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*Original Article*

# Black Seed (*Nigella sativa*) Powder Supplementation Prevented Oxidative Stress and Cardiac Fibrosis in Isoprenaline Administered Rats

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**Abstract:**
Black seed is a well-established herbal medicine that possesses intense anti-inflammatory and antioxidant activity. Additionally, black seed is rich in polyphenolic compounds. The present study was undertaken to examine the efficacy of black seed powder supplementation on myocardial infarction, and fibrosis in the heart of isoprenaline (ISO) administered rats. Five equal groups of thirty-five matured Long Evans male rats were created. ISO was administered twice a week at a dose of 50 mg/kg S.C. for two weeks. Powdered black seed was mixed with ground food and provided every day for two weeks. After completing the treatment period, every rat was sacrificed, and blood and organs were collected. The blood plasma, heart, and kidney tissue homogenates were assayed to determine the level of various biochemical parameters, and oxidative stress indicators. Inflammatory cell infiltration, and fibrosis were also assessed by histological staining. Increased concentrations of different indicators of oxidative stress including malondialdehyde (MDA) and nitric oxide (NO) were seen in ISO-administered rats. Powdered black seed supplement decreased the MDA and NO level in ISO administered rats. Black seed-treatment in ISO-administered rats also improved the endogenous antioxidant catalase and SOD activities. Furthermore, treatment with black seed markedly ameliorated inflammatory cells infiltration; fibrosis in the heart and decreased myeloperoxidase (MPO) and CK-MB activity in plasma of ISO-administered rats. The finding of this study implies that treatment with black seed powder prevents oxidative stress in the kidneys and heart in ISO-administered rats. The protective effect may be exerted due to the antioxidant compounds present in black seed powder.

**Keywords:** cardiac fibrosis; isoprenaline; black seed; oxidative stress; myocardial infarction

## 1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally [1, 2]. In developed countries about 50% of the mortality rate is due to cardiovascular disease. In developing countries, the epidemic of CVD is becoming increasingly prevalent. In Singapore, for instance, in the two decades between the 1960s and 1980s, the death rate from coronary heart disease become doubled [3]. The main risk factors were associated with the emerging incidence of CVD in developing countries such as smoking, salt intake, sedentary nature of lifestyle, high-calorie intake, etc. [4]. The prevalence of CVD has been estimated to be nearly three percent in 200, and up to 10% in recent years, indicating an emerging prevalence in India [5, 6]. In Bangladesh, the exact prevalence of CVD is unknown. The prevalence of coronary artery diseases

(CAD) has been outlined to be 0.33% to 19.6% in various studies in Bangladesh [7, 8].

Myocardial infarction (MI) is the death of cardiomyocyte due to an ischemic insult [9]. The ability of clinicians to identify cardiomyocyte death has been significantly improved by the introduction of highly sensitive biomarkers, such as cardiac troponins. In certain healthy individuals, mildly persistent elevations in troponin levels may be caused by the slow natural turnover of cardiomyocytes. The normal heart has a significant capacity to control the production of reactive oxygen species (ROS) by means of inhibitory enzymatic pathways (catalase, glutathione peroxidase, and superoxide dismutase, for instance) [10, 11]. The tumor necrosis factor (TNF)-α, a pro-inflammatory cytokine that is also secreted in the site of infracted myocardium and may contribute to the stimulation of cytokine generation in the infarct zone of the heart through mono nuclear cells infiltration [12].

Isoproterenol (ISO), a β1-adrenergic agonist, has been employed as a model substance in rats to trigger infarct-like lesions as well as various other animal species. It is also reported to be associated with oxidative stress in the heart tissues that cause the heart muscle infarct-like necrosis [13]. ISO is known to generate lipid peroxidation (LPO), which is a factor that leads to irreparable damage to the cardiac membrane, as well as free radical generation, and thus favors the deposition of myocardial lipids and fibrosis [14]. Thus, natural antioxidants may be useful in the scavenging of free radicles and oxidative stress in ISO induced MI in rats.

