Article

Evaluation of *Terminalia arjuna* Bark Powder Supplementation on Isoprenaline-Induced Oxidative Stress and Inflammation in the Heart of Long Evans Rats, Understanding the Molecular Mechanism of This Old Medicinal Plant

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Abstract: This study was conducted to determine the effect of *Terminalia arjuna* bark powder supplementation on the oxidative stress of the cardiovascular system. Isoprenaline (ISO) was administered to the rats to develop the cardiac hypertrophy and myocardial infarction (MI). *Terminalia arjuna* bark powder was mixed with the food powder and provided for two weeks. At the end of the experiment, all rats were sacrificed and tissue samples were collected. ISO administration in rats increased the oxidative stress markers such as malondialdehyde (MDA), nitric oxide (NO), advanced oxidation protein product (APOP), and myeloperoxidase (MPO) in plasma and heart. *Terminalia arjuna* bark powder lowered the MDA, NO, and AOPP concentration level in ISO administered rats. Additionally, *Terminalia arjuna* restored the antioxidant enzymes (catalase and SOD) activities. Gene expression of antioxidant enzymes and inflammatory markers in the heart were studied. *Terminalia arjuna* restored Nrf-2, HO-1, HO-2, catalase, SOD, and GPx gene expression in the heart of ISO administered rats. ISO induced increased transcription levels of inflammatory genes such as IL-1, IL-6, TNF-α, TGF-β, iNOS, and NF-κB, which were decreased by *Terminalia arjuna* bark powder. Histopathology was checked and hematoxylin and eosin and Sirius red staining were performed on heart sections. ISO administration resulted in mononuclear cells infiltration and collagen deposition in the heart which were lowered by *Terminalia arjuna* bark powder. In conclusion, this study suggests that the *Terminalia arjuna* bark powder alleviated the oxidative stress by restoring the antioxidant genes and prevented the increase in inflammatory markers in the heart of ISO administered rats.

Keywords: *Terminalia arjuna*; isoprenaline; catalase; superoxide dismutase; oxidative stress

1. Introduction

The term “cardiovascular disease” (CVD) encompasses a wide range of conditions affecting the heart and blood vessels [1]. The development and progression of cardiovascular disease have been linked to inflammation and increased oxidative stress [2]. Oxidative stress may be a result of the elevated generation of oxidants or the weakened cellular defense systems. Lipid peroxidation is one of several oxidative consequences caused by forming a strong oxidant close to cell membranes. When oxygen combines with peroxyl radicals, lipid hydroperoxidation, membrane disruption, and the production of toxic compounds like malondialdehyde (MDA) occur [3]. Basic defense systems have evolved in organisms to counteract reactive oxygen species (ROS) production and injury. Superoxide dismutase (SOD), catalase, and glutathione are the detoxification systems found to be most active in the heart [4–6]. An acute state of myocardial necrosis, myocardial infarction (MI), is brought on by a disparity between coronary blood supply and myocardial demand [7]. The role of inflammation in mediating the damage to heart tissue following an ischemia event is critical. By entering the infarcted zone, neutrophils can potentially release proteolytic enzymes and produce ROS that can damage cardiac cells [8]. The pro-inflammatory cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6) have been shown to be elevated when the myocardium is injured or triggered by adrenergic receptors. After myocardial infarction, pro-inflammatory cytokines and other cytokines

like transforming growth factor-β (TGF-β) have been linked to the onset of tissue repair and wound healing [9]. However, increased ROS, ILs and TGF-β may trigger fibrosis development in the myocardium and pose potential threat for cardiac failure [10]. Isoprenaline (ISO), a β-adrenoceptor agonist, has been shown to cause myocardial infarction (MI) when administered in large doses. This is because isoprenaline undergoes auto-oxidation, releasing highly cytotoxic free radicals known to initiate the peroxidation of membrane phospholipids, severely damaging the cardiac membrane. Thus, ISO is commonly used in the development of animal models in rats since it causes MI in rats [11,12].

Prolonged inflammation may reduce blood antioxidant levels due to the chronic generation of increased ROS. Antioxidants can prevent cardiovascular disease by neutralizing reactive oxygen species (ROS) [13]. A previous report showed that antioxidant rich ratmontchi (*Flacourtia indica*) fruits extract may protect the heart in ISO induced oxidative stress in rats [14]. It has been observed that antioxidant treatment can prevent heart injury in rats by lowering lipid peroxidation and by improving antioxidant enzymes [15]. *Terminalia arjuna*, also known as arjun, is a member of the *Combretaceae* family and used as a putative cardioprotective agent. The Indo-sub-Himalayan regions of Uttar Pradesh, Southern Bihar, Chota Nagpur, Burma, Madhya Pradesh, Delhi, and the Deccan area are home to the arjuna tree, a tall deciduous tree that may grow to a height of 60–80 feet and is often found beside rivers, streams, and dry water bodies. Sri Lankan and Mauritian woods are also home to this species [16]. *Terminalia arjuna'*s bark, leaves, and fruits have been utilized in traditional medicine to treat a variety of ailments [17]. The *Terminalia arjuna* tree is revered in India for its medicinal bark, which has been utilized there for thousands of years [18]. The inotropic effect of *Terminalia arjuna* is thought to originate from the saponin glycosides present in the plant. In contrast, the flavonoids/phenolics are thought to provide antioxidant and vascular stimulating activity, validating the multifaceted role in cardio-protection. Triterpenoids are chemical ingredient present in the *Terminalia arjuna* bark, which are responsible for the cardio-active effects [19,20]. According to previous research, the bark has a substantial inotropic and hypotensive effect, implying the improvement of coronary blood flow and shields the heart from ischemic damage. Mild diuretic, hypolipidemic, antithrombotic, and prostaglandin E-enhancing effects have also been detected [21]. Ischemic mitral regurgitation (IMR) is a major cause of morbidity and mortality after an acute myocardial infarction. In healthy volunteers under the age of 70, administration of the arjun bark powder resulted in a decrease in IMR, an increase in early echocardiographic phases and late atrial phase of ventricular filling ratio (E/A) ratio, and a decrease in anginal frequency [22]. The production of reactive oxygen species is critical in the development of several illnesses. Moderate free radical scavenging activity was shown with arjungenin, and its glucoside, both of which were isolated from *Terminalia arjuna* stem bark. The antioxidant capacity of arjungenin was evaluated using the 1-1 diphenyl-2-picrylhydrazyl (DPPH) assay [23]. In response to lipopolysaccharide stimulation of macrophages, terminoside A, an oleanane triterpene isolated from the acetone fraction of the ethanolic extract of *Terminalia arjuna* stem bark, has been shown to impede nitric oxide (NO) generation and reduce inducible nitric oxide synthase (iNOS) levels. Atherosclerosis, heart failure, ischemic cardiomyopathy, and myocardial necrosis have all been linked to increase NO production by nitric oxide synthase [24]. An increase in coronary blood flow was seen after injecting an aqueous bark extract into an isolated rabbit heart preparation [25]. Arjuna bark extract has been shown to lower both total cholesterol (TC) and triglyceride levels in prior animal studies [26]. Increased hepatic clearance of cholesterol, down-regulation of lipogenic enzymes, and inhibition of HMG-CoA reductase are postulated to cause the hypolipidemic effect [27]. Research into the effects of the bark extract on cardiac and hepatic LPO in albino rats revealed a possible role on thyroid hormones (suppression of thyroid function) in the improvement of these symptoms [28]. However, there was a lack of investigation on the impact of *Terminalia arjuna* bark extract on cardiac dysfunction due to oxidative stress. In light of this literature, this experiment examined the cardioprotective effect of *Terminalia arjuna* bark powder supplementation in ISO administered rats.

