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ORIGINAL RESEARCH

# The hepatoprotective effects of *Hypericum perforatum* L. on hepatic ischemia/reperfusion injury in rats

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**Abstract** Little is known about the effective role of *Hypericum perforatum* on hepatic ischemia–reperfusion (I/R) injury in rats. Hence, albino rats were subjected to 45 min of hepatic ischemia followed by 60 min of reperfusion period. *Hypericum perforatum* extract (HPE) at the dose of 50 mg/kg body weight ($HPE_{50}$) was intraperitonally injected as a single dose, 15 min prior to ischemia. Rats were sacrificed at the end of reperfusion period and then, biochemical investigations were made in serum and liver tissue. Liver tissue homogenates were used for the measurement of malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPx) levels. At the same time alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were assayed in serum samples and compared statistically. While the ALT, AST, LDH activities and MDA levels were significantly increased, CAT and GPx activities significantly decreased in only I/R-induced control rats compared to normal control rats ($p < 0.05$). Treatment with $HPE_{50}$ significantly decreased the ALT, AST, LDH activities and MDA levels, and markedly increased activities of CAT and GPx in tissue homogenates compared to I/R-induced rats without treatment–control group ($p < 0.05$). In oxidative stress generated by hepatic ischemia–reperfusion, *H. perforatum* L. as an antioxidant agent contributes an alteration in the delicate balance between the scavenging capacity of antioxidant defence systems and free radicals in favour of the antioxidant defence systems in the body.

**Keywords** *Hypericum perforatum* · Ischemia/reperfusion · Liver · Oxidative stress · Rat

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## Introduction

Hepatic ischemia–reperfusion (I/R) injury is an important pathological process leading to systemic and hepatic damage after circulatory shock, hepatic trauma, transplantation or hepatic surgery (Daglar et al. 2009). Hepatic I/R injury is a result of a series of complex mechanisms including free oxygen radicals activated by energy depletion and failure of oxygen delivery to the vital tissues in the ischemic period (Yaylak et al. 2008).

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The increased production of reactive oxygen species (ROS) during I/R injury results in consumption and depletion of endogenous antioxidants. In this situation, the cells require exogenous antioxidant to protect them from ROS-induced damage (Korkmaz and Kolankaya 2010).

For several years, medicinal plants have been used in developing countries as alternative treatments and they are an important aspect of health management (Noumi et al. 2010; Jin et al. 2013). Many plant extracts and their components like essential oils, flavonoids and phenolic compounds isolated from plants have been shown to exert biological activity such as anti-microbial, anti-inflammatory, antidepressant and antioxidant activity (Mukherjee et al. 2000; Noumi et al. 2010; Ebrahimzadeh et al. 2010). Especially, phenolic compounds have been reported to have a capacity to scavenge free radicals and their antioxidant activities are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Padma et al. 2011).

The genus *Hypericum* L., a member of the *Guttiferae* (*Hypericaceae*) family, contains about 400 species in the world, about 80 species in Turkey, all are small herbaceous perennials. From a medicinal viewpoint, *Hypericum perforatum* L. (HP) is the major species of this genus (Ozturk et al. 2009). HP popularly called St. John's wort (Coskun et al. 2006), is one of the most important pharmaceutical herbs and its extract contains flavonoids and phenolic acids, which have been demonstrated exerting very efficient anti-inflammatory effects and a free radical scavenging activity in animal model of acute inflammation (Castro et al. 2012). In Turkey, HP is used as medicine against ulcers, diabetes mellitus, common cold, gastrointestinal disorders, jaundice, hepatic and biliary disorders (Uzbay et al. 2007). Also, this plant generally used in the treatment of depression in many countries and recent research show that antidepressants have antioxidant effects against immobilization stress (Ozturk et al. 2007; Sagratini et al. 2008; Arokiyaraj et al. 2011). In 1992, Ozturk et al. have reported hepatoprotective activity of its alcoholic extracts on rodent species (Ozturk et al. 1992). Also, HP has been reported to possess marked antimicrobial, antiviral, antitumoral and anti-HIV/anti-AIDS activities (Ozturk et al. 2007).