*Nigella sativa* (*N. sativa*) is a spice plant belonging to the family of *Ranunculaceae* [15]. It is a grassy annual plant with green to blue flowers and black seeds. For skin eruptions, paralysis, hemiplegia, back discomfort, rheumatoid arthritis, and other inflammatory illnesses, the fixed oil derived from N. sativa seeds is beneficial [16, 17]. Diabetes has been treated with the help of plant extracts. Because N. sativa extract inhibits hepatic gluconeogenesis and has insulinotropic qualities, it is effective in lowering blood sugar [18-20]. Moreover, some N. sativa extracts and their components may reduce blood pressure by blocking calcium channels [21, 22] and had a strong inhibitory effect on the isolated guinea pig heart's contractility and heart rate [23, 24]. It was also discovered that *N. sativa* seeds and their component, thymoquinone, lower serum cholesterol, triglyceride, and glucose levels as well as platelet and leukocyte counts [24, 25]. It was demonstrated that the whole oil and crushed seeds of *N. sativa* reduced the levels of cholesterol, triglycerides, prolactin, and glucose in the healthy female subjects [26]. *N. sativa* seed extract also possesses antioxidant activity in scavenging free radicles in various in vitro system. *N. sativa* seed extract treatment may also prevent inflammation and oxidative stress in experimental animals. Considering the beneficial role of *N. sativa* seed extract in various experimental diseases conditions, this study was conducted to assess the possible advantages in preventing inflammation and oxidative stress in ISO administered rats.

## 2. Materials and Methods

### *2.1 Chemicals and reagents*

From Samarth Life Sciences Pvt. Ltd. (Mumbai, India), the isoprenaline ampoule (solution) was supplied. From Sigma Chemical Company (USA), thiobarbituric acid (TBA) has been purchased for the detection of malondialdehyde. Purchased from J. I. Baker (USA) were metaphosphoric acid and trichloroacetic acid (TCA). 50, 50-dithiobis-2-nitrobenzoate (Elman's reagent) was acquired from Sigma Aldrich (USA); sodium hydroxide was purchased from Merck (Germany); and creatine kinase muscle/brain (CK-MB) assay kits were acquired from DCI diagnostics (Budapest, Hungary). In this experiment, standard and analytical grade chemicals and reagents were utilized exclusively.

### *2.2 Black seed sample collection and powder preparation*

Fresh black seeds were collected from a local market in Dhaka, Bangladesh. Black seed was authenticated from the National Herbarium located in Mirpur, Dhaka (Accession Number DACB 94799). The black seeds were ground into coarse powder in a kitchen grinder. Treatment of the experimental rats was done with this coarse powder.

*2.3 Animals for experiment*

From North South University's animal home, 35 male Long Evans rats weighing between 200 and 220 g at twelve to fifteen weeks of age were received. Each rat was kept in a separate cage with a 12-hour light and day cycle and a 24 ± 2 °C temperature control. There was plenty of food and water available to all of the rats. The experimental method had been approved by the Institutional Animal Care and Use Committee (IACUC); the authorization number is 2022/OR-NSU/IACUC/0304.

Five groups of seven rats each were used to examine the impact of Nigella sativa seed powder on isoproterenol-induced cardiac dysfunction in the animals.

- GROUP-I (Control): For two weeks, they were provided with regular water supplies and laboratory-prepared food.
- GROUP-II (ISO): Received ISO, which was given twice a week for two weeks at a dose of 50 mg/kg S.C. In addition, they were provided with regular water and laboratory-grade food for two weeks.
- GROUP-III (ISO + BS 0.5%): Received ISO, administered at a dose of 50 mg/kg *S.C.* twice a week for two weeks, and black seed powder 0.5% w/w given in powder food every day for two weeks and normal water.
- GROUP-IV (ISO + BS 1%): Received ISO, administered at a dose of 50 mg/kg *S.C.* twice a week for two weeks, and black seed powder 1% w/w given in powder food every day for two weeks and normal water.
- GROUP-V (ISO + BS 2.5%): Received ISO, administered at a dose of 50 mg/kg *S.C.* twice a week for two weeks, and black seed powder 2.5% w/w given in powder food every day for two weeks and normal water.

Daily tracking was done on food intake, water consumption, and body weight. Every single rat was sacrificed after 14 days via intra peritoneal injection of 90 mg/kg of ketamine hydrochloride. Soon after the sacrifice, internal organs including the kidney and heart were taken away along with blood. For the histological evaluation, each organ's tissue was weighed and stored in neutral buffered formalin (pH 7.4). For the purposes of further investigation, the tissues were stored at -20°C. Blood samples were centrifuged at 4000 rpm to separate the plasma, which was subsequently frozen at -20°C for further analysis.