2. Methods and Materials

2.1. Chemicals and Reagents

Creatine kinase- Muscle Brain (CK-MB), creatinine, and uric acid kits were purchased from Clinchem (Budapest, Hungary). Di-sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, potassium chloride, glacial acetic acid, thiobarbituric acid, and dimethyl sulfoxide were collected from LOBA Chemie (Mumbai, India). Ethanol, xylene, and formaldehyde were obtained from Merck (Darmstadt, Germany). Isoproterenol was bought from Sigma Aldrich (St. Louis, USA). Tri-sodium citrate, sulfanilamide, N-(1-Naphthyl) ethylenediamine, potassium iodide, hydrogen peroxide, DTNB-Ellman's reagent or (5, 5′-dithiobis-(2-nitrobenzoic acid), hydrochloric acid (HCl), *o*-dianisidine and pentobarbital were also obtained from Merck (Darmstadt, Germany). Adrenaline was obtained from Incepta Pharmaceuticals Limited, Bangladesh.

2.2. Plant Materials

The arjun bark was collected from the North South University campus and authenticated by the expert of Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen was preserved for future reference (DACB-99123). The bark was cut into small pieces and dried in the open air. The dried bark was then grinded into coarse powder. This powder was supplemented with food to treat the ISO administered rats.

2.3. Animals Used for Experiments

Long-Evans male rats were used in this investigation. There were 24 rats, divided into four groups. The rats were about eight weeks old and weighed between 180 and 190 g. All rats were obtained from North South University's animal house facilities, which is part of the Department of Pharmaceutical Sciences. Each rat was housed in a standard isolated closet for a 12-h day/night cycle, in an air conditioned room (25 °C, 45% humidity). Standard chow diet and tap water supply were scrupulously maintained throughout the experiment. The Institutional Animal Use Ethical Committee (IACUC) approved the study protocol (Approval number—2024/OR-NSU/IACUC/0104).

2.4. Treatment Protocols

For this experiment, four groups were made: The control group, the ISO group (given 50 mg/kg ISO), the Control + arjun group (received arjun powder 2.5% in chow food, *W*/*W*), the ISO + arjun group (given 50 mg/kg ISO and received arjun powder 2.5% in chow food, *W*/*W*). Body weight was recorded on a daily basis, and the final body weight was reported on the 14th. On the day 14th, all rats were sacrificed with a high dose of pentobarbital (approximately 90 mg/kg), administered into the peritoneal region of each rat. Following the sacrifice, organs (heart and kidney) were taken and the moist weight of the organs was recorded. There were three sections for analysis: one for histology purposes, one for biochemical purposes and the other part was for gene expression analysis. The tissues for the histological sections were stored in neutral buffered formalin (pH 7.4), while the tissues part for biochemical analysis was frozen at −20 °C in a refrigerator. The third part was preserved carefully for mRNA extraction, at −80 °C in a refrigerator. Plasma was also extracted from blood at 4000 rpm for 15 min before being transferred to 1.5 mL microcentrifuge tubes and frozen at −20 °C for future experiments.

2.5. Heart Tissue Processing

Heart tissue was chosen for homogenization, therefore phosphate buffer with a pH 7.4, was used. These tissues were centrifuged at 8000 rpm, 4 °C for 15 min. The supernatants from the tubes were collected and preserved at −20 °C for further analysis. Clear supernatant was used to determine enzymatic activities and protein assays.

2.6. Oxidative Stress Markers Assay Procedures

2.6.1. Lipid Peroxidation Assay as Malondialdehyde (MDA)

Malondialdehyde (MDA) was determined to assess lipid peroxidation in plasma and tissues using the previously published method [29,30]. A standard curve was prepared and the MDA unit was stated as nmol/g tissue.

2.6.2. Nitric Oxide Assay in Plasma and Tissue

The level of nitric oxide (NO) was determined by measuring nitrate, and a previously reported method was used [29,30]. The absorbance of solutions at 540 nm was measured in comparison to a blank solution. The NO level was expressed in nmol/g tissue and was measured using a standard curve.

2.6.3. Advanced Oxidation Protein Product (AOPP) Assay

The degree of advanced oxidation protein product (AOPP) was evaluated using the previously described method by Sagor et al. (2015) and Ulla et al. (2017) [29,30]. In the experiment, chloramine-T standard was served in various concentrations as a positive control, and a negative control was represented by 0.2 mL acetic acid and 2 mL phosphate buffer saline. The absorbance of chloramine-T was measured at 340 nm and ranged from 0 to 100 nmol/mg. As a result, the APOP concentration unit was given as nmol mg^{-1} chloramine-T equivalents.

2.6.4. Myeloperoxidase (MPO) Activity Assay in the Heart Tissue

Myeloperoxidase (MPO) activity was measured using dianisidine-H_2O_2 system in 96-well plates [31]. For this test, three chemicals were used: H_2O_2, *o*-dianisidine dihydrochloride, and potassium phosphate buffer. The reagent amounts were as follows: potassium phosphate buffer was about 50 mM with a pH of 6, H_2O_2 was around 0.15 mM, and *o*-dianisidine dihydrochloride was about 0.53 mM. MPO absorbance was measured at 460 nm. The MPO unit was represented as U/min/mg protein.

2.7. Antioxidant Enzyme Activity Analysis

2.7.1. Catalase (CAT) Activity Assay

Catalase (CAT) activity assay protocol was explained in detail in previously published literature by Sagor et al. (2015) and Ulla et al. (2017) [29,30]. One unit of catalase activity is defined as a change in absorbance of 0.01 and expressed as units per minute.

2.7.2. Superoxide Dismutase (SOD) Activity Assay

To execute superoxide dismutase assay, the previously reported method was used [29,30]. The plasma and homogenized heart and renal tissue supernatant was used in this SOD activity assay. The capability of inhibition of epinephrine auto-oxidation was measured which was expressed as unit/mg and 50% inhibition is defined as one unit of enzyme activity.

2.8. Uric Acid and Creatinine Level Determination in Plasma

To analyze uric acid and creatinine level in plasma, the corresponding assay kits were used. All assay methods were carried out in accordance with the manufacturer's supplied instructions. The absorbance of uric acid was measured at 505 nm while creatinine absorbance was determined at 490 nm.

2.9. Analysis of Inflammation Regulatory Genes and Relative Oxidative Stress Levels by Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

The heart left ventricular tissues were used for the extraction of mRNA for qRT-PCR analysis using a previously published procedure [14]. Total mRNA isolation, cDNA synthesis, and qRT-PCR were done to measure the relative mRNA expression for inflammation and oxidative stress related genes. With the primer 3 online software, forward and reverse primers were designed and employed in this study. The genes and primers are given in Table 1. To standardize the relative transcript levels of each target gene, the housekeeping gene β-actin was employed.

Table 1. The forward and reverse sequence of the primer applied in qRT-PCR.

