is a non-selective agonist of all adrenergic receptors, including the major subtypes \(\alpha_1, \alpha_2, \beta_1, \beta_2\), and \(\beta_3\). The binding of epinephrine to these receptors triggers a number of metabolic changes\(^3\). Endogenous plasma adrenaline concentrations in resting adults have been reported normally to be less than 10 mg/L, but may increase by 10-fold during exercise and by 50-fold or more during times of stress. Therefore, adrenaline is an endogenous stress inducer.

*Terminalia arjuna* (TA) Wight & Arn. belonging to the family Combretaceae, is distributed throughout the greater part of India, Burma and Sri Lanka. Different parts, particularly its fruit and bark are used as a human consumable component in water, milk and other drinks to maintain good health\(^4\). Chemical analyses showed that the entire plant is full of compounds like tannin, saponin, ester, sugar, steroids, acids and minerals etc\(^5\). Experimental and clinical studies revealed the beneficial effects of this plant against various diseases by exerting its effect as gastroprotective \(^6\) as well as anti-mutagenic activities \(^7\). Its aqueous bark extract showed a novel protection mechanism in several *in vitro* systems like RBC, liver tissue, heart mitochondria as discussed in our previous publications\(^8,9,10\) and its antioxidant mechanisms were also established\(^10\). But till date there is no evidence regarding the hepato-protective activity of the bark.
aqueous extract of TA against adrenaline-induced hepatotoxicity. Therefore, the present study aims to find out the hepatoprotective effects of bark aqueous extracts of TA with different doses against adrenaline-bi-tertarate-induced hepatotoxicity.

Herein, we provide evidence that aqueous bark extract of TA has potential to provide protection against adrenaline-induced oxidative stress mediated damages in rat liver and this protection may be exerted through antioxidant mechanism(s).

MATERIALS AND METHODS

Chemicals
Sodium pyruvate, isocitrate, succinate, 2-ketoglutarate and bovine serum albumin (BSA) were purchased from SRL Chemicals, Mumbai, India. Adrenaline bitartrate was procured from Vulcan laboratories, India. Thiobarbituric acid (TBA) was procured from Spectro Chem., India. All other chemicals used were of analytical grade.

Animals
Male albino rats of Wister strain, weighing 150–200 g were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of environment and forests, Government of India. All the experimental protocols had the approval of the Institutional Animal Ethics Committee (IAEC) of the Department of Physiology, University of Calcutta (approval no IAEC-III/Proposal/DB-01/2013 dated 22.03.2013).

Preparation of aqueous extract of bark of TA
5gm of TA bark powder was dissolved in 25ml of double distilled water. After proper mixing it was kept with cotton plugging for overnight (approximately 16 hours). Then it was centrifuged twice at 1300g for 10 minutes. Then the supernatant was collected and lyophilized. The yield of the aqueous extract of Terminalia arjuna from 5gm of TA bark powder was 10%.

Induction of hepatic damages with adrenaline-bi-tertarate: a dose-response study
During experiment, the animal room was maintained at a temperature of 25±1°C, humidity 50±10% and a 12-h light/dark cycle and the rats were allowed to access standard diet containing 18% protein (casein) and water ad libitum for 7 days (quarantine period). The 18% protein diet was considered as an adequate dietary protein level, which was used on earlier occasions.

Hepatic damages were induced in rats by sub-cutaneous (s.c.) injection of adrenaline-bi-tertarate at the dose of 0.3mg/kg body weight. Briefly, male rats were divided into eight groups. Each group of animals comprised of 6 rats. The rats of the first group constituted the vehicle-treated control. The rats of the second group were injected with sub-cutaneous (s.c.) injection of adrenaline at the dose of 0.3mg/kg body weight. The rats of the third, fourth and the fifth group were orally fed, respectively, with different doses of aqueous bark extract of TA (10, 20, 40 mg/kg body weight) for 17 consecutive days where water was used as the vehicle. The rats of sixth, seventh and eight groups were orally fed different doses of aqueous bark extract of TA (10, 20, 40 mg/kg body weight) for 17 consecutive days and injected with sub-cutaneous (s.c.) injection of adrenaline at the dose of 0.3mg/kg body weight pior for last 10 days. After the completion of treatment the animals were sacrificed by cervical dislocation following mild ether anaesthesia. The liver tissues were surgically extirpated after carefully opening the thoracic and abdominal cavity and were washed thoroughly in cold saline. The saline was soaked properly with a piece of blotting paper from the tissues and the tissues were stored at -20°C for further biochemical analyses. Prior to sacrifice, the blood was collected by cardiac puncture for the preparation of the serum.