*2.4 Induction of Myocardial Infarction*

Rats were given subcutaneous injections of 50 mg/kg of isoproterenol (ISO) hydrochloride, dissolved in physiological solution, to induce an experimental myocardial infarction.

*2.5 Preparing a Tissue Sample for Oxidative Stress Marker Evaluation*

To separate the supernatant, the heart and kidney tissues were homogenized in 10% phosphate buffer saline (pH 7.4) and centrifuged at 8000 rpm for 30 minutes at 4°C. After being collected, the supernatants were utilized in enzymatic and protein analyses.

*2.6 Determination of Lipid Peroxidation (LPO) Marker as Malondialdehyde (MDA) and Nitric Oxide (NO)*

*2.6.1 Malondialdehyde (MDA) estimation*

To assess lipid peroxidation, MDA concentrations in tissues and plasma were examined. Thiobarbituric acid reactive substances (TBARS) were produced in the reaction mixture with thiobarbituric acid and a previously established test procedure were used to measure lipid peroxidation [27-29]. In concise, 2 milliliters of TBA-concentrated acetic acid-HCl reagent (1:1:1) was added with 0.1 milliliters of liver tissue homogenate or plasma, and the mixture was allowed to cool in a water bath for fifteen minutes. The clear supernatant's absorbance was determined using an ELISA plate reader and a reference blank set at 535 nm. MDA was expressed in terms of nmol/mL for tissues and plasma.

*2.6.2 Estimation of nitric oxide (NO)*

A previously established assay protocol was used to measure the concentration of NO in tissues and plasma, which was a Griess-illosvoy reaction-based assay approach [27, 30]. A pink color chromophore was produced during 150 minutes of 25 °C incubation of the reaction mixture containing PBS, the reagent and the tissue homogenates or the plasma. At 540 nm, the absorbance was measured in relation to a comparable blank solution. The NO levels have been determined and represented as nmol/mL or nmol/g of tissue using a standard curve.

*2.7 Catalase determination*

The enzyme catalase is responsible for detoxifying $H_2O_2$, generated during oxidative stress. The catalase enzyme activity was measured using a method that has been previously documented [27, 31]. Absorbance changes were detected in the reaction mixture consisting of 0.1 mL of enzyme extract, 5.9 mmol of hydrogen peroxide, and 50 mmoL of phosphate buffer (pH 5.0), which were measured at 240 nm. A 0.01 units/min change in absorbance was taken to represent one unit of CAT activity.

*2.8 Estimation of SOD enzyme activity*

Using previously published techniques, SOD activity in plasma, kidney, and heart tissue homogenates was measured [27]. Phosphate buffer saline (PBS) and aliquots of tissue homogenates were added, and the reactivity of epinephrine was seen at 480 nm for one minute at intervals of 15 seconds. A separate run of a control sample without tissue homogenate was performed. In this work, a 50% decrease in the auto-oxidation of the assay-used epinephrine is interpreted as one unit of SOD activity.

*2.9 Myeloperoxidase (MPO) activity estimation*

The activity of MPO, a tissue inflammatory marker, was measured using a modified dianisidine-$H_2O_2$ based assay technique [29, 32]. Once samples (10 µg of protein as tissue homogenate) were added into the PBS mixture containing H2O2 (0.15 mM) and o-dianisidine dihydrochloride (0.53 mM), the absorbance of the reaction mixture at 460 nm was determined. MPO/mg protein was used to express the MPO activity [27].

*2.10 Assessment of Biochemical Parameters CK-MB*

A kit for measuring creatine kinase-MB activity was implemented to assess CK-MB levels in plasma following the manufacturers supplied protocol.

*2.11 Histopathological Studies*

The tissues of the heart and kidneys were preserved in neutral buffered formalin (NBF, 10% v/v) for a week. Before being covered in paraffin wax, these preserved tissues were subjected to a progressive xylene treatment. The 5-micron-thick tissue slices were cut with a microtome and placed on glass slides. Each section was de-paraffinized with xylene and then went through a series of progressive alcohol dehydration and rehydration procedures. After that, the slices were stained with hematoxylin and eosin to illustrate the basic architecture of the tissue as well as the infiltration of inflammatory cells. Sirius red was additionally used to stain tissue sections which highlights the deposition of collagen. All of the images were snapped at a 40× magnification using a light microscope (Axioscope, Carl Zeiss) [33-35].