Name of gene	Type	Sequence
Nrf-2	Forward	5′-CCC AGCACA TCC AGACAGAC-3′
	Reverse	5′-TATCCAGGGCAAGCGACT C-3′
Heme oxigenase-1 (HO-1)	Forward	5′-TGCTCGCATGAACACTCTG-3′
	Reverse	5′-TCCTCTGTCAGCAGTGCCT-3′
Heme oxigenase-2 (HO-2)	Forward	5′-CACCACTGCACTTTACTTCA-3′
	Reverse	5′-AGTGCTGGGGAGTTTTAGTG-3′
MnSOD	Forward	5′-GCTCTAATCACGACCCACT-3′
	Reverse	5′-CATTCTCCCAGTTGATTACATTC-3
Catalase	Forward	5′-ATTGCCGTCCGATTCTCC-3′
	Reverse	5′-CCAGTTACCATCTTCAGTGTAG-3′
Glutathione peroxidase (GPx)	Forward	5′-GGGCAAAGAAGATTCCAGGTT-3′
	Reverse	5′-GGACGGCTTCATCTTCAGTGA-3′
IL-1	Forward	5′-ATGCCTCGTGCTGTCTGACC-3′
	Reverse	5′-CCATCTTTAGGAAGACACGGGTT-3′
IL-6	Forward	5′-AGCGATGATGCACTGTCAGA-3′
	Reverse	5′-GGTTTGCCGAGTAGACCTCA-3′
TNF-α	Forward	5′-ATGTGGAACTGGCAGAGGAG-3′
	Reverse	5′-CCACGAGCAGGAATGAGAAGAG-3′
TGF-β	Forward	5′-AAGAAGTCACCCGCGTGCTA-3′

	Reverse	5′-TGTGTGATGTCTTTGGTTTTGTC-3′
iNOS	Forward	5′-TGGTCCAACCTGCAGGTCTTC-3′
	Reverse	5′-CAGTAATGGCCGACCTGATGTTG-3′
NF-κB	Forward	5′-TGTGAAGAAGCGAGACCTGGAG-3′
	Reverse	5′-GGCACGGTTATCAAAAATCGGATG-3′
®-Actin	Forward	5′-GCGAGAAGATGACCCAGATC-3′
	Reverse	5′-GGATAGCACAGCCTGGATAG-3′

2.10. Histopathological Staining of the Heart of ISO Administered Rats

Initially, heart tissues were fixed in neutral buffered formalin. After being fixed, all of the tissues were embedded in paraffin. Following that, these tissues were sectioned at 5 μm with a rotary microtome, and the sliced sections were saved for staining with hematoxylin and eosin and Sirius red. Hematoxylin and eosin staining was used to reveal inflammatory cells in the heart. On the other hand, Sirius red staining was employed to assess the level of collagen deposition in the heart. After staining, photographs were taken with a Zeiss Axioscope microscope at 40× magnification.

2.11. Statistical Analysis

The mean ± standard error of the mean (SEM) was used to express all values. The results were calculated and determined using the graph pad prism program (Version 9). One-way ANOVA was used for statistical analysis and Tukey test was used as a post hoc test for multiple comparisons among the groups involved in this study. A significance level of $p < 0.05$ was adopted for all results.

3. Results:

3.1. Effect of Terminalia arjuna Bark Powder on Total Heart, Left Ventricle (LV), Right Ventricle (RV) and Kidneys Wet Weight in ISO Administered Rats

In all groups of rats, ISO administration significantly increased the heart weight in rats compared to the control rats ($p < 0.05$). The result of this study demonstrates that the *Terminalia arjuna* bark powder decreased the wet weight of the entire heart (total heart weight) ($p < 0.001$) in ISO administered rats (Figure 1A). However, the wet weight of the left ventricle was not lowered in ISO administered rats by the *Terminalia arjuna* bark supplementation (Figure 1B). The heart and LV weights in control+ *Terminalia arjuna* group was not significantly impacted by the *Terminalia arjuna* bark powder supplementation (Figure 1). The kidney wet weight in Control, ISO, Control + *Terminalia arjuna* and ISO+ *Terminalia arjuna* group of rats were not significantly altered by the ISO administration as well as *Terminalia arjuna* bark supplementation (Figure 1C).

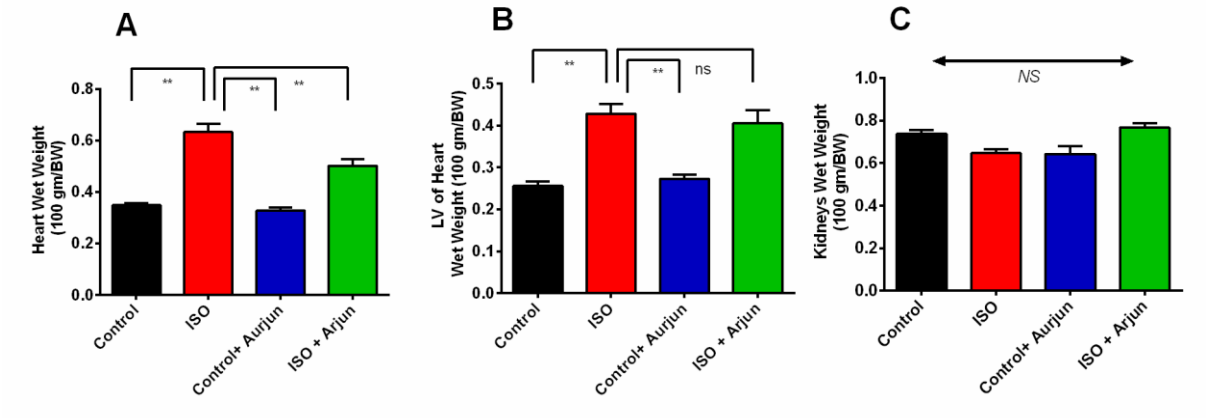

Figure 1. Effect of *Terminalia arjuna* bark powder supplementation on total heart (**A**), left ventricular (**B**) and kidney wet weights (**C**) in ISO administered rats. Data are expressed as mean± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$, ns—not significant.

3.2. Effect of Terminalia arjuna Bark Extract on MDA in Plasma, Heart of ISO Administered Rats

As a result of lipid peroxidation, the first parameter, called MDA, was found to be considerably higher in the plasma and heart of ISO administered rats than in the controls ($p < 0.001$) (Figure 2A,B). The ISO + *Terminalia arjuna* group showed considerably decreased MDA concentrations in the heart and plasma compared to the ISO group, ($p < 0.001$) (Figure 2A,B). The MDA levels in the plasma and heart did not change in the Control + *Terminalia arjuna* group compared to control rats (Figure 2A,B).

