

Investigation the effect of the alcoholic extract of *Zingiber officinale* on liver enzymes and oxidative stress during ischemia reperfusion injury

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Summary:

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 *Zingiber officinale*. Additionally, it has been presented that *Zingiber officinale* has protective effect against ischemia reperfusion injury to various organs. Therefore, it seems possible that the administration of *Zingiber officinale* might protect the liver against the ischemia reperfusion injury.

Objectives : To determine whether *Zingiber officinale* 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 *Zingiber officinale* treatment group (Group 3). All rats underwent hepatic ischemia for 60 min followed by 60 min period of reperfusion. Rats were internally infused with only 0.9% saline solution in group 2. Rats in group 3 received alcoholic extract of *Z. officinale* (200 mg/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), lactate dehydrogenase (LDH) oxidative stress levels of reactive oxygen species (ROS), nitric oxide (NO) and lipid peroxidation (LPO) levels were determined.

Results: The levels of liver enzymes and Oxidative stress in group 3 were significantly lower than those in the group 2.

Conclusion: Our results suggest that *Zingiber officinale* treatment protects the rat liver against hepatic ischemia-reperfusion.

Keywords : *Zingiber*, liver enzymes, oxidative stress ,ischemia.

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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]. It is well known that ischemia/reperfusion (I/R) generates metabolic and structural hepatic damage, and may be due to trauma, sepsis, liver transplantation[2] or hepatic pedicle clamping during liver surgery[3]. This remains a significant problem for surgical procedures, and also remains limitation of liver transplantation[4]. Oxygen free radicals, produced

on reperfusion, play a critical role in the injury caused by ischemia-reperfusion[5]. Reactive oxygen radicals lead to an inflammatory response and tissue damage by activating some mediators. It can also directly damage cell components[6]. 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[5,7-9]. Various therapeutic effects, such as antioxidant, anti-inflammatory, anticancer[10], antihistaminic[11], 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[12]. *Zingiber officinale* Roscoe or ginger, member of the family of Zingiberaceae, is widely used as a spice. Moreover, it is used in Asian traditional medicine for various purposes including stomach ache [13], nausea and diarrhea,

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and joint and muscle pain [14]. In addition to neuroprotective effect [15], and anxiolytic effect [16].

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 Zingiber officinale 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 reperused by opening the clamp, and reperfusion was achieved for 60 min. Zingiber officinale was given to the rats in treatment group, before ischemia and before reperfusion at a dose received alcoholic extract of Z. officinale (200 mg/kg) intraperitoneally. We chose the dose of this agent according to reported studies about I/R and Zingiber officinale as this dose has been shown to be effective in previous studies. 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: a) liver enzymes : 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 using an autoanalyser (aeroset® Abbott Laboratories, Chicago, IL). Statistical calculations were performed by using EXCEL (Microsoft) and SPSS. b) Oxidative stress indices: The left hemisphere was used to detect ROS according to a previously published method with minor modifications[17]. nitric oxide (NO) measured as total nitrite [18] and lipid peroxidation (LPO) as Thio Barbituric Acid Reactive Substances [19].

Results:

As expected, ischemia reperfusion (I/R) caused production of oxygen free radicals has been reported in ischemic reperused 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 Zingiber officinale 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 Zingiber officinale , treatment group were significantly lower than those in the I/R and control groups. They were significantly higher in the I/R group than those in the control group. The results are summarized in Table.

Table 1. clinical parameters in control, I/R and I/R + Zingiber officinale , rats (n=12, mean± SD)

Clinical-parameters	Control	I/R	I/R + Z. officinale	P
AST (U/L)	134 ± 18	963 ± 242	668±118	0.001
ALT (U/L)	84 ± 14	707 ± 192	493± 106	0.001
LDH (U/L)	524 ± 172	3892 ± 549	2848 ± 473	0.001

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase
Significances against controls: *P<0.001

as indicated by increased oxidative stress levels of ROS, NO and LPO (Table2) while oxidative stress, NO and LPO levels in the Zingiber officinale 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.

Table 2. Oxidative stress in control, I/R and I/R + Zingiber officinale , rats (n=12, mean± SD)

Oxidative-stress in	control	I/R	I/R + Zingiber officinale	P
NO (µmol/ml) In Serum	14.02± 0.5	26.72 ± 1.4	18.07 ± 1.6	0.001
LPO (nmol/ml) In Serum	0.34±0.003	1.04 ± 0.03	0.71 ± 0.07	0.001

NO: nitric oxide , LPO: lipid peroxidation

Discussion:

Our results demonstrated for the first time that Z. officinale could protect hepatic ischemic damage in a rat model of focal cerebral ischemia. Moreover, it also reduced cognitive deficits induced by hepatic ischemia. Surprisingly, Z. officinale could increase the neurons’ density in hippocampus and improved the spatial memory. Although the neurodegeneration in hippocampus is reported to be associated with the spatial memory deficit [20,21], Our results also demonstrated that Z. officinale at dose of 200 mg/kg body weight could mitigate the liver infarct volume and could decrease oxidative stress by decrease the activity of (NO) nitric oxide in serum and decrease of (LPO) lipid peroxidation level in all areas mentioned earlier. Therefore, the neuroprotective effect of Z. officinale extract might be related to its antioxidant effect.

In conclusion, our data suggested that Z. officinale possessed the protective effect against hepatic ischemia induced by the occlusion of right middle cerebral artery. It could attenuate the memory impairment, neurodegeneration, and brain infarct volume in this condition. The cognitive enhancing effect and neuroprotective effect of Z. officinale appeared to show almost the same magnitude as the positive control groups used in this study. Moreover, Z. officinale also showed multiple sites of action. Therefore, Z. officinale at the correct dose is a potential novel candidate for developing food supplement against hepatic ischemia. However, further clinical trial study is still required.

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