*2.12 Statistical Analysis*

For every test parameter in the data computation, the Mean ± Standard error of mean (SEM) was used. Graph Pad Prism (Version 9) was utilized in this study to analyze all the data. A One-way ANOVA and a Tukey test were conducted for the comparison of means among the groups used in this study. At $p < 0.05$, all differences were determined to be significant.

## 3. Results

### *3.1 Effect of black seed powder supplementation on Oxidative Stress Parameters (MDA and NO) in Plasma, Heart, and Kidney Tissue Homogenates of ISO-Administered Rats*

While comparing the plasma, heart, and kidney tissue homogenates to the control rats, ISO treatment resulted in a significant ($p$<0.05) rise in the levels of the oxidative stress marker MDA (**Figure 1**). All three doses (0.5%, 1%, and 2.5%) of black seed supplementation decreased the plasma MDA level significantly**($p$<0.05)** compared to the ISO administered rats (**Figure 1A**). However, the dose of 1% and 2.5% reduced the MDA level much more efficiently than the dose of 0.5% in the heart and kidney of the ISO-administered rats (**Figure 1B, 1C**).

Similarly, When ISO was given to the rats, their NO concentrations were significantly higher than those of the control group **(Figure 2)**. All three black seed doses (doses 0.5%, 1%, and 2.5%) were able to reduce the NO level significantly **($p$<0.05)** in ISO-administered rats **(Figure 2)**. Plasma NO concentrations were reduced to near normal by all three doses **(Figure 2A)**. However, the heart NO levels were decreased more effectively by the 1% dose of black seed **(Figure 2B)** whereas the 2.5% dose of black seed was able to reduce the kidney NO level more **(Figure 2C)** in ISO-administered rats.

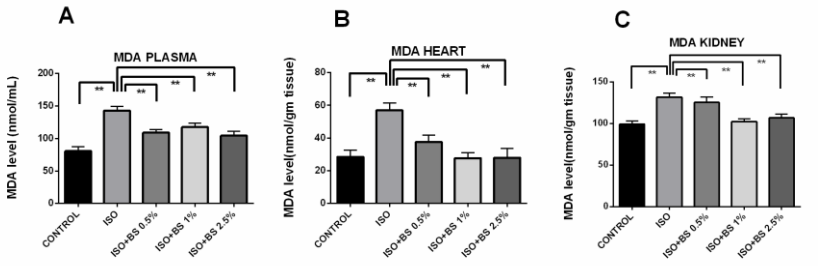


**Figure 1:** The effect of supplementing with black seed powder on the oxidative stress measure MDA in ISO-administered rats' plasma, heart, and kidney tissue homogenates. N = 7 in each group, and values are shown as mean ± SEM. A *post hoc* analysis using a one-way ANOVA and Tukey testing was performed. At $p < 0.05$, values are deemed significant.

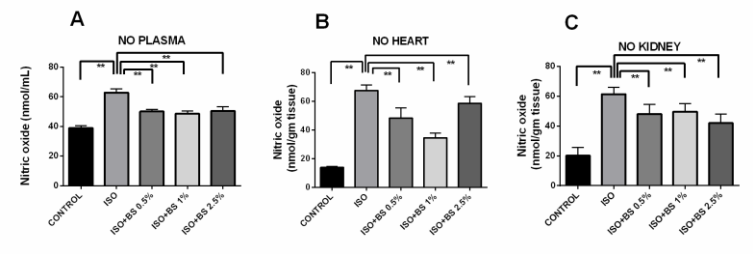


**Figure 2:** Impact of supplementing with black seed powder on markers of oxidative stress NO in ISO-administered rat plasma, heart, and kidney tissue homogenates. The values are shown as mean ± SEM, with 7 rats in each group. As a *post hoc* analysis, a one-way ANOVA with Tukey tests was conducted. When a value is $p < 0.05$, it is deemed significant.

