Effect Nigella Sativa (Black Seed) on Liver Enzymes in Ischemia Reperfusion Injury

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ABSTRACT:

BACKGROUND:

Hepatic ischemia-reperfusion (I/R) 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 hepatic I/R injury. Various therapeutic effects have been described for *Nigella sativa.* Additionally, it has been presented that *Nigella sativa (orientalis)* oil has protective effect against ischemia reperfusion injury to various organs. Therefore, it seems possible that the administration of *Nigella sativa (orientalis)* oil might protect the liver against the ischemia reperfusion injury .

OBJECTIVE:

To determine whether *Nigella sativa (orientalis)* oil prevents hepatic ischemia-reperfusion injury to the liver.

METHODS:

Thirty-six rats were divided into three groups as control (Group 1), I/R group (Group 2), and *Nigella* sativa oil (NS) treatment group (Group 3). All rats underwent hepatic ischemia for 60 min followed by 60 min period of reperfusion. Rats were intraperitoneally infused with only 0.9% saline solution in group 2. Rats in group 3 received NS oil (0.2 mL/kg) intraperitoneally, before ischemia and before reperfusion. Blood samples were harvested from the rats, and then the rats were sacrificed. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were determined.

RESULTS:

The levels of liver enzymes in group 3 were significantly lower than those in the group 2.

CONCLUSION:

Our results suggest that *Nigella sativa* oil treatment protects the rat liver against to hepatic ischemia-reperfusion injury.

KEY WORDS : nigella sativa, liver enzymes, ischemia.

INTRODUCTION:

It is well known that ischemia/reperfusion (I/R) generates metabolic and structural hepatic damage, and may be due to trauma, sepsis, liver transplantation[1]or hepatic pedicle clamping during liver surgery[2]. This remains a significant problem for surgical procedures, and also remains limitation of liver transplantation[3].Oxygen free radicals, produced on reperfusion, play a critical role in the injury caused by ischemia-reperfusion[4]. Reactive oxygen radicals lead to an inflammatory response and tissue damage by activating some mediators. It can also directly damage cell components[5]. Several attempts to reduce these mechanisms have been reported in the literature. Protection against reperfusion Injury can be induced by assorted treatments including administration of antioxidants and anti-inflammatory drugs[4,6,7,8].Various therapeutic effects, such as antioxidant, anti-inflammatory, anticancer[9],antihistaminic[10],antibacterial effects[11] have been described for *Nigella sativa(orientalis).* Additionally, it has been shown that *Nigella sativa* has protective effect against ischemia reperfusion injury to various organs[12,13,14].

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MATERIALS AND METHODS:

Thirty-six male Wistar rats weighting 200-230g 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, control group (Group 1), 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. After the abdomen was shaved and disinfected, a midline incision was made and rats underwent either sham surgery or ischemia-reperfusion. Ischemia was carried out by exposing the afferent and efferent blood vessels and then clamping for 60 min with a microvascular "bulldog" clamp Sixty minutes later, the ischemic liver was reperfused by opening the clamp, and
eperfusion was achieved for 60 min. *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 re-ported studies about I/R and *Nigella sativa oil*, as this dose has been shown to be effective in previous studies[15,16].Rats in the I/R group were infused only with saline. At the end of the procedures, the rats were killed and blood. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities were measured for evaluating the liver functions.

Biochemical analyses

Plasma was used to measure AST, ALT and LDH as indicative parameters of hepatic
function. The plasma activities of AST, ALT and LDH were estimated by commercially available kits. Statistical calculations were performed by using EXCEL and SPSS. The results are expressed as mean±SEM. Differences between the group means were analysed for significance using the Independent Student's *t*- and Mann-Whitney *U*- tests. In all cases a *P* value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION:

As expected, ischemia reperfusion (I/R) caused production of oxygen free radicals has been reported in ischemic reperfused liver, leading to tissue damage and this is an unavoidable process in liver transplantation[4], as indicated by increased levels of ALT, AST, and LDH(Table1) while Plasma ALT, AST, and LDH levels in the Nigella sativa treatment group were significantly lower than those in the I/R group. They were significantly higher in the I/R group than those in the control group. The results are summarized in Table 1.

Plasma ALT, AST, and LDH levels in the Nigella sativa treatment group were significantly lower than those in the I/R and control groups ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively, and $P < 0.01$ for all). They were significantly higher in the I/R group than those in the control group ($P < 0.01$ for all). The results are summarized in Table1.

Table1: Clinical parameters in control, I/R and I/R +NS oil rats (n=12, mean± SD).

Clinical parameters	control	I/R	I/R + NS oil	P
AST (U/L)	134 ± 18	963 ± 242	548±104	0.001
ALT (U/L)	84 ± 14	707 ± 192	313± 116	0.001
LDH (U/L)	524 ± 172	3892 ± 549	2325 ± 623	0.001

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase

Thymoquinone, the active constituent of *Nigella sativa* seeds, is a pharmacologically active quinone, which possesses several properties including analgesic and anti-inflammatory actions[17]. It has been reported that thymoquinone prevents oxidative injury in various *in vitro* and *in vivo* studies in rats[18,19]. It has been suggested that thymoquinone may act as an antioxidant agent and prevents membrane lipid peroxidation in tissues[20]. The mechanism of action is still largely unknown. But, it seems these effects may be related to inhibition of eico-sanoid generation, namely thromboxane B2 and leucotri- enes B4 (by inhibiting cyclooxygenase and 5-lipooxygen- ase, respectively), and membrane lipid peroxidation[13]. Moreover, it has been demonstrated that *Nigella sativa* can significantly prevent hepatotoxicity[21] and might

have protective effects against nephrotoxicity induced by either disease or chemicals[13]. 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(22). Similarly, black seed oil was shown to be an effective adjuvant for the treatment of patients with allergic diseases(23). In another clinical study, significant benefits of *Nigella sativa* extract in the treatment of acute tonsillopharyngitis was shown(24). Also, it was shown that *Nigella sativa* has anti- epileptic effects in children with refractory seizures(25).Therefore, it seems possible that the administration of *Nigella sativa* might protect the liver against the ischemia reperfusion injury.An excessive production of oxygen free radicals has been reported in ischemic reperfused liver, leading to tissue damage, and this is an unavoidable process in liver transplantation and in the surgical procedures in which the Pringle maneuver is used(4).

CONCLUSION:

Our results suggest that *Nigella sativa (orientalis)* oil treatment protects the rat liver against to hepatic ischemia-reperfusion injury.

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