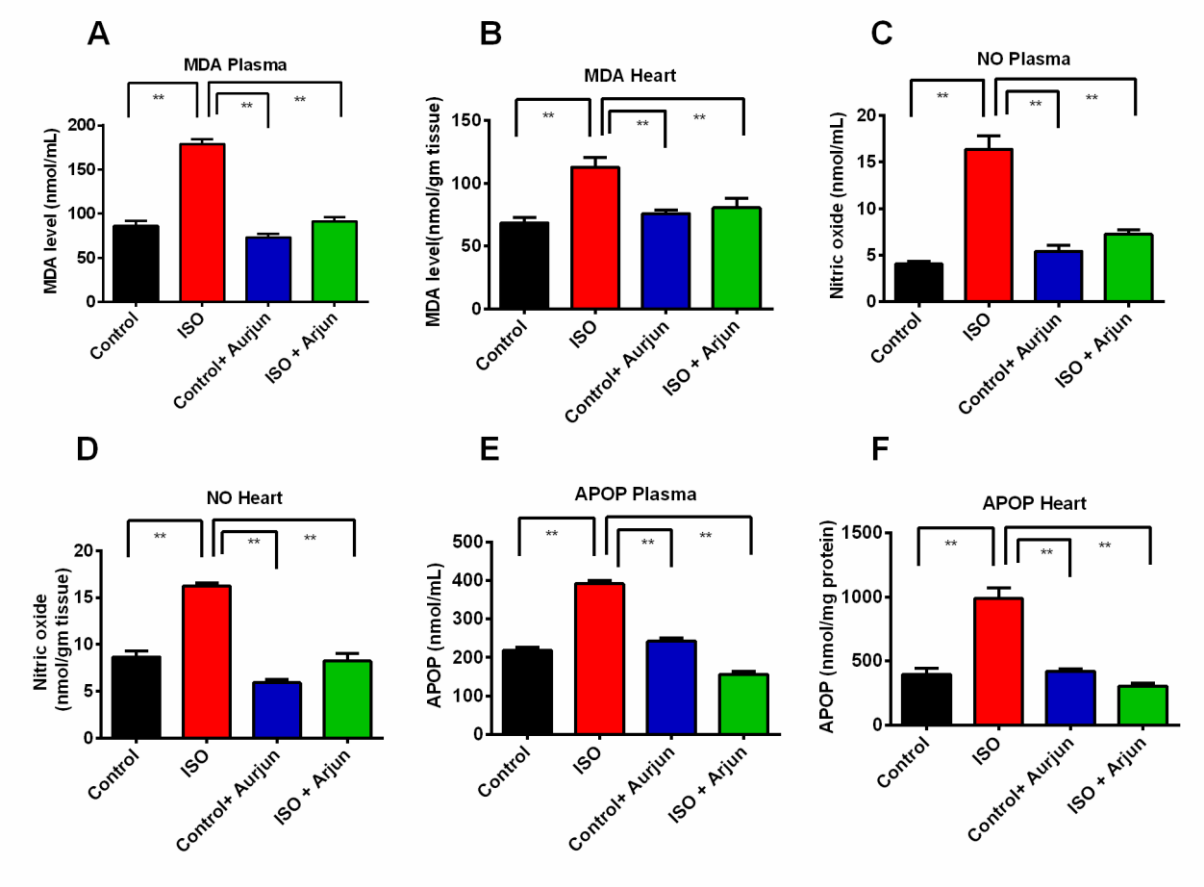

Figure 2. Effect of *Terminalia arjuna* bark powder supplementation on oxidative stress parameters such as MDA, NO and APOP level in plasma and heart of ISO administered rats. The picture represented the following data, MDA plasma (A), MDA Heart (B), NO plasma (C), NO heart (D), APOP plasma (E) and APOP heart (F). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$.

The next oxidative stress parameter measured was NO. When compared to the control group, the NO concentration in the heart and plasma was increased significantly ($p < 0.001$) in the ISO administered group (Figure 2C,D). The rats in the ISO + *Terminalia arjuna* group showed lower NO concentrations in the heart and plasma ($p < 0.001$) compared to the ISO administered rats. This data demonstrated that even after administering ISO, *Terminalia arjuna* bark powder may return the NO concentrations to normal level (Figure 2C,D). Moreover, in comparison to the control group, the Control + *Terminalia arjuna* did not alter the NO concentration in plasma, heart and kidneys ($p < 0.001$) (Figure 2C,D).

AOPP is another parameter of oxidative stress. The AOPP level in plasma and tissues was increased significantly in ISO administered rats compared to the control group (Figure 2E,F). *Terminalia arjuna* bark powder supplement in ISO administered rats showed significantly declined levels of AOPP in plasma, and heart ($p < 0.001$) compared to the rats which were only administered with ISO (Figure 2E,F). Control + *Terminalia arjuna* group did not show any changes in AOPP level in plasma, and heart compared to the control rats (Figure 2E,F).

3.3. Effect of Terminalia arjuna Bark Powder on SOD, Catalase and Glutathione in Plasma and Heart of ISO Administered Rats

The SOD enzymatic activity was significantly lowered in plasma and heart of ISO administered rats compared to the controls ($p < 0.001$) (Figure 3A,B). On the other hand, the *Terminalia arjuna* bark powder recovered the SOD enzyme activity considerably ($p < 0.001$) in the plasma and heart of ISO administered rats. This data suggests that *Terminalia arjuna* bark powder can enhance the SOD action in ISO administered rats (Figure 3A,B). In comparison to the control group, the plasma and cardiac SOD enzyme activities were not altered in the Control + *Terminalia arjuna* group ($p < 0.001$) (Figure 3A,B).

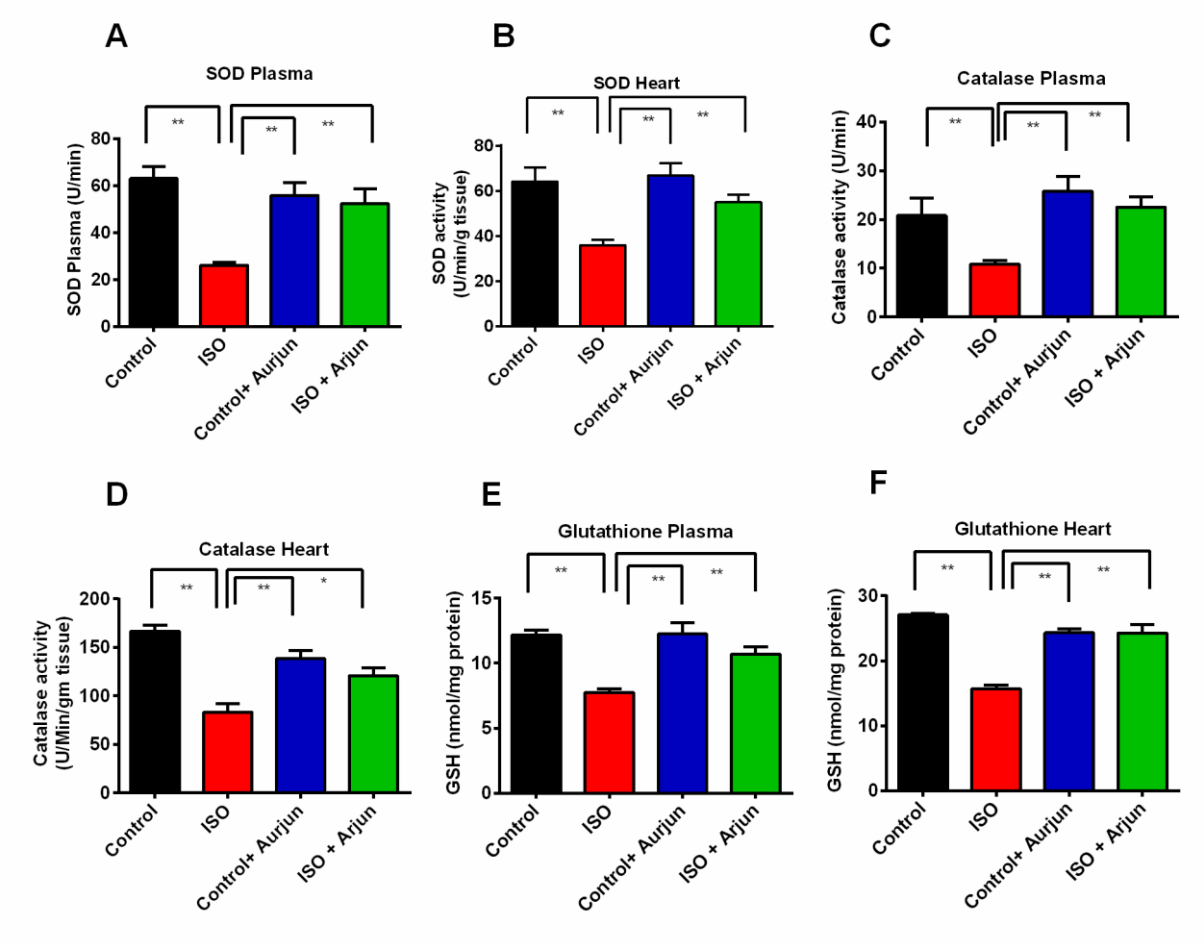

Figure 3. Effect of *Terminalia arjuna* bark powder supplementation on SOD and catalase activity and GSH concentration in plasma and heart of ISO administered rats. The picture represented the following data, SOD plasma (A), SOD Heart (B), catalase plasma (C), catalase heart (D), glutathione plasma (E) and glutathione heart (F). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. * means $p < 0.05$ and ** sign means $p < 0.001$.

