# Effect of Trigonella Foenum-Graecum L. (Fenugreek) 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 Trigonella. Additionally, it has been presented that *Trigonella* has protective effect against ischemia reperfusion injury to various organs. Therefore, it seems possible that the administration of *Trigonella* might protect the liver against the ischemia reperfusion injury.

**OBJECTIVE:**

To determine whether *Trigonella foenum-graecum* L. (Fenugreek) prevents hepatic ischemia-reperfusion injury to the liver.

**METHODS:**

Thirty-six rats were divided into three groups as, (Group 1) control group, (Group 2)I/R group and (Group3) *Trigonella foenum-graecum* L. (Fenugreek) treatment group. All rats underwent hepatic ischemia for 60 min followed by 60 min period of reperfusion. Rats were internal infused with only 0.9% saline solution in group 2. Rats in group 3 received hydro alcoholic - extracted Trigonella foenum-graecum L. (Fenugreek) (500 mg/kg)internal, 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 *Trigonella foenum-graecum* L. (*Fenugreek) treatment* protects the rat liver against hepatic ischemia-reperfusion injury.


***KEY WORDS*** : trigonella, liver enzymes.

## INTRODUCTION:

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer,diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging, etc. [(1),] A free radical is defined as any atom or molecule possessing unpaired electrons. The primary oxygen derived free radicals are superoxide anion ($O_2^{\cdot}$), hydroxyl ($OH^{\cdot}$), hydroperoxyl ($OOH^{\cdot}$), peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals and non free radicals are hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), ozone ($O_3$) and singlet oxygen ($^1O_2$). These reactive intermediates are collectively termed as reactive oxygen species (ROS).Similarly, reactive nitrogen species (RNS) are mainly nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^{\cdot}$) and nitrogen dioxide ($NO_2$). Free radicals can cause a wide range of toxic oxidative reactions like initiation of the per oxidation of the membrane lipids leading to the accumulation of lipid peroxides, direct inhibition of mitochondrial respiratory chain enzymes, fragmentation or random cross linking of molecules like DNA, enzymes and proteins which ultimately leads to cell death [2] ROS can be formed in living organisms by both endogenous and exogenous sources. Endogenous sources of free radicals include normal aerobic

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respiration, paroxysms and stimulation of polymorph nuclear leucocytes and macrophages. The exogenous sources include ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA [4]' Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include super oxide dismutase, catalase, and glutathione peroxides. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources [5] proposed for use in the treatment of various human diseases [6]. There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butyl hydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage and mutagenesis Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals [8] Hence, nowadays search for natural antioxidant source is gaining much importance. It is well known that ischemia/reperfusion (I/R) generates metabolic and structural hepatic damage, and may be due to trauma, sepsis and liver transplantation [9] or hepatic pedicle clamping during liver surgery [10]. This remains a significant problem for surgical procedures, and also remains limitation of liver transplantation [11].Oxygen free radicals(produced on reperfusion), play a critical role in the injury caused by ischemia-reperfusion[12]. Reactive oxygen radicals lead to an inflammatory response and tissue damage by activating some mediators. It can also directly damage cell components [13]. 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 [14].Various therapeutic effects, such as: antioxidant, anti-inflammatory, anticancer [15], antihistaminic [16]and antibacterial effects[17].Living organisms have developed a comprehensive array of endogenous antioxidant defences to prevent free radical formation or limit their damaging effects. In the retina, for example,we used extracts of fenugreek (greek clover, greek hay; *Trigonella foenum),* an annual plant belonging to the Fabaceae. The leaves are used as a vegetable, whereas its brownish seeds are used as a spice and for treatment of diabetes, fever and abdominal colic[18].