*3.2 Effect of black seed powder supplementation on Antioxidant Enzyme (Catalase, SOD) Activities in Plasma, Heart, and Kidney of ISO-administered Rats*

By lowering the catalase activities in the ISO group ($p$<0.05) relative to the control group, ISO treatment significantly reduced the cellular antioxidant capacities (**Figure 3**). Therapy with the black seed with all three doses (0.5%, 1%, and 2.5%) was able to increase the plasma CAT activities significantly in ISO-administered rats compared to the ISO group, where the dose of 1% elevated the plasma CAT level the most (**Figure 3A**). Heart catalase activity was not increased significantly with any of the doses of black seed powder (**Figure 3B**). In the case of kidney CAT activity, only the 2.5% dose of black seed powder could increase the level by a significant amount **($p$<0.05)** (Figure 3C).

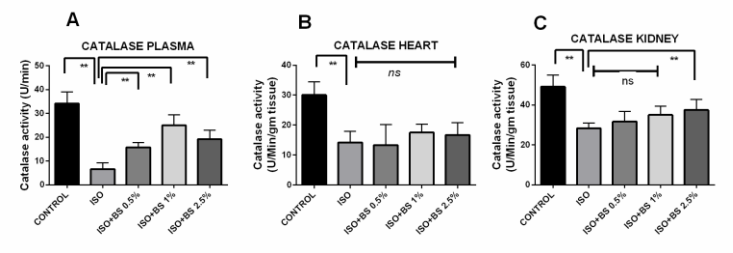


**Figure 3:** Impact of black seed powder on the plasma, heart, and kidney catalase activity of rats given ISO. The data is displayed as mean ± SEM. N in every group is 7. As a *post hoc* analysis, a one-way ANOVA with Tukey tests was carried out. When a value is $p < 0.05$, it is deemed significant.

Moreover, ISO administration significantly lowered the SOD activity in plasma, heart, and kidneys **($p$<0.05)** compared to the control rats (**Figure 4)**. Black seed powder (doses 0.5%, 1%, and 2.5%) supplementation significantly **($p$<0.05)** increased the SOD activity in the plasma of ISO-administered rats **(Figure 4)**. But only the dose of 1% and 2.5% could significantly **($p$<0.05)** increase the heart and kidney SOD activity in ISO-administered groups compared to the ISO group **(Figure 4)**; the 0.5% dose could not increase the level significantly in ISO administered rats **(Figure 4B, 4C)**. Interestingly, the black seed 1% dose worked better in increasing heart SOD activity (**Figure 4B**), and the 2.5% dose worked better in increasing the kidney SOD activity (**Figure 4C**).

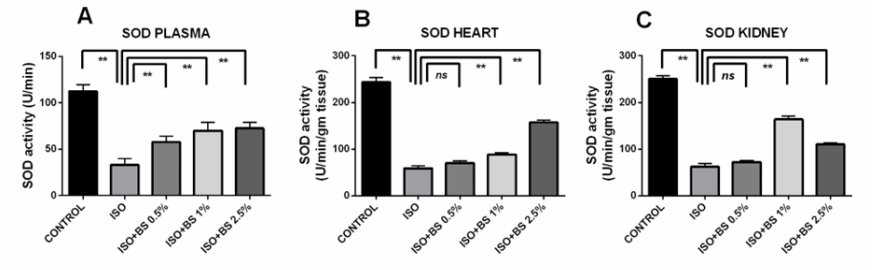


**Figure 4:** Effect of black seed powder on SOD activities in plasma, heart, and kidneys of ISO-administered rats. Values are presented as mean ± SEM. N = 7 in each group. As a *post hoc* analysis, a one-way ANOVA with Tukey tests was conducted. When a value is $p < 0.05$, it is considered to be significant.

*3.3 Effect of black seed powder supplementation on Cardiac Markers MPO and CK-MB Activities in Plasma, Heart, and Kidney of ISO-administered Rats*

ISO administration highly increased the activity of plasma CK-MB significantly **($p<0.05$)** in comparison with control rats. Black Seed (doses 0.5%, 1%, and 2.5%) treatment normalized the CK-MB activity significantly **($p<0.01$)** in the plasma compared to the ISO group (**Figure 5**).

Additionally, when contrasted with the control group, ISO treatment significantly ($p<0.05$) enhanced the MPO level in the kidney and heart **(Figure 6)**. Black seed powder (doses 0.5%, 1%, and 2.5%) treatment significantly (***p*<0.01**) normalized the MPO activity in the heart and kidneys of ISO-administered rats compared to the ISO group (**Figure 6**).