The ISO administered rats showed improvement of catalase activity in the plasma and heart compared to the controls ($p < 0.001$) (Figure 3C,D). Rats in the ISO + *Terminalia arjuna* group exhibited a considerable ($p < 0.001$) improvement of catalase enzyme activity in both plasma and the heart when compared to the ISO group (Figure 3C,D). The Control + *Terminalia arjuna* group also showed normal catalase enzyme activity in the heart and plasma (Figure 3C,D).

GSH is an additional indicator of antioxidant enzymes. The concentration of GSH in the heart and plasma was reduced significantly ($p < 0.001$) in ISO administered rats in comparison to the control group (Figure 3E,F). ISO administered rats given *Terminalia arjuna* bark powder supplement showed considerably ($p < 0.001$) restored plasma and cardiac GSH concentrations than the ISO group (Figure 3E,F). When compared to the control rats, the GSH level in the plasma and heart did not alter in the Control + *Terminalia arjuna* group (Figure 3E,F).

3.4. Effect of Terminalia arjuna Bark Powder Supplementation on CK-MB Activities in the Plasma and MPO Activity in the Heart of Isoprenaline (ISO) Administered Rats

Rats given ISO showed higher levels of CK-MB activities in the plasma ($p < 0.001$) when compared to the control group (Figure 4A). Rats treated with *Terminalia arjuna* in ISO administered rats showed significantly ($p < 0.001$) lower CK-MB activity in the plasma (Figure 4A).

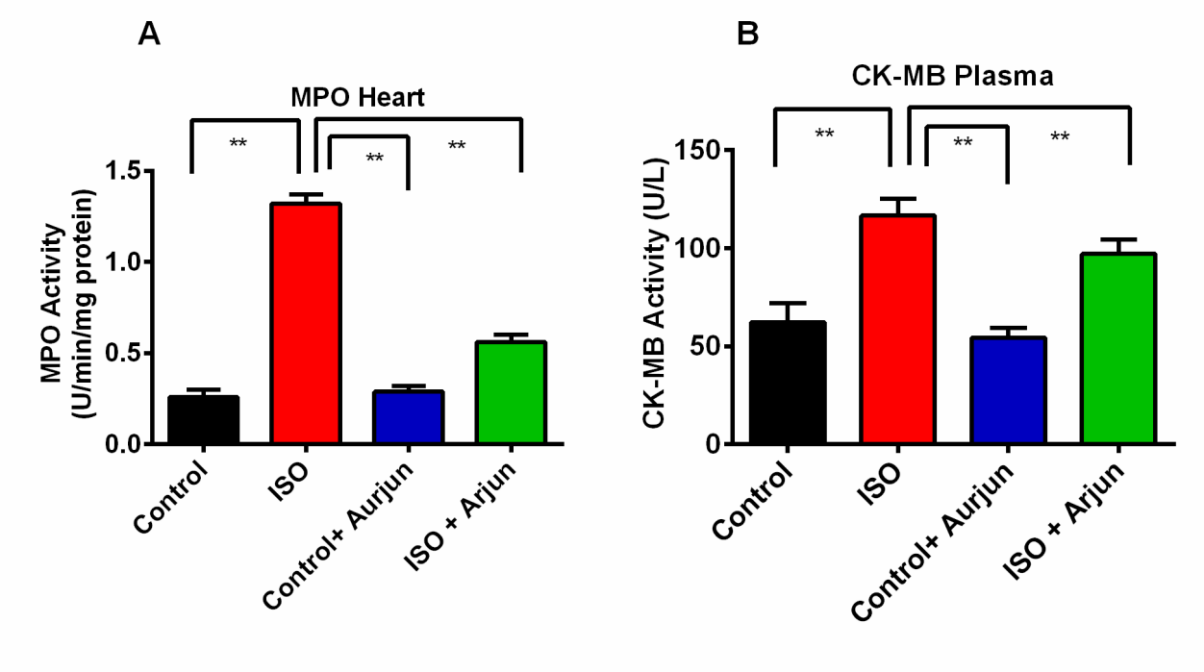

Figure 4. Effect of *Terminalia arjuna* bark powder supplementation on MPO and CK-MB activity in ISO administered rats. The picture represented the following data, MPO heart (A), and CK-MB plasma (B). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$.

ISO administration in rats resulted in significantly increased MPO activities in ISO administered rats significantly ($p < 0.05$) compared to control rats. *Terminalia arjuna* bark powder supplementation in ISO administered rats showed considerably decreased MPO activity ($p < 0.001$) than ISO administered rats (Figure 4B). *Terminalia arjuna* bark powder supplementation did not change the MPO activities in control+ *Terminalia arjuna* rats (Figure 4B).

3.5. Effect of Terminalia arjuna Bark Powder Supplementation on Antioxidant Genes Expression in the Heart of ISO Administered Rats

The ISO administered rats showed lower Nrf-2 transcript levels in the heart than control rats (Figure 5). *Terminalia arjuna* bark powder supplementation restored the Nrf-2 expression in the heart of ISO administered rats significantly (Figure 5). A considerable ($p < 0.001$) up-regulation of HO-1 and HO-2 transcript levels was also seen in ISO administered rats, which received the *Terminalia arjuna* bark powder supplementation (Figure 5). Moreover, ISO administered rats showed lower gene expression for endogenous antioxidant enzymes including SOD, catalase, and GPx compared to the control rats (Figure 5). Additionally, the *Terminalia arjuna* bark powder supplementation in ISO-administered rats resulted in a considerable restoration in the gene expression of those antioxidant enzymes (Figure 5).