Measurement of Serum glutamate oxaloacetate transaminase (SGOT), total lactate dehydrogenase (LDH T), lactate dehydrogenase 5 (LDH5) activities
Serum GOT activity was 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-dinitrophenylhydrazine (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 lactate dehydrogenase (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, which destroys all isoforms except LDH for 30 min according to the method of Strittmatter (1965) with some modifications. The serum lactate dehydrogenase 5 (LDH-5) 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, which destroys all isoforms except LDH-5 for 30 min according to the method of Strittmatter (1965) with some modifications.

Measurement of hepatic tissue lipid peroxidation (LPO) level, reduced glutathione (GSH) content, total glutathione (TSH) content and protein carbonyl (PCO) content
The lipid peroxides in the hepatic tissue homogenates were determined separately as thioarbituric acid reactive substances (TBARS) according to the method of Buege and Aust with some modifications as adopted by Bandopadhyay et al. The reduced...
glutathione (GSH) content (as acid soluble sulphydryl) and total glutathione content of the hepatic tissue homogenates were estimated separately by its reaction with DTNB (Ellman’s reagent) following the method of Sedlak et al, 196817 with some modifications by Dutta et al, 201418. Protein carbonyl content was estimated by DNPH assay 19. The values were expressed as nmoloes / mg protein.

**Measurement of the activities of Cu-Zn superoxide dismutase (Cu-Zn SOD), Catalase, Mn superoxide dismutase (Mn SOD), Glutathione reductase (GR), Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) of rat hepatic tissue**

Copper–zinc superoxide dismutase (Cu-Zn-SOD) activity was measured by Hematoxylin autooxidation method of Martin et al, 198720 with some modifications as adopted by Mishra et al, 201321. The enzyme activity was expressed as units /mg protein. Catalase activity was assayed by the method of Beers et al, 195222 with some modifications as adopted by Chattopadhyay et al, 200023. The enzyme activity was expressed as µmoles of H2O2 consumed/ mg protein. Manganese superoxide dismutase (Mn-SOD) activity was measured by pyrogallol autooxidation method24 modified by Rudra et al, 201425. The glutathione reductase activity was measured according to the method of Krohne-Ehrich et al, 197726. The specific activity of the enzyme was calculated as units/ mg protein. The glutathione peroxidase activity was measured according to the method of Paglia and Valentine, 196727 with some modifications as adopted by Chattopadhyay et al, 200023. The glutathione-S-transferase activity of the rat cardiac tissue was measured spectrophotometrically according to Habig et al, 197428.

**Indirect assessment of the generation of superoxide anion free radical (O2−) by xanthine oxidase (XO) and xanthine dehydrogenase (XDH)**

Xanthine oxidase activity of rat hepatic tissue was assayed by measuring the conversion of xanthine to uric acid following the method of Greenlee et al, 196429. Xanthine dehydrogenase (XDH) activity was measured by following the reduction of NAD+ to NADH according to the method of Strittmutter, 196530 with some modifications30. The enzyme activity was expressed as milli units/ protein.

**Measurement of the activities of pyruvate dehydrogenase and Kreb’s cycle enzymes**

Pyruvate dehydrogenase activity of rat hepatic tissue was measured spectrophotometrically according to the method of Chretien et al, 195531, with some modifications. Isocitrate dehydrogenase activity of rat hepatic tissue was measured according to the method of Duncan et al, 197932 by measuring the reduction of NAD+ to NADH at 340 nm with the help of a UV–VIS spectrophotometer. Alpha-Ketoglutarate dehydrogenase activity of rat hepatic tissue were measured spectrophotometrically according to the method of Duncan et al, 197932. Likewise, succinate dehydrogenase activity of rat hepatic tissue was measured spectrophotometrically by following the reduction of potassium ferricyanide [K3Fe(CN)6] at 420 nm according to the method of Veeger et al, 196933 with some modifications34.

