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PROTECTIVE EFFECTS OF *NIGELLA SATIVA* (BLACK SEED) ON ISCHEMIA-REPERFUSION INDUCED MYOCARDIAL INJURIES

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ABSTRACT

Ischemia-reperfusion (I/R) model of myocardial infarction injury may occur in a variety of clinical settings and this remains a significant problem. Oxygen free radicals, produced on reperfusion have been shown to play a major role in I/R injury. Various therapeutic effects have been described for *Nigella sativa.* Additionally, it has been presented that *Nigella sativa* has protective effect against ischemia reperfusion injury to various organs. Therefore, it seems possible that the administration of *Nigella sativa* might protect the heart against the ischemia reperfusion injury.

The present study was undertaken to evaluate the cardioprotective potential of *Nigella sativa (black seed)* in the ischemia-reperfusion (I/R) model of myocardial infarction (MI). Thirty-six rats were divided into three groups as control (Group 1), I/R group (Group 2), and *Nigella sativa* treatment group (Group 3). All rats underwent ischemia. Thirty-six male Wistar rats weighting 200-230 g were used in this experimental study. All animals were maintained under standard conditions. Rats were deprived of food, but not water, for 24 h before surgery.Animals were divided into three groups, sham group (Group 1), ischemia (45 min)-reperfusion (60 min) I/R group (Group 2), and *Nigella sativa (orientalis) oil* treatment group (Group 3). All rats were anesthetized with 40-50 mg/Kg of thiopental sodium. *Nigella sativa* oil was given to the rats in treatment group, before ischemia and before reperfusion at a dose of 0.2 mL/Kg by intraperitoneal route. We chose the dose of this agent according to reported studies about I/R and *Nigella sativa oil*, as this dose has been shown to be effective in previous studies[14,15]. Rats in the I/R group were infused only with saline.

The study showed that the *Nigella sativa (orientalis)* oil contain: glycosides, saponins, tannins, phenolic compounds, resins, alkaloids, proteins and flavonoids. The levels of GSH, SOD, GSHPx, CAT enzymes in treatment group were significantly higher than those in the group I/R, but MDA in treatment group were lower than in the I/R group. Our results suggest that *Nigella sativa* (black seed) treatment protects the rat heart against ischemia-reperfusion model of myocardial infarction (MI).

Key words: *Nigella sativa*, Ischemia-reperfusion, Myocardial infarction.

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INTRODUCTION

Oxidative stress defines that, the level of Reactive Oxygen Species (ROS) exists in excess of antioxidant defenses. This imbalance in the redox milieu results in a switch from ROS-stimulated ambient signaling processes to ROS-mediated pathophysiological consequences. Oxidative stress has been implicated in the installation and progression of several degenerative diseases via DNA mutation, protein oxidation and/or lipid peroxidation. In the vasculature, oxidant stress may result from either over production of ROS and/or a decrease in antioxidant capacity when either predominates in the vessel wall, the net result is ROS-mediated decrease in bioavailable nitric oxide and oxidative modification of lipids and proteins leading to impaired vasomotor reactivity, inflammation and dysregulated cell proliferation[1]. Cardiac ischemia is a condition in which blood flow and oxygen supply are insufficient to the heart muscle. The main cause of cardiac ischemia is narrowed coronary arteries. When arteries are narrowed, there is less blood and oxygen supply to the heart muscles. Cardiac ischemia leads to coronary heart disease, angina pectoris, myocardial infarction, heart failure and ultimately heart attack[2]. Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts. Particularly chemical constituent of antioxidant activity can be found at high concentration in plants and can be responsible for their preventing effects in various degenerative diseases, including cancer, neurological and cardiovascular diseases. Thus, the antioxidant properties of plants have full range of perspective applications in human health care[3].

Nigella sativa Linn. is indigenous to the Mediterranean region but has been cultivated into other parts of the world including Saudi Arabia, northern Africa and parts of Asia. The plant is known by names, such as black cumin (English), blck-caraway seeds (USA) and shonaiz (Persian)[4]. Different pharmacological effects such as isulinotropic[5], hypoglycemic[6], anticancer[7], antinociceptive, anti-inflammatory[8], hepatoprotective[9], neuroprotective, antihistamine, antiulcer[10] and bronchodilator[11] activities have been reported for this plant. Black cumin has been traditionally used in the Indian continent, Arabian countries and Europe for culinary and medicinal purposes as a natural remedy for a number of illnesses and conditions that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza. The seeds or its oil are used as a carminative, diuretic, lactagogue and vermifuge[12]. *N. sativa* seeds contain 36%-38% fixed oils, proteins, alkaloids, saponin and 0.4%-2.5% essential oil. The fixed oil is composed mainly of unsaturated fatty acids. The essential oil was analyzed using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%- 57.0%),

p-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), t-anethole (0.25%-2.3%), 4-terpineol (2.0%-6.6%) and longifoline (1.0%-8.0%). Thymoquinone readily dimerizes to form dithymoquinone. Four alkaloids have been reported as constituents of *N. sativa* seeds.