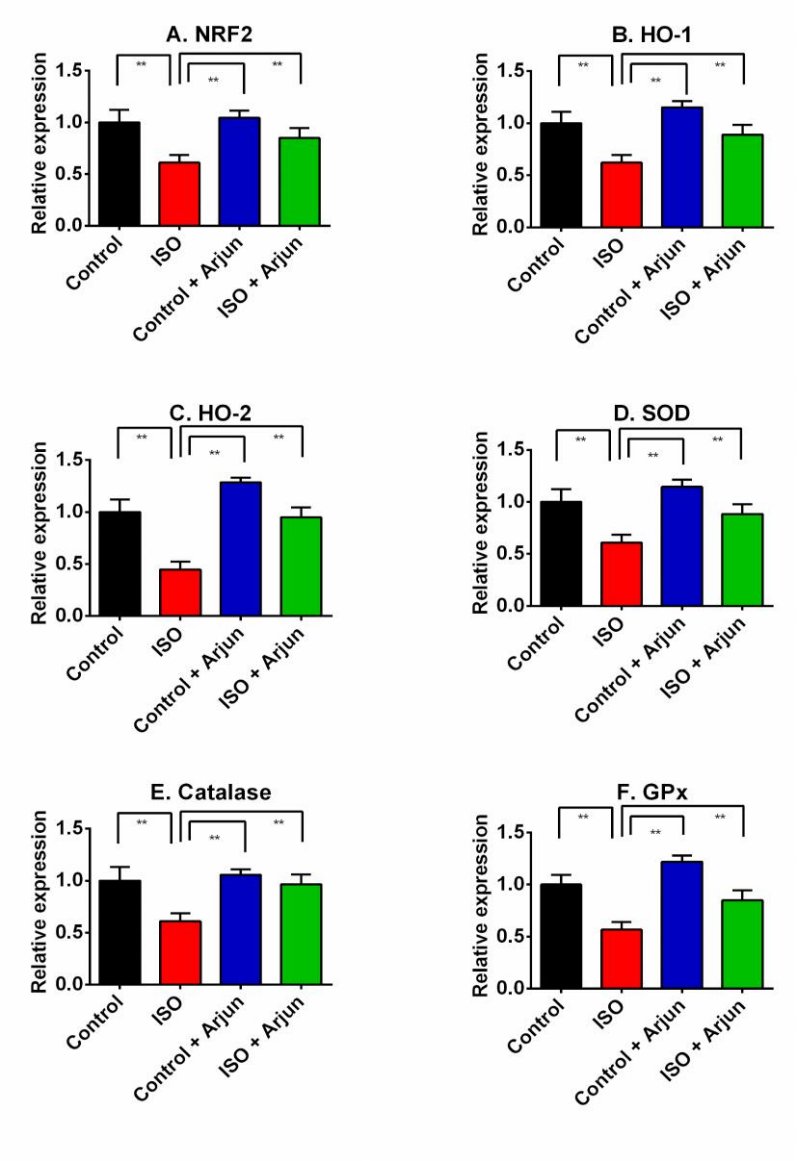

Figure 5. Effect of *Terminalia arjuna* bark powder supplementation on mRNA expression related antioxidants in the heart of ISO administered rats. The picture represented the following data, Nrf-2 (A), HO-1 (B), HO-2 (C), SOD (D), catalase (E) and GPx (F). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$.

3.6. Effect of Terminalia arjuna Bark Powder Supplementation on Inflammatory Genes Expression in the Heart of ISO Administered Rats

This study assessed the expression of six genes, including interleukin-1 (IL-1), interleukin-6 (IL-6), transforming growth factor beta-1 (TGF-β1), tumor necrosis factor alpha (TNF-α), nuclear factor kappa B (NF-κB), and inducible nitric oxide synthase (iNOS), that cause inflammation and fibrosis in the heart of ISO administered rats (Figure 6). The heart showed considerably ($p < 0.001$) higher levels of IL-1, IL-6, and TNF-α gene expression in ISO administered rats compared to the control rats (Figure 6). *Terminalia arjuna* bark powder supplementation in ISO-administered rats resulted in a lower level of these gene expression in the heart compared to the ISO administered rats (Figure 6).

Again, rats given ISO doses showed considerably higher expression of TGF-β1, iNOS, and NF-κB in the heart than the control group (Figure 6). *Terminalia arjuna* bark powder supplementation lowered the expressions of all these pro-inflammatory and inflammatory genes in the heart of ISO administered rats significantly ($p < 0.05$) (Figure 6).

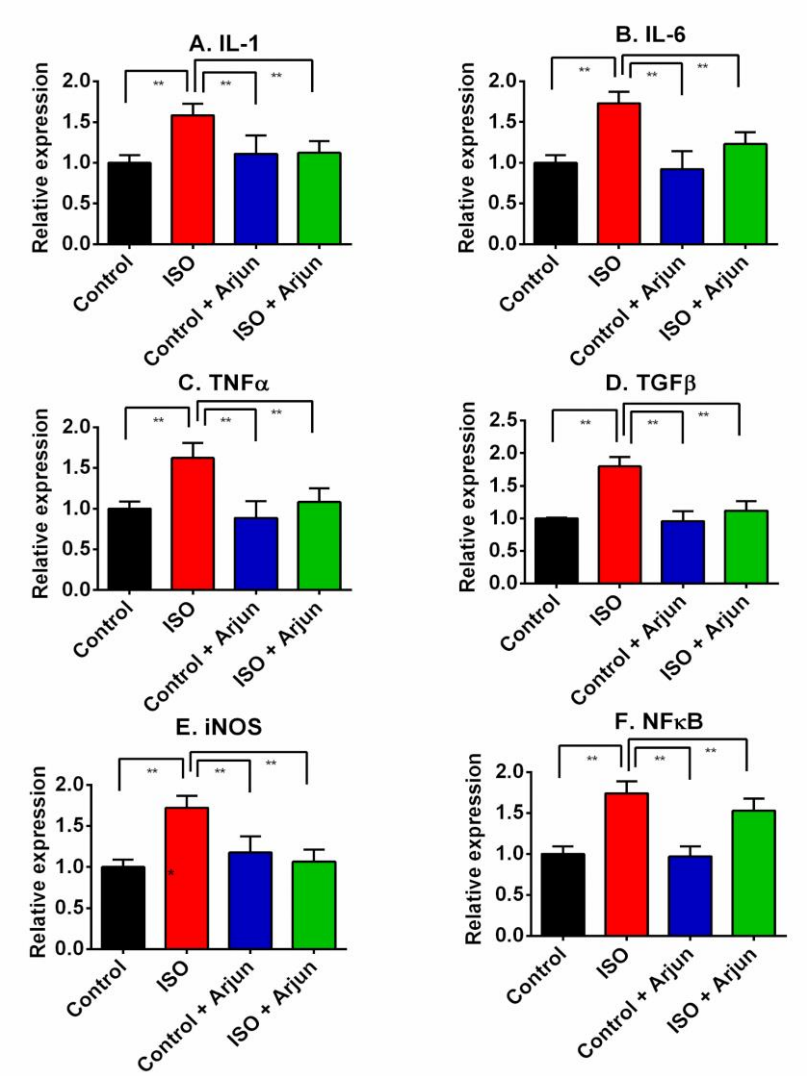

Figure 6. Effect of *Terminalia arjuna* bark powder supplementation on mRNA expression related to inflammation in the heart of ISO administered rats. The picture represented the following data, IL-1 (A), IL-6 (B), TNF-α (C), TGF-β (D), iNOS (E) and NF-κB (F). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$.

3.8. Effect of Terminalia arjuna Bark Powder Supplementation on Plasma Uric Acid and Creatinine Level in ISO Administered Rats

The plasma uric acid concentration was measured for every group present in this study. The uric acid concentration was increased significantly ($p < 0.001$) in ISO administered rats than that of the control group (Figure 7A). As compared to the ISO group, the uric acid plasma concentration was found considerably lower ($p < 0.001$) in rats, which received *Terminalia arjuna* bark powder supplementation (Figure 7A). The Control + *Terminalia arjuna* group did not exhibit any anomalies in plasma uric acid concentration (Figure 7A).

Moreover, the ISO administered rats showed higher levels of plasma creatinine concentration ($p < 0.001$) compared to the control rats (Figure 7B). *Terminalia arjuna* bark powder supplementation lowered the creatinine plasma levels significantly ($p < 0.001$) in the ISO + *Terminalia arjuna* group (Figure 7B). Creatinine plasma concentrations were found normal in the Control + *Terminalia arjuna* group compared to the control group (Figure 7B).