**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, 199534. Cytochrome c oxidase activity was determined spectrophotometrically by following the oxidation of reduced cytochrome c at 550 nm according to the method of Goyal et al, 199534.

**Tissue morphological studies**

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, 201435. Sections of liver tissue (5 µm thick) was prepared. The tissue sections were stained with hematoxylin–eosin stain. The tissue sections were examined under Leica microscope and the images were captured with a digital camera attached to it.

**Estimation of protein**

The protein content of the different samples was determined by the method of Lowry et al, 195136.

**Statistical evaluation**

Each experiment was repeated at least three times. Data are presented as means ± S.E. Significance of mean values of different parameters between the treatments groups were analyzed using one way 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 AND DISCUSSION**

**Serum parameters**

Table 1 showed significant alterations in the activities of serum biomarkers following treatment of rats with adrenaline (+P < 0.001 vs. control). These alterations of enzymes activities in serum were found to be protected significantly (**P < 0.001) when the rats were pre-treated with TA extract (20mg/ml), indicating the ability of this aqueous extract to protect the hepatic tissue against oxidative stress. A dose-dependent protection was found by the aqueous bark extract of TA against the adrenaline-induced alterations in serum biomarkers. The aqueous bark extract of TA itself had no effect on the serum biomarkers. Activity of SGOT was significantly higher in adrenaline treated rats indicating liver dysfunctions39. Of the five isozymes, LDH-
The values are expressed as Mean ± S.E.; Adr 0.3mg/kg: rats treated with adrenaline at the dose of 0.3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of Terminalia arjuna at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.

Oxidative stress biomarkers
The sub-chronic administration adrenaline-bi-tertarate can causes generation of Reactive Oxygen Species (ROS) which can attack of protein, lipid, mitochondria, DNA molecules and other organelles of cell and brings about oxidative damage. Excessive generation of ROS by the auto-oxidation of adrenaline or by Fenton type reaction as well as Haber-Weiss reaction may occur in the hepatocytes. So, it may causes the oxidative stress in case of prolong administration and has the potential to cause various hepatic disorders.

Table 2, showed a significant increase in hepatic LPO level and the level of protein carbonyl following the treatment of rats with adrenaline (*P < 0.001 vs. control). This elevated level of lipid peroxidation products and protein carbonyl level were found to be significantly protected (***P < 0.001) when the rats were pre-treated with TA extract (20mg/ml), indicating the ability of this aqueous extract to protect the hepatic tissue against oxidative stress-induced changes due to adrenaline. On the other hand, significant decrease was observed in hepatic reduced GSH and total glutathione content following the treatment of rats with adrenaline (*P < 0.001 vs. control). This decreased level of reduced and total GSH content was found to be significantly protected (***P < 0.001) when the rats were pre-treated with TA extract (20mg/ml) and adrenaline. A dose-dependent protection was found by the aqueous bark extract of TA against the adrenaline-induced alterations in oxidative stress biomarkers. The aqueous bark extract of TA itself had no effect on the oxidative stress biomarkers.

5 predominates in the liver. Any significant increase in serum LDH-5 activity specifically indicates liver diseases such as hepatic dysfunction due to oxidative stress.  