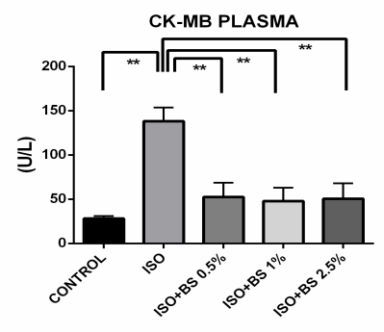


**Figure 5:** Impact of supplementing with black seed powder on CK-MB activity in rats given ISO plasma. Results are shown as mean ± SEM, with 7 participants in each group. As a *post hoc* analysis, a one-way ANOVA with Tukey tests was conducted. When a value is $p<0.05$, it is referred significant.

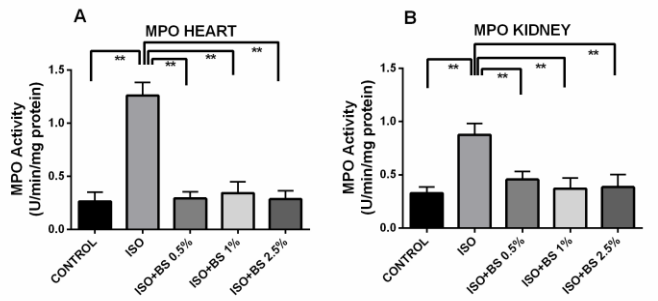


**Figure 6:** Impact of supplementing with black seed powder on MPO activity in the kidney and heart of rats given ISO. The data is shown as mean ±SEM, with 7 rats in each group. As a *post hoc* analysis, a one-way ANOVA with Tukey tests was conducted. When a value is $p < 0.05$, it is deemed significant.

*3.4 Effect* of black seed powder supplementation *on Histological Changes in the Heart and Kidney of ISO-administered Rats*

*3.4.1 Histological assessment by Hematoxylin and Eosin staining (H & E staining) and Sirius red staining of heart section.*

Necrosis, edema, and inflammation were not present from the control group's cardiac histology, which revealed an intact and homogenous histoarchitecture (**Figure 7A**). On the other hand, the ISO-administered group displayed pivotal necrosis of the heart muscle fibers with inflammatory cells infiltration, edema, and increased extracellular matrix deposition along with other degenerative changes (**Figure 7B**). ISO + black seed

(doses 1%, and 2.5%) groups showed reduced cardiac damage and inflammation in the heart **(Figure 7C, D)**. Reduced inflammatory cell infiltration, edema, and comparatively reduced cell necrosis showed potential protection of the black seed powder from myocardial injury. Also, the black seed treatment groups exhibited less extracellular matrix deposition (**Figure 7G, 7H**) compared to the ISO administered rats (**Figure 7 F**).

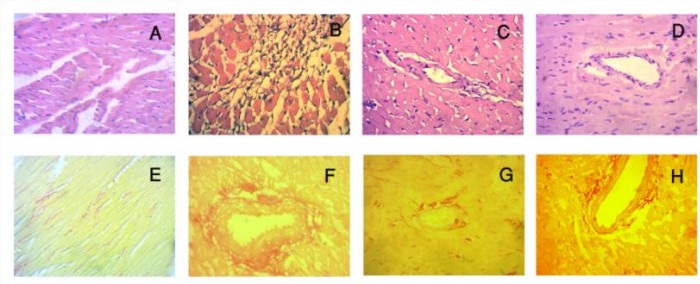


**Figure 7:** Effect of black seed powder supplementation on heart histopathology on ISO-induced rats on 40x microscopic field. Upper panel showed H&E staining, A. Control; B. ISO; C. ISO+ 1% BS; D. ISO+ 2.5% BS. Lower panel showed Sirius red staining, E. Control; F. ISO; G. ISO +1% BS; H. ISO + 2.5% BS.

*3.4.2 Histological assessment by Hematoxylin and Eosin staining (H & E staining) and Sirius red staining in kidney section.*

The kidney tissues of the control rats had normal histoarchitecture and no signs of necrosis or edema when examined histologically with H&E staining **(Figure 8A**). The ISO group showed an unusual, visible infiltration of excess inflammatory, severe tubular necrosis, mononuclear cells, edema, and dilation between renal tubules (**Figure 8 B**). Black Seed treatment tends to minimize the severity of inflammatory cell accumulation and tubular necrosis **(Figure 8C, 8D).** The black seed treatment groups also exhibited less extracellular matrix deposition and fibrosis (**Figure 8 G and 8H)** compared to the ISO administered rats (**Figure 8F**).