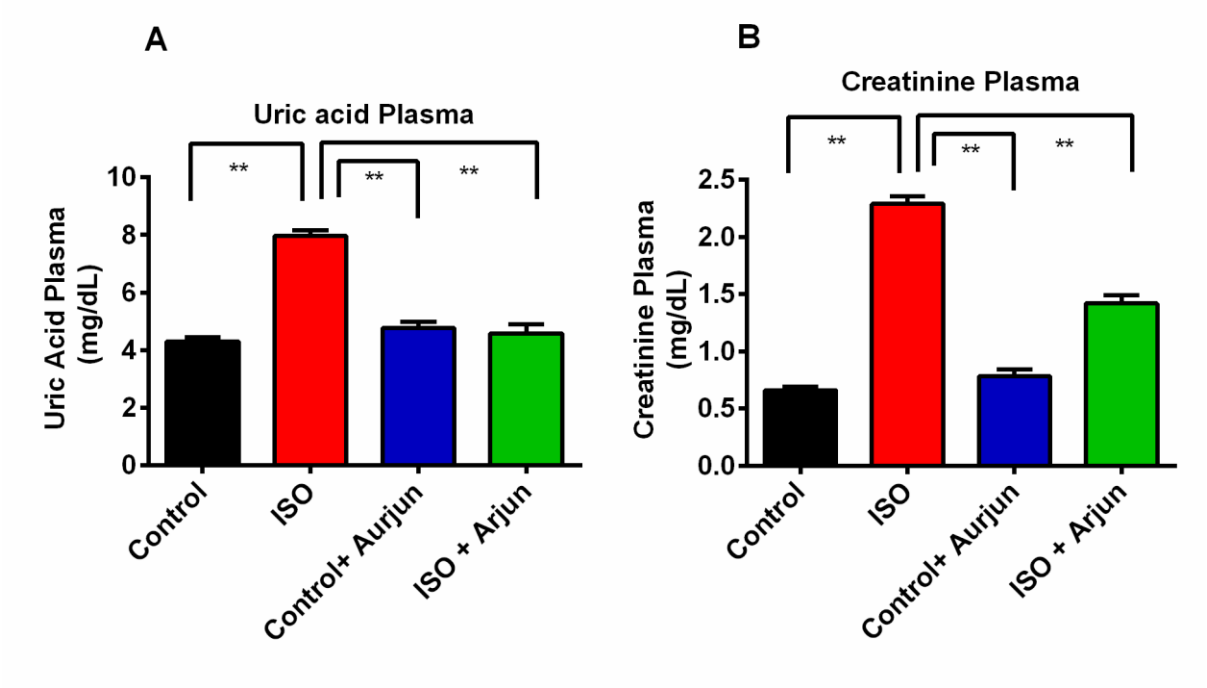

Figure 7. Effect of *Terminalia arjuna* bark powder supplementation on uric acid and creatinine level in plasma of ISO administered rats. The picture represented the following data, uric acid plasma (A) and creatinine plasma (B). Data are expressed as mean ± standard error of mean, $n = 6$. Statistical analysis was done by One-way ANOVA followed by Tukey post hoc test for the comparison between the groups used in this study. Statistical significance was considered as $p < 0.05$ in all cases. ** sign means $p < 0.001$.

3.9. Effect of Terminalia arjuna Bark Powder Supplementation on Histological Assessment in the Heart of ISO Administered Rats

To check the abnormalities of the cardiac tissues due to ISO administration, the hematoxylin and eosin (H and E) staining as well as Sirius red staining were performed. H and E staining revealed that ISO administered rats showed increased scar formation, inflammatory cells infiltration and myocytes hypertrophy in the heart compared to the control rats (Figure 8). *Terminalia arjuna* bark powder supplementation lowered these pathological changes in the heart of ISO administered rats (Figure 8). *Terminalia arjuna* bark powder supplementation did not produce any deleterious changes in the heart of control rats (Figure 8).

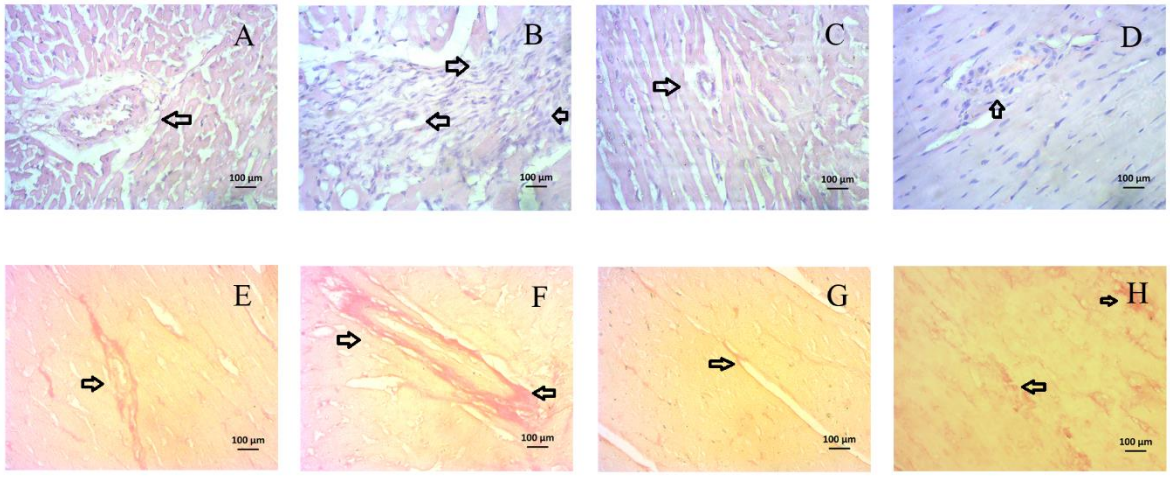

Figure 8. Effect of *Terminalia arjuna* bark powder supplementation on cardiac structure and fibrosis level in ISO administered rats. Upper panel Hematoxylin and eosin staining, (**A**) control; (**B**) ISO; (**C**) Control + *Terminalia arjuna*; (**D**) ISO + *Terminalia arjuna*. Lower panel- Sirius red staining, (**E**) control; (**F**) ISO; (**G**) Control+ *Terminalia arjuna;* (**H**) ISO + *Terminalia arjuna*. The arrow head showed the inflammatory cells infiltration and collagen deposition in the heart section.

Sirius red staining in the heart section demonstrated increased levels of collagen deposition and fibrosis in the left ventricle of ISO administered rats compared to the control rats (Figure 8). *Terminalia arjuna* bark powder supplementation decreased the collagen deposition and fibrosis in the heart of ISO administered rats (Figure 8). This staining also revealed that *Terminalia arjuna* bark powder supplementation did not alter the normal architecture of the heart (Figure 8).

4. Discussion

Oxidative stress is considered as a key regulator for the development of cardiac dysfunction in case of MI. This investigation showed that ISO administration in rats developed lipid peroxidation and oxidative stress in the heart. The antioxidant enzymes function also declined because of the ISO administration. *Terminalia arjuna* bark powder supplementation prevented the lipid peroxidation and improved the oxidative stress in ISO administered rats. Moreover, the inflammation and cardiac fibrosis was also prevented by the *Terminalia arjuna* bark powder supplementation.

Oxidative stress is particularly responsible for the tissue damage in the heart and therefore an efficient treatment is necessary to reduce this stress. MDA is a final product of oxidative stress in the tissues. In our experiment, ISO raised MDA concentration in tissues, which was decreased by *Terminalia arjuna* bark powder supplementation. This result is also consistent with the prior study showing that *Terminalia arjuna extract* reduced MDA levels due to its antioxidant activity [32]. Another marker related to oxidative stress is NO. In this study, ISO administration in rats raised NO concentration, which was lowered by *Terminalia arjuna* bark powder supplementation. In a prior investigation, rotenone was used to induce neurotoxicity accompanied by elevated NO level which was reduced with *Terminalia arjuna* extract therapy [33]. Severe oxidative stress may lead to the development of protein oxidation and generate dysfunctional essential proteins. AOPP is thus considered as another component of the oxidative stress. This investigation showed that ISO administration in rats caused increased AOPP levels in the plasma and tissues. This finding is supported by previous report suggests that ISO administration may increase the AOPP level in kidney tissues [34]. *Terminalia arjuna* bark powder supplementation reduces AOPP concentration in the plasma and tissues in ISO-administered rats.