Table 1. Protective effect of aqueous extract of bark of T. arjuna against adrenaline-induced alteration in the serum biomarkers

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (IU/L)</th>
<th>LDH-Total (IU/L)</th>
<th>LDH-5 (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.43±0.120</td>
<td>0.26±0.009</td>
<td>0.17±0.009</td>
</tr>
<tr>
<td>Adr 0.3mg/kg</td>
<td>11.46±0.088*</td>
<td>0.54±0.009*</td>
<td>0.23±0.009*</td>
</tr>
<tr>
<td>TA10mg/kg</td>
<td>8.2±0.115</td>
<td>0.26±0.006</td>
<td>0.15±0.006</td>
</tr>
<tr>
<td>TA20mg/kg</td>
<td>8.4±0.115</td>
<td>0.27±0.006</td>
<td>0.15±0.006</td>
</tr>
<tr>
<td>TA40mg/kg</td>
<td>8.36±0.145</td>
<td>0.25±0.009</td>
<td>0.17±0.009</td>
</tr>
<tr>
<td>TA10mg/kg+ Adr 0.3mg/kg</td>
<td>8.3±0.088</td>
<td>0.26±0.012</td>
<td>0.22±0.012</td>
</tr>
<tr>
<td>TA20mg/kg+ Adr 0.3mg/kg</td>
<td>9.63±0.088*</td>
<td>0.41±0.006*</td>
<td>0.17±0.006*</td>
</tr>
<tr>
<td>TA40mg/kg+ Adr 0.3mg/kg</td>
<td>7.93±0.088</td>
<td>0.34±0.009</td>
<td>0.17±0.007</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± S.E.; Adr 0.3mg/kg: rats treated with adrenaline at the dose of 3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of Terminalia arjuna at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.

Table 2. Protective effect of aqueous extract of bark of T. arjuna against adrenaline-induced alteration in the oxidative stress biomarkers

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (nmoles TBARS/mg protein)</th>
<th>GSH (nmoles GSH/mg protein)</th>
<th>TSH (nmoles of TSH/mg protein)</th>
<th>PCO (nmoles of carbonyl/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.57±0.010</td>
<td>14.63±0.080</td>
<td>102±1.154</td>
<td>0.91±0.007</td>
</tr>
<tr>
<td>Adr 0.3mg/kg</td>
<td>0.90±0.006*</td>
<td>22.46±0.088*</td>
<td>86±1.154*</td>
<td>1.33±0.067*</td>
</tr>
<tr>
<td>TA10mg/kg</td>
<td>0.58±0.009</td>
<td>14.36±0.185</td>
<td>103.33±0.666</td>
<td>0.93±0.006</td>
</tr>
<tr>
<td>TA20mg/kg</td>
<td>0.56±0.011</td>
<td>14.7±0.100</td>
<td>102.66±1.452</td>
<td>0.88±0.009</td>
</tr>
<tr>
<td>TA40mg/kg</td>
<td>0.59±0.009</td>
<td>14.7±0.057</td>
<td>104.33±0.881</td>
<td>0.90±0.033</td>
</tr>
<tr>
<td>TA10mg/kg+ Adr 0.3mg/kg</td>
<td>0.72±0.009</td>
<td>18.06±0.066</td>
<td>92.33±0.881</td>
<td>1.07±0.033</td>
</tr>
<tr>
<td>TA20mg/kg+ Adr 0.3mg/kg</td>
<td>0.62±0.011**</td>
<td>15.26±0.088**</td>
<td>97±0.577**</td>
<td>0.80±0.009**</td>
</tr>
<tr>
<td>TA40mg/kg+ Adr 0.3mg/kg</td>
<td>0.56±0.009</td>
<td>13.53±0.088</td>
<td>97.66±1.154</td>
<td>0.81±0.006</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± S.E.; Adr 0.3mg/kg: rats treated with adrenaline at the dose of 3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of Terminalia arjuna at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.
Antioxidant enzymes activities

Table 3 showed significant alterations in the activities of hepatic antioxidant enzymes following treatment of rats with adrenaline (*P < 0.001 vs. control). These alterations of the activities of antioxidant enzymes in the hepatic tissue were found to be protected significantly (***P < 0.001) when the rats were pre-treated with TA to protect the hepatic tissue against oxidative stress-induced changes due to adrenaline. Adrenaline treatment caused a significant increase in the generation of O2•- free radical in hepatic tissue following the treatment with adrenaline at the dose of 0.3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of Terminalia arjuna at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.