## MATERIALS AND METHODS:

**Preparation of extracts and Chemical detection of the plant components:**
The Fenugreek seed sample was collected from the local market of Iraq . The sample *T. Foenum-graecum* seeds were identified by College of Pharmacy, University of Baghdad. Dry fenugreek seed (1kg) was cleaned and ground into coarse powder. 80%Ethanol and 20% water were used for extraction method. The extracts were filtered. The residue was re-extracted twice under the same condition to ensure complete extraction.

The chemical components of the prepared hydro alcoholic extract were detected as shown in table .1.They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids [19,20]and proteins [21].

**Experimental Design:**
Thirty-six male Wistar rats weighting 200-230gwere 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,(Group1) control group, (Group2) I/R group, and (Group3) *Trigonella foenum-graecum* L. (Fenugreek) treatment group. 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 reperfusion was achieved for 60 min. *Trigonella foenum-graecum* L. (Fenugreek) was given to the rats in treatment group, before ischemia and before reperfusion at a dose of hydro alcoholic - extracted *Trigonella* (500 mg/kg) internal. We chose the dose of this agent according to

reported studies about I/R and *Trigonella foenum-graecum* L. (Fenugreek) , as this dose has been shown to be effective in previous studies. Rats in I/R group were infused only with saline. At the end of the procedures, the rats were killed then blood taken and measured. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities were measured for evaluating the liver functions.

**BIOCHEMICAL ANALYSIS:**

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 .

**HISTOPATHOLOGICAL STUDIES**

At the end of the experiment, liver tissue was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. The sections were examined under light microscope after hematoxylin and eosin staining and photomicrographs were taken.

**RESULTS AND DISCUSSION :**

The results showed in Table.1, the extract gave positive tests for glycosides, proteins, saponins, tannins, resins , various phenolic compounds alkaloids and flavonoids ) similar results are also obtained by other studies

**Table1: Chemical components analysis for hydro alcoholic extract of *Trigonella foenum-graecum* L**

| Components | Reagents | Note | Result<br>hydro alcoholic - extract |
|---|---|---|---|
| Glycosides | Iodine test<br>Molish test<br>Benedict test | Blue ppt.<br>Violet ring<br>Orange ppt. | +Ve<br>+Ve<br>+Ve |
| Proteins | Folin-Ciocalteau reagent | Blue color | +Ve |
| Saponins | Fast stirring<br>Mercuric Chloride | Dense foam for long time<br>White ppt. | +Ve<br>+Ve |
| Phenolic compounds | 1%Aqueous<br>Ferric chloride | Green ppt. | +Ve |
| Tannins | 1%Aqueous<br>Ferric chloride<br>1%Lead acetate | Green ppt.<br><br>Preface yellow ppt. | +Ve<br><br>+Ve |
| Resins | Ethanol + Boiling + D.w. | turbidity | +Ve |
| Flavonoids | 1%aqueous<br>Ferric chloride<br>Ethanol hydroxide alcohol | No Green ppt.<br><br>No Yellow ppt. | +Ve<br>+Ve |
| Alkaloids | Mayer's reagent<br>Wagner reagent<br>Picric acid | white ppt.<br>Brown ppt.<br>Yellow ppt. | +Ve<br>+Ve<br>+Ve |

The total phenolic compounds may contribute directly to the antioxidant action [22]and therefore it is necessary to investigate total phenol content.The Radical scavengers may directly react and quench the peroxide radicals to terminate the per oxidation chain reaction and improve the quality and stability of food product. The stable DPPH radical has been used to evaluate antioxidants.

for their radical quenching capacity and to understand and their antioxidant mechanism. The Fenugreek extract was evaluated for radical

scavenging activity against α,α-□Diphenyl-β-□picryl-hydrazyl (DPPH).The decrease in absorbance of DPPH radical is caused by the reaction between antioxidant molecule and radical which results in the scavenging of the radical by hydrogen donation.

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[12], as indicated by increased levels of ALT, AST, and LDH(Table 2) while Plasma ALT, AST, and LDH levels in the Trigonella, treatment group were significantly lower than those in the I/R group. They were significantly higher in I/R group than those in the control group.