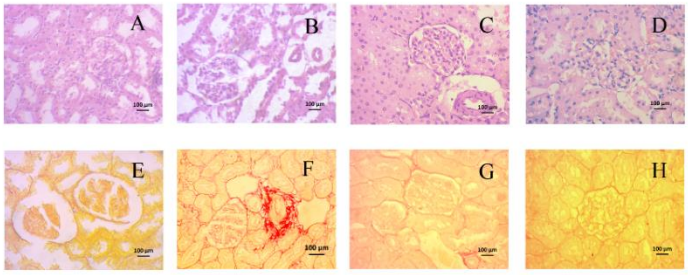


**Figure 8:** Effect of black seed powder supplementation on kidney histopathology of ISO-administered rats. The magnification used are 40x. Upper panel denotes H and E staining, A, Control; B. ISO; C, ISO+ 1% BS; D, ISO+ 2.5% BS. Lower panel denotes Sirius red staining, E, control; F. ISO; G, ISO+ 1% BS; H, ISO + 2.5% BS.

## 4. Discussion

Myocardial infarction is one of the major causes of death all over the world despite the advances in diagnosis and treatment[36]. Our study was conducted to find a more natural and regular basis solution for the MI and related oxidative stress. Likewise to MI, the larger dose of ISO causes morphological and functional changes in the heart muscle [37]. Due to catecholamines' oxidative metabolism, ISO also generates an excessive number of free radicals. There is emerging research suggesting that cardiac toxicity of ISO develops through oxidative stress process [38]. The findings of this study, application of black seed to the heart of rats given ISO reduced inflammation and oxidative stress.

Massive concentrations of reactive oxygen species, including superoxide, hydrogen peroxide, and nitric oxide, are generated during myocardial infarction and contribute to cardiac tissue damage [39]. ISO may undergo auto-oxidation and produce quinones which react with $O_2$ to produce superoxide anions ($O_2^{\cdot-}$) and $H_2O_2$ which leads in excessive formation of free radicals and finally lipid peroxidation [40]. The peroxidation of phospholipids found in the membrane by free radicals triggers abnormalities in the permeability of the renal and cardiac membranes, which in turn promotes an intracellular calcium overload and long-term tissue damage [41]. In the current research, the MDA level was significantly higher in the ISO-administered group rather than the control group. Rats given ISO had considerably reduced MDA levels after undergoing black seed therapy.

Additionally, it was found that ISO-administered rats showed higher levels of nitric oxide [42]. It has been observed that myocardial infarcted hearts generate more nitric oxide (NO) and more expression of inducible nitric oxide synthase (iNOS) [43]. Moreover, β-adrenergic stimulation controls iNOS and substantially increases NO production[44]. When additional reactive species (ROS) like superoxide are present, elevated nitric oxide concentrations induce nitrosative stress and produce the strong oxidant molecule peroxynitrite (-ONOO-)[45]. In this study in particular, the NO levels in the kidney, heart, and plasma were significantly elevated in the ISO group in comparison to the control group. In rats given ISO, the administration of black seed markedly inhibited the increase in nitric oxide levels. All these reductions in the MDA and NO levels might indicate the antioxidant properties of the black seed.

Antioxidant is the defense mechanism limiting the free radicals initiating tissue damage. Catalase (CAT) and superoxide dismutase (SOD) are such free radical scavenging enzymes or antioxidants which are normally present at the tissues [46]. ISO-induced myocardial damage is also associated with a decrease in these endogenous antioxidants CAT and SOD in the heart and kidneys which are structurally and functionally impaired by the free radicals, resulting in damage to the cardiac and renal cells [47]. In the present experiment, rats given ISO exhibited noticeably reduced CAT and SOD activity in their plasma, hearts, and kidney tissues. Rats treated with ISO had considerably higher levels of plasma SOD and catalase activity after receiving black seed therapy. This increase in the plasma CAT and SOD activity might indicate the moderate antioxidant stimulant activity of the black seed in the plasma.