Increased oxidative stress is a direct consequence of declined antioxidant enzymes function in the tissues. Several antioxidant systems are available to fight against the free radicals production and oxidative stress. Catalase and SOD activities are measured for assessing the antioxidant capacities in tissues. It was demonstrated that ISO reduced the antioxidant enzyme activities such as SOD and catalase [14,15]. Declined SOD and catalase could be a result of decreased gene expression in the tissues level. Previous reports suggest that SOD and catalase gene expression were found decreased in the heart of ISO administered rats [14]. *Terminalia arjuna* bark powder supplementation restored the SOD and catalase activities by stimulating the gene expression in the heart of ISO administered rats. These findings are in line with previous investigational report suggesting that *Terminalia arjuna* boosted antioxidant activity in lymphoma bearing AKR mice [35].

ISO administration in rats also developed cardiac damage and oxidative stress as discussed above. Significant hypertrophy and increased wet weight of heart in ISO administered rats were observed in this study. The cardiac damage is confirmed by the elevation of CK-MB activities in plasma of ISO administered rats. Previous reports also suggest that ISO administration develop MI like symptoms and cardiac damage which is evident by the raised CK-MB activities [14]. *Terminalia aurjuna* powder supplementation prevented the cardiac damage and normalized the CK-MB activities in plasma of ISO administered rats. Previous investigation showed that antioxidant rich plant extracts may prevent cardiac damage and lowered the CK-MB activities suggesting the cardiac protection of antioxidants [14]. *Terminalia aurjuna* also possess strong antioxidants which may be responsible for the cardiac protection in ISO administered rats.

Nuclear factor erythroid 2–related factor 2 (Nrf-2), heme oxygenase 1 (HO-1), and heme oxygenase 2 (HO-2), which are mostly related to oxidative stress, were all investigated in this study. The Nrf-2 gene senses the oxidative stress and enhances the expression of other antioxidant genes such as SOD, catalase and glutathione peroxidase (Gpx) etc. to counter the oxidative stress. Thus, decline expression of Nrf-2 may further jeopardize the antioxidant system in the tissues. In this study, ISO administration in rats showed declined Nrf-2 with its other regulator genes such as HO-1 and HO-2 [14]. This phenomenon can be seen in other studies showing that increased oxidative stress may lead to the decline of Nrf-2 expression [34,36]. In this study, *Terminalia arjuna* bark powder enhanced the Nrf-2 transcript levels in the heart, which were reduced by ISO administration. In this investigation, ISO reduced the transcript level of HO-1, which was regained by *Terminalia arjuna* bark powder supplementation. This report again supported by a previous investigation showed that *Terminalia arjuna* bark powder

supplementation boosted catalase, SOD, and GPx enzyme activities, which was exacerbated by the administration of N-nitrosodiethylamine [37].

Interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS), transforming growth factor beta (TGF-β), and nuclear factor kappa B (NF-κB) are important inducers for inflammation and fibrosis in tissues. In this investigation, ISO administration raised IL-1 and IL-6 transcription factors, which were normalized by *Terminalia arjuna* bark powder supplementation. This finding is in consonance with the previous one showing that IL-6 was downregulated by the treatment of *Terminalia arjuna* extract [38]. TNF-α is another inflammatory agent that was raised by ISO administration. However, *Terminalia arjuna* bark powder supplementation decreased TNF- expression in the heart of ISO administered rats which was also supported by a previous study [32]. In this investigation, *Terminalia arjuna* bark powder supplementation also normalized the elevated levels of iNOS, TGF β, and NF-κB transcript levels in ISO administered rats. It is evident that *Terminalia arjuna* extract decreased iNOS in murine macrophages [39]. MPO, which serves as an important marker for inflammation in the tissues, is particularly present in the neutrophils. In this study, ISO boosted the MPO activities in the heart. *Terminalia arjuna* bark powder supplementation reduces increased MPO activity in rats given ISO. *Terminalia arjuna* extract prevented the rise of MPO activity in rotenone induced neurotoxicity [40].

Cardiac fibrosis is evident in case of oxidative stress and in ISO administered rats [36]. Inflammatory cell infiltration and inflammatory mediators may influence the cardiac fibroblast to increase the production of extracellular matrix proteins, mainly collagen [41]. The genes expression of IL-1, IL-6 and TNF-α are highly correlated with the production of cardiac fibrosis in MI and in ISO administered rats [42]. These inflammatory gene expressions may raise the TGF-β signaling which is the master regulator of fibrosis development and also cardiac hypertrophy [43]. Enlarged heart and fibrosis scar leads to a dysfunctional heart and ultimately turn into heart failure. In this study, histological assessment confirms that ISO administration may induce mononuclear cell infiltration in the heart and causes collagen deposition as a marker of fibrosis. *Terminalia arjuna* bark powder prevented the mononuclear cells infiltration and collagen deposition in the heart of ISO administered rats. This finding is supported by previous report showed that inflammatory gene expression and TGF-β signaling may jeopardize the cardiac function which may be modulated by *Terminalia arjuna* bark extract [44].

In this investigation, plasma creatinine and uric acid were also measured. ISO administration in rats raised plasma creatinine and uric acid levels. In this study, it was found that creatinine and uric acid levels were normalized/reduced in rats given ISO by *Terminalia arjuna* bark powder supplementation. These findings are correlated with a previous study reporting that *Terminalia arjuna* bark powder supplementation reduces both serum creatinine and uric acid levels in cyclosporine A- induced cardiotoxicity in rats [45].

5. Conclusions

This investigation revealed the protective effect of *Terminalia arjuna* bark powder in the heart of ISO administered rats through biochemical and histological assessment. The molecular mechanism of the protective effect in the heart of ISO administered rats were also elucidated in this study probably by suppressing the mediators of oxidative stress and inflammation. However, more clinical and preclinical research is warranted for elucidating the benefits in human. There should also be a toxicological assessment, required for the safety assurance of *Terminalia arjuna* bark use. Considering these aspects, it could be a future choice of medicine for the treatment of cardiac disorders associated with inflammation and oxidative stress.

Author Contributions: The concept and design of this study was generated by N.S. and M.A.A. M.A.A. also trained M.A., N.R., K.A., P.S., S.S. (Sumaia Sarif), K.F.M., I.J. and S.S. (Shahnaz Siddiqua) on all the research related activities and supervised and coordinated the whole study. M.A., P.S., N.R., and S.S. (Sumaia Sarif) carried out animal handling, animal experimentation and animal sacrifice. M.A., N.R., P.S., K.F.M., I.J. and S.L. also performed the biochemical analysis. K.A., S.S. (Sumaia Sarif), I.J. and S.S. (Shahnaz Siddiqua) performed the histological analyses. M.A., P.S., N.R., I.J. and F.K. performed the gene expression analysis. Statistical analysis and result interpretation were done by M.A.A., N.S., K.A. and F.K. The draft manuscript was prepared by N.S., M.A.A., K.A., and F.K., that was read and approved by all authors. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable

Data Availability Statement: All experimental data of this study are stored in the hard disk drive of laboratory computer which will be attainable upon request.

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