The purpose of the present study was to know the safe and potent cardioprotective effect of *Nigella sativa* (black seed) against ischemia/reperfusion induced myocardial damage in rats.

EXPERIMENTAL

Materials and methods

Chemical detection of the plant components

The *Nigella sativa (orientalis)* oil sample was collected from the local market of Iraq. The sample *Nigella sativa (orientalis)* oil were identified by College of Pharmacy, University of Baghdad.

The chemical components of the *Nigella sativa (orientalis)* oil extract were detected as shown in Table 1. They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids and proteins[13].

Thirty-six male Wistar rats weighting 200-230 g were used in this experimental study. All animals were maintained under standard conditions. Rats were deprived of food, but not water, for 24 h before surgery. Animals were divided into three groups, sham group (Group 1), ischemia-reperfusion I/R group (Group 2), and *Nigella sativa* (orientalis) oil treatment group (Group 3). All rats were anesthetized with 40-50 mg/Kg of thiopental sodium. *Nigella sativa* oil was given to the rats in treatment group, before ischemia and before reperfusion at a dose of 0.2 mL/kg by intraperitoneal route. We chose the dose of this agent according to reported studies about I/R and *Nigella sativa oil*, as this dose has been shown to be effective in previous studies[14,15]. Rats in the I/R group were infused only with saline.

Surgical procedure

The ischemia-reperfusion injury was produced in rat heart based on Buerke's description with modifications[16,17]. The rats were placed on a warm board to control the body temperature at 37°C for surgery. The chest was opened at the left fourth intercostal space. The pericardium was incised and the left atrium appendage was elevated to expose the left anterior descending (LAD) coronary artery. A 6-0 silk suture was passed around the LAD coronary artery, and the ends of suture were threaded through a small vinyl tube to form a snare. The thoracic cavity was covered with saline-soaked gauze to prevent the heart

from drying, ischemia was established by tightening the suture from both ends with fixed weight. The animals then underwent 45 min of ischemia. Reperfusion was introduced by releasing the snare gently for a period of 60 min. The sham control animals were subjected to the entire surgical procedure described above, except the introduction of LAD ligation and release. At the end of reperfusion,

Biochemical studies

A ten-percent homogenate of myocardial tissue was prepared in 50 mM phosphate buffer, pH 7.4 and an aliquot was used for the assay of malondialdehyde according to the method described by[18]. The homogenate was centrifuged at 7000 rpm for 15 minutes and the supernatant was used for the estimation of the biochemical parameters: glutathione[19]; glutathione peroxidase[20], superoxide dismutase[21], catalase[22] and protein[23]. Statistical calculations were performed by using Excel (Microsoft) and SPSS.

RESULTS AND DISCUSSION

The chemical components analysis of *nigella sativa* oil is given in Table 1. The extract gave positive tests for (glycosides, proteins, saponins, tannins, resins, various phenolic compounds alkaloids and flavonoids) similar results were also obtained by other studies.

Table.1: Chemical components analysis for *Nigella sativa* oil

Components	Reagents	Note	Result extract
Glycosides	Iodine test	Blue ppt.	ve+
	Molish test	Violet ring	ve+
	Benedict test	Orange ppt.	ve+
Proteins	Folin-Ciocalteau reagent	Blue color	ve+
Saponins	Fast stirring	Dense foam for long time White ppt.	ve+
	Mercuric chloride		ve+
Phenolic compounds	Aqueous % 1 Ferric chloride	Green ppt.	ve+
Tannins	Aqueous % 1 Ferric chloride	Green ppt.	ve+
	Lead acetate %% 1	Preface yellow ppt.	ve+

Cont…

Components	Reagents	Note	Result extract
Resins	Ethanol + Boiling + D.w.	turbidity	ve+
Flavonoids	Aqueous % 1	Green ppt.	ve+
	Ferric chloride	Yellow ppt.	ve+
	Ethanol hydroxide alcohol		
Alkaloids	Mayer's reagent	White ppt.	ve+
	Wagner reagent	Brown ppt.	ve+
	Picric acid	Yellow ppt.	ve+