Generation of superoxide anion free radical (O2•-): an indirect assessment

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 ROS generation in liver tissue was aggravated after the treatment with adrenaline of rats by the endogenous formation of •OH. Table 4 showed significant generation of O2•- in hepatic tissue following the treatment of rats with adrenaline (*P < 0.001 vs. control). This generation of superoxide anion free radical in hepatic tissue were found to be prevented significantly (***P < 0.001) when the rats were pre-treated with TA extract (20mg/ml), indicating the ability of this aqueous extract of TA to protect the hepatic tissue against ROS mediated damage due to adrenaline. A dose-dependent protection was found by the aqueous bark extract of TA against the adrenaline-induced alterations in O2•- generation. The aqueous bark extract of TA itself had no effect on the O2•- generation.

Table 4. Protective effect of aqueous extract of bark of T. arjuna against adrenaline-induced increase in the generation of superoxide anion free radical (O2•-)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Xanthine oxidase activity (milli units/min/mg protein)</th>
<th>Xanthine dehydrogenase activity (milli units/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.82±0.011</td>
<td>0.096±0.001</td>
</tr>
<tr>
<td>Adr 0.3mg/kg</td>
<td>1.40±0.057</td>
<td>0.15±0.010</td>
</tr>
<tr>
<td>TA10mg/kg</td>
<td>0.82±0.011</td>
<td>0.096±0.002</td>
</tr>
<tr>
<td>TA20mg/kg</td>
<td>0.826±0.012</td>
<td>0.097±0.002</td>
</tr>
<tr>
<td>TA40mg/kg</td>
<td>0.81±0.005</td>
<td>0.097±0.002</td>
</tr>
<tr>
<td>TA10mg/kg+ Adr 0.3mg/kg</td>
<td>1.03±0.033</td>
<td>0.136±0.006</td>
</tr>
<tr>
<td>TA20mg/kg+ Adr 0.3mg/kg</td>
<td>0.93±0.006</td>
<td>0.106±0.006</td>
</tr>
<tr>
<td>TA40mg/kg+ Adr 0.3mg/kg</td>
<td>0.906±0.006</td>
<td>0.103±0.006</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± S.E.; Adr 0.3mg/kg: rats treated with adrenaline at the dose of 0.3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of Terminalia arjuna at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg : rats co-treated with aqueous bark extract of Terminalia arjuna at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.
Table 5. Protective effect of aqueous extract of bark of *T. arjuna* against adrenaline-induced alteration in the activities of pyruvate dehydrogenase and some of the Kreb’s cycle enzymes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pyruvate dehydrogenase activity (Units/mg protein)</th>
<th>Isocitrate dehydrogenase activity (Units/mg protein)</th>
<th>a-ketoglutarate dehydrogenase activity (Units/mg protein)</th>
<th>Succinate dehydrogenase activity (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.23±0.088</td>
<td>32.5±0.763</td>
<td>10.16±0.120</td>
<td>58.66±0.333</td>
</tr>
<tr>
<td>Adr 0.3mg/kg</td>
<td>9.2±0.115*</td>
<td>27.36±0.366*</td>
<td>7.16±0.033*</td>
<td>40.66±0.881*</td>
</tr>
<tr>
<td>TA10mg/kg</td>
<td>14.17±0.120</td>
<td>32.66±0.284</td>
<td>10.36±0.066</td>
<td>56±1.154</td>
</tr>
<tr>
<td>TA20mg/kg</td>
<td>14.37±0.145</td>
<td>33±0.115</td>
<td>9.86±0.033</td>
<td>57±0.577</td>
</tr>
<tr>
<td>TA40mg/kg</td>
<td>14.16±0.088</td>
<td>33.2±0.115</td>
<td>9.76±0.033</td>
<td>56.33±0.666</td>
</tr>
<tr>
<td>TA10mg/kg+ Adr 0.3mg/kg</td>
<td>11.27±0.120</td>
<td>30.2±0.115</td>
<td>8.16±0.088</td>
<td>52±1.154</td>
</tr>
<tr>
<td>TA20mg/kg+ Adr 0.3mg/kg</td>
<td>12.8±0.057**</td>
<td>31.66±0.088**</td>
<td>8.86±0.033**</td>
<td>55.5±0.5**</td>
</tr>
<tr>
<td>TA40mg/kg+ Adr 0.3mg/kg</td>
<td>13.8±0.057</td>
<td>32.4±0.208</td>
<td>9.83±0.033</td>
<td>56.06±0.066</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± S.E.; Adr 0.3mg/kg: rats treated with adrenaline at the dose of 0.3mg/kg of body weight; TA10-40mg/kg: rats treated with aqueous bark extract of *Terminalia arjuna* at the dose of 10-40mg/kg of body weight respectively; TA10mg/kg+ Adr 0.3mg/kg: rats co-treated with aqueous bark extract of *Terminalia arjuna* at the dose of 10mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA20mg/kg+ Adr 0.3mg/kg: rats co-treated with aqueous bark extract of *Terminalia arjuna* at the dose of 20mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; TA40mg/kg+ Adr 0.3mg/kg: rats co-treated with aqueous bark extract of *Terminalia arjuna* at the dose of 40mg/kg of body weight and with adrenaline at the dose of 0.3mg/kg of body weight; * P < 0.001; As compared to control values using ANOVA; **As compared to adrenaline-induced values using ANOVA.