Plasma ALT, AST, and LDH levels in the *Trigonella foenum-graecum* L. (Fenugreek) , treatment group were significantly lower than those in I/R and control groups. They were significantly higher in I/R group than those in the control group. The results are summarized in Table 2

**Table 2 :Clinical parameters in control, I/R and I/R + *Trigonella foenum-graecum* L. (Fenugreek) ,rats (n=12, mean± SD)**

| Clinical parameters | control | I/R | I/R + *Trigonella,* | 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.

Although oxygen is critical for life and for maintenance of metabolic processes, reactive metabolites of oxygen may be toxic to cells. In particular, the cellular damage that occurs secondary to ischemia may be exacerbated by the sudden reintroduction of oxygen into tissues during reperfusion, triggering free radical cascades that overwhelm endogenous free radical scavengers. Here we show, for the first time, an exhaustion of the endogenous free radical scavenger, reduced Glutathione (GSH).

In this context, it is noteworthy that the application of *Trigonella foenum-graecum* L. (Fenugreek) test substances was effective in reducing the detrimental effects of I/R. Particularly, as the ameliorating effects of *Trigonella foenum-graecum* L. (Fenugreek) extracts were very similar to that of, the medic use of such cheap and locally available plan extracts appears to be recommendable in developing countries where access to expensive drugs is restricted. However, it should also be pointed out that lutein showed superior efficacy in all respects tested, it blocked the I/R-induced malondialdehyde (MDA) accumulation and GSH exhaustion almost completely, [23,24]. Thus, the future therapeutic use of lutein deserves further experiments and clinical trials. 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 [12].

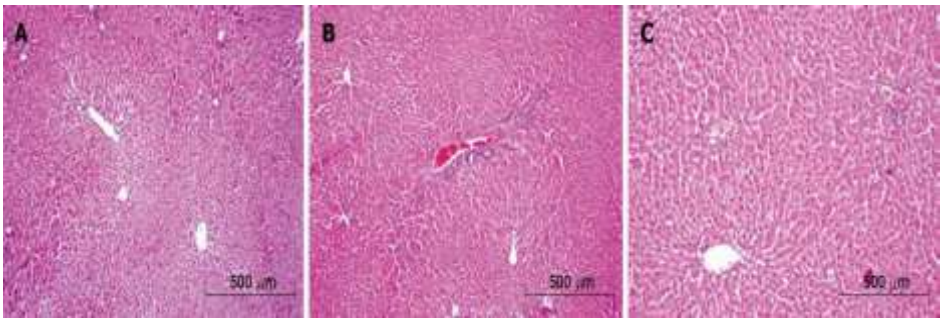


**Figure 1 A: Normal liver tissue(Group 1) B: Histopathological findings 60 min after I/R(Group 2); C: Histopathological findings 60 min after I/R + *Trigonella*(Group 3).**

In histopathological evaluation, there were no pathological changes in liver tissue of the sham group (Group 1). Liver specimens from rats after ischemia- reperfusion exhibited focal necrosis and infiltration of leukocytes (Group 2). *Trigonella* treatment significantly decreased these pathological changes (Group 3). Histological tissue damage was milder in the *Trigonella* treatment group than that in the control group.