A significant decrease in GSH levels ($p < 0.05$) as well as in the activities of GSHPx, CAT ($p < 0.05$) and an increase in MDA level ($p < 0.05$) were observed in the control I/R group as compared to sham group (Table 2). NS oil treatment resulted in a significant repletion of these biochemical markers compared to the control I/R group. A marked restoration in antioxidant enzyme GSHPx ($p < 0.05$), SOD ($p < 0.05$). NS oil also markedly reduced lipid peroxidation ($p < 0.05$) as evidenced by reduction in MDA levels as compared to control I/R group (Table 2). NS oil treatment however failed to restore the activities of CAT and GSH level significantly as compared to control I/R group (Table 2).

Table 2: Clinical parameters in control, I/R and I/R + + NS oil, rats (n=12, mean ± SD)

Parameters	Sham	I/R	I/R + NS oil (0.2 mL/Kg)
GSH	1.83 ± 0.48	0.54 ± 0.03	1.59 ± 0.09
SOD	7.89 ± 1.98	3.48 ± 0.97	4.89 ± 1.08
GSHPx	0.35 ± 0.09	0.15 ± 0.04	0.31 ±0.07
CAT	22.37 ± 2.05	15.09 ± 2.06	19.13 ± 4.76
MDA	65.24 ± 5.1	76.22 ± 9.68	67.58 ± 7.19

GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase;
GSHPx: Glutathione peroxidase. MDA: Malondialdehyde.
The values are expressed as mean ± SD.

Ischemia initiated by occlusion of a main coronary artery leads to a complex series of cellular events that can result in myocardial cell death. while reperfusion can make salvage of ischemic tissue, it may also contribute to myocardial injury[24]. Reperfusion can

accelerate necrosis in irreversibly injured myocytes because of an increase in cell swelling, disruption of cell ultrastucture, formation of contraction bands, and deposition of intra-mitochondrial calcium phosphate granules[25].

Some mechanisms have been proposed to give details the myocardial damage observed after ischemia and reperfusion. Recent studies have demonstrated that production of free radicals by neutrophils, monocytes and endothelial cells contribute to myocardial cell injury[26]. Free radicals have been shown to initiate lipid peroxidation resulting in an alteration of membrane integrity, fluidity and Permeability[27]. We observed a significant elevation in MDA levels in the control I/R group as compared to sham group. Besides MDA, a significant decrease in myocardial GSH and endogenous antioxidant enzymes (SOD, CAT and GSHPX,) further confirms myocardial oxidative stress because of disruption of endogenous antioxidant network, as observed in the study, the myocardium may be more susceptible to any ischemia-reperfusion injury.

NS oil exhibited significant antioxidant activity as it restored GSH levels, GSHPx activity and reduced lipid peroxidation compared to control I/R.

Thymoquinone compound, the active part of *Nigella sativa* seeds, is a pharmacologically active quinone, which possesses several properties including analgesic and anti-inflammatory actions[28]. It has been reported that thymoquinone prevents oxidative injury in various *in vitro* and *in vivo* studies in rats[29,30]. It has been suggested that thymoquinone may act as an antioxidant agent and prevents membrane lipid peroxidation in tissues[31]. The mechanism of action is still largely unknown. But, it seems these effects may be related to inhibition of eico- sanoid generation, namely thromboxane B_2 and leucotri-enes B_4, and membrane lipid peroxidation[32]. Moreover, it has been demonstrated that *Nigella sativa* can significantly stop hepatotoxicity[33] and might have protective effects against nephrotoxicity induced by either disease or chemicals[32]. But, the exact mechanism is not clear. There are also several clinical studies. In one study, the prophylactic effect of boiled extract of *N. sativa* on asthmatic disease was examined[34]. Similarly, black seed oil was shown to be an effective adjuvant for the treatment of patients with allergic diseases[35]. In another clinical study, significant benefits of *Nigella sativa* extract in the treatment of acute tonsillopharyngitis was shown[36]. Also, it was shown that *Nigella sativa* has anti-epileptic effects in children with refractory seizures[37]. Therefore, it seems possible that the administration of *Nigella sativa* might protect the heart against the ischemia reperfusion injury. An excessive production of oxygen free radicals has been reported in ischemic reperfused heart leading to tissue damage, and this is an unavoidable process in heart transplantation and in the surgical procedures in which the Pringle maneuver is used[38].

CONCLUSION

Our results suggest that *Nigella sativa* (black seed) treatment protects the rat heart against ischemia-reperfusion model of myocardial infarction (MI).

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