Activities of pyruvate dehydrogenase and some of the Kreb’s cycle enzymes

Mitochondria are the major source of ROS production in cells. Table 5 showed significant alterations in the activities of hepatic pyruvate dehydrogenase and Kreb’s cycle enzymes following the treatment of rats with adrenaline (*P < 0.001 vs. control). These alterations of enzymes’ activities in hepatic tissue were found to be protected significantly (*P < 0.001) when the rats were pre-treated with TA extract (20mg/ml), indicating the ability of this aqueous extract of TA to protect the hepatic mitochondrial energy metabolizing enzymes against oxidative stress-induced changes due to adrenaline. The aqueous bark extract of TA itself had no effect on the Kreb’s cycle enzymes activities. The dose-dependent effect is evident and shown in table 5.

Activities of mitochondrial respiratory chain enzymes

Table 5 showed significant alterations in the activities of hepatic mitochondrial respiratory chain enzymes activities following the treatment of rats with adrenaline (*P < 0.001 vs. control). These alterations of enzymes’ activities in hepatic tissue were found to be significantly protected (*P < 0.001) when the rats were pre-treated with adrenaline and TA extract (20mg/ml), indicating the ability of this aqueous extract of TA to protect the hepatic tissue mitochondrial respiratory chain enzymes against oxidative stress-induced changes due to adrenaline. The aqueous bark extract of TA itself had no effect on the respiratory chain enzymes activities. Here also, the dose-dependent effect of aqueous bark extract of TA is evident and shown in table 6.
Tissue morphological studies

Histological studies shows marked damage in adrenaline treated hepatic tissues of rats. There were clear signs of degenerative changes indicating liver necrosis. Mild dilatation of central vein, mild inflammatory cell infiltration in portal tract and mild congestion of sinusoids were observed in HE stained liver tissue. No such damage was observed in aqueous bark extract of TA treated rats. These histological alterations of hepatic tissue due to adrenaline were found to be protected significantly (**P < 0.001) when the rats were pre-treated with TA extract (20mg/ml). [Figure 1]

Figure 1: Tissue morphological studies of liver tissue using H & E Stain [400X]
CONCLUSION

It is well established that various polyherbal formulation is beneficial against hepato-toxicity. Our study also establishes the fact that the aqueous bark extract of TA which may have antioxidant nutrient has potent ameliorative capability against adrenaline-induced oxidative stress in liver of experimental rats [Figure 2]. The aqueous extract of bark of TA contains phytochemicals (such as phenolics, flavonoids etc) which possess antioxidant activity. So, it can be assumed that these phytochemicals may be responsible for the protective effects of the aqueous extract of TA against adrenaline-induced oxidative stress in \textit{in vivo} model. Our results may have future therapeutic relevance.

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