## CONCLUSION:

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

## REFERENCES:


1. Bandyopadhyay U, Das A and Bannerjee R K : Reactive oxygen species Oxygen Damage and pathogenesis. *Curr Sci* 1999;77:658-66.
2. Halliwell B and Gutteridge J.M.C : *Free radicals in Biology and Medicine*. Edition 3, Oxford University Press: Oxford 1999:23-27.
3. Irshad M and Chaudhuri P.S : Oxidant-antioxidant system Role and significance in human body. 2002.
4. Fang Y, Yang S and Wu G: Free radicals, antioxidants and nutrition. *Nutrition* 2002;18:872-79.
5. Lee J, Koo N and Min DB : Reactive oxygen species aging and antioxidative nutraceuticals.*CRFSFS.* 2004;3:21-33.
6. Cuzzocrea S, Riley DP, Caputi AP and Salvemini D : Antioxidant therapy A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. *Pharmacol. Rev* 2001;53:135-59.
7. Grice HC : Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and Gastrointestinal tract. *Food Chem Toxicol* 1986; 24: 1127-1130.
8. Formica J.V. and Regelson W: Review of the biology of quercetin and related bioflavonoids. *Food Chem Toxicol* 1995;33:1061-80.
9. Shin T, Kuboki S, Huber N, Eismann T, Galloway E, Schuster R, Blanchard J, Pritts TA, Lentsch AB. Activation of peroxisome proliferator-activated receptor-gamma during hepatic ischemia is age-dependent. J Surg Res.2008;147:200-5.
10. van Gulik TM, de Graaf W, Dinant S, Busch OR, Gouma DJ. Vascular occlusion techniques during liver resection. Dig Surg. 2007; 24:274-81.
11. He XS, Ma Y, Wu LW, Wu JL, Hu RD, Chen GH, Huang JF. Dynamical changing patterns of glycogen and enzyme histochemical activities in rat liver graft undergoing warm ischemia injury. World J Gastroenterol.2005;11:2662-65.
12. Hassan-Khabbar S, Cottart CH, Wendum D, Vibert F, Clot JP, Savouret JF, Conti M, Nivet-Antoine V. Postischemic treatment by trans-resveratrol in rat liver ischemia-reperfusion: a possible strategy in liver surgery. Liver Transpl.2008;14:451-59.
13. Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA$_r$Pina E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. J SurgRes .2008;147:153-59.

**14.** Polat KY, Aydinli B,rPolat O, Aydin U, Yazici P, OztiukG,Gimdogdu C, Kiziltunc A. Hie protective effect of aprotinin and alpha-tocopherol on ischemia-reperfusion injury of the rat liver. Transplant Proc .2008;40:63-68.

**15.** Khalife KH, Lupidi G. Nonenzymatic reduction of diymoquinone in physiological conditions. Free RadicRes .2007;41:153-61.

**16.** Kanter M, Coskun O, Uysal H. The antioxidative and antihistamine effect of Nigella sativa and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage. *Arch Toxicol*. 2007;80:217-24.

**17.** Morsi NM. Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria.*ActaMicrobiolPol*.2000; 49:63-74.

**18.** Vats V, Yadav SP, Biswas NR, Grover JK .Anti-cataract activity of *Pterocarpusmarsupium* bark and*Trigonellafoenum-graecum* seeds extract in alloxan diabetic rats. J Ethnophar- macol 2004;93:289-94.

**19.** Jassim,A.M.N. , "Study of Some *Eucalptus Rostrata* Leaves Components and Effect of Its Extract on Different Microorganism Al-Mustansirya J.Sci. 2005;16: 62-71 .

**20.** Mohammed,M.T, "Study of Some *Vinca Rosea l.* (Apocynaceae) LeavesComponents and Effect of Its Extract on Different Microorganisms",Al-Mustansirya J.Sci. 2007;18: 28-36.

**21.** Plummer,D.T , An Introduction of Practical Biochemistry , 1978;2ed:145-46 , McGRAw-HILL Book Co.,England,.

**22.** Awika JM, Rooney LW, X. Wu, RL. Priorand L. Cisneros-Zevallos :.J *Agric Food Chem* 2003;51:6657.

**23.** Alves-Rodrigues A, Shao A .The science behind lutein.ToxicolLett.2004;150:57-83.

**24.** Sundelin SP, Nilsson SE .Lipo- fuscin-formationin retinal pigment epithelial cells is educed by antioxidants. Free RadicBiol Med.2001;31:217-25.