Additionally, the cardiac marker creatinine-kinase MB (CK-MB) significantly increased, according to our investigation. CK-MB is used to help diagnose acute MI which is a type of protein that is mostly present in cardiac muscle cells [48]. When cardiac muscle damage or cell necrosis occurs, it causes the cardiac cells to release the protein in the blood, thus the plasma CK-MB level elevates [49]. Damage to the heart muscle is caused by ISO therapy, which also raises plasma CK-MB levels [50]. In this study, the ISO-treated group showed a significant rise in the CK-MB level in comparison with the control group. Treatment with black seed at doses of 0.5%, 1%, and 2.5% were able to significantly decrease the CK-MB level in ISO-administered rats. This reduction in the level may be due to the cardio protective effect of the black seed.

Furthermore, previous research revealed significantly high MPO enzyme levels and activity in ISO-induced MI in animals [51]. Myeloperoxidase (MPO) is an enzyme found in the lysosomes of polymorphonuclear leukocytes and monocytes [52]. Patients suffering coronary artery disease had significantly higher MPO levels, according to early research [53]. The early threat of MI is independently determined by the plasma MPO enzyme level alone [54]. By releasing free radicals, RNS, and hypochlorous acid, MPO increases the reactivity of $H_2O_2$. All these products and MPO promote protein nitration, lipid peroxidation, and further oxidative changes in acute MI [51]. This study showed that the activity of MPO increased significantly in both heart and kidneys in the ISO-treated group. However, all three groups receiving treatments with black seed at 0.5%, 1%, and 2.5% doses in the ISO-administered rats showed significantly decreasing the MPO activity. This might be due to the anti-inflammatory, cardio and reno-protective activity of the black seed.

To analyze the onset of cardiac failure after delivering an ISO injection, and to find out the protective activity of black seed, distinct morphological studies of the heart and kidney tissues using different staining were

performed. The cardiac histological results showed that ISO administration caused cardiac cell necrosis, edema, high infiltration of inflammatory cells, and collagen deposition in the heart. Following myocardial infarction, monocyte migration to the scar site of the injured tissue may be triggered by cardiomyocyte necrosis and lipid peroxidation-mediated oxidative stress [55]. Due to the damage, collagen deposition occurred to protect the damaged tissue, producing scar tissue [56]. Black seed administration was successful in preventing inflammation and cardiac damage by reducing the monocyte accumulation. It also prevented collagen deposition, indicating the cardio protective activity of the treatment.

Renal histological analysis also showed inflammation, glomerular abnormalities and broken tissue lining of the proximal and distal tubules were visible in the kidney section of ISO administered rats. Black seed supplementation in the ISO group minimized these abnormal conditions in the kidneys. So, it could be assumed that black seed showed protective activity in the kidneys. Also, tubular and glomerular necrosis, dilation between the renal tubules, collagen deposition, and fibrosis were observed in the ISO group, whereas nothing such degradations were observed in the black seed-treated rats. This result are line with previous report suggest that thymoquinone treatment may prevent renal damage and collagen deposition in LDL-receptor deficient mice [57].

Black seed and its components are used in traditional system of medicine for many years due to its nontoxic nature [58]. In a previous study, it seems that thymoquinone at dose levels (2.5, 5.0, 10.0 mg/kg body weight for 28 days repeatedly) is well tolerated by male and female rats and showed no sign of toxicity [59]. Earlier report also suggests that human volunteers received *N. sativa* oil (5 mL/day) for 26 days did not show any significant harmful effect on hepatic, renal, or gastrointestinal system [60].

## 5. Conclusions

This study reveals that black seed powder supplementation showed a significant cardio-protective effect against ISO-induced myocardial infarction in rats. Furthermore, this research offered experimental proof that supplementing with black seed powder increased antioxidant enzyme levels and decreased lipid peroxidation after exposure to high doses of ISO. These biochemical findings were further confirmed by histopathological examination of hearts and kidneys. The potential preventive benefit of black seed may be linked to the augmentation of the myocardial antioxidant defense system.

**Author Contributions:** The concept and design of this study was generated by NS and MAA. MAA also trained SS, SAE, SS, HCG, MSA, SR and MTH on all the research related activities and supervised and coordinated the whole study. SS, SAE, SS, and MTH carried out animal handling, animal experimentation and animal sacrifice. SS, HCH, SAE and SR also performed the biochemical analysis. SS, SS and HCG performed the histological analyses. Statistical analysis and result interpretation were done by MSA NS, and MAA. The draft manuscript was prepared by NS, MAA, MSA, and SS. All authors have read and agreed to this version of the manuscript.

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