To our knowledge, there are few studies about the hepatoprotective effects of *Hypericum perforatum* extract (HPE) as an antioxidant agent against liver injury. Hence, the purpose of the present study was to examine the protective role of HPE against I/R injury in rat liver. For this aim, malondialdehyde (MDA) levels, catalase (CAT) and glutathione peroxidase (GPx) activity in the liver tissue homogenates and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities in the serum were measured and compared statistically.

## Materials and methods

### Plant materials

*Hypericum perforatum* L. was collected from Türkmen Mountains, upper parts of Kalabak, 1300m, Eskişehir, Turkey (OUFE (Eskişehir Osmangazi University Herbaria Catalog Number) 10337). The plant was identified according to Flora of Turkey and the East Aegean Islands (Robson 1967, 1988; Donmez 2000).

### Preparation of extracts

Extraction procedures were applied as described elsewhere (Ozturk et al. 2009). Dried leaves of *H. perforatum* L. were ground and extracted with petroleum ether in a Soxhlet apparatus. Fat-free air-dried material was extracted with methanol: water (70:30, v/v) at 40 °C, 30 min four times. The extract was concentrated to dryness in vacuum and the aqueous solution was lyophilized.

### Animals

Twenty-one adult Sprague-Dawley rats (weighing 210–230 g) were provided from TICAM (Medical and Surgical Experimental Research Centre, Eskisehir Osmangazi University). They were housed in polycarbonate cages in an air-conditioned room (lights on 7 a.m.–7 p.m., 22 ± 2 °C and 45–50 % humidity) and were fed with laboratory pellet chow and water was given ad libitum during the experimental period. All procedures were conducted in conformity with the Institutional Ethical Committee for Animal Care and Use at Eskisehir Osmangazi University (Protocol No.: 157/2010) and the international guidelines on the ethical use of animals (NIH publications no.: 80–23).

### Experimental protocols

The rats were randomly divided into three groups (each containing 7 animals);

Group 1: Normal control (NC) was made up of non-operated rats that received no treatment,
Group 2: I/R control (I/R-C or I/R + saline) were operated rats with no treatment,
Group 3: (I/R + $HPE_{50}$) were operated rats that received single dose 50 mg/kg bw HPE,

HPE solutions used for the treatment were intraperitoneally (ip) injected as a single dose (dissolved in saline, 2 ml/kg bw volume), 15 min before ischemia operation. In all surgical operations; the rats were anesthetized with intramuscular injection of ketamine hydrochloride at the dose of 70 mg/kg bw (Ketalar, Eczacibasi Turkey) and xylazine hydrochloride at the dose of 10 mg/kg bw (Rompun, Bayer, Istanbul, Turkey).

Under anaesthesia, a midline laparotomy was made using minimal dissection. Total hepatic ischemia was induced for 45 min by clamping the hepatic artery, the portal vein, and the bile duct using a vascular clamp. During the period of ischemia 0.5 ml of saline was given ip. Albino rats were subjected to 45 min of hepatic ischemia followed by 60 min of reperfusion period (Sener et al. 2003).

### Biochemical analysis

In present study, biochemical investigations were made in serum and liver tissue. The ALT, AST and LDH levels in serum were immediately measured with a commercial kit (Biolabo, Maizy, France) using an auto analyzer (Airone 200 RA; Crony Instruments, Rome, Italy). The serum ALT, AST and LDH levels were expressed as "U/L".

MDA was measured by thiobarbituric acid reaction as a lipid peroxidation product according to the method of Uchiyama and Mihara (1978). CAT activity was determined using ammonium molybdate–hydrogen peroxide reaction as described previously by Góth (1991). The amount of total protein in the tissue was measured with a total protein kit that was prepared according to the Biuret method. The hepatic MDA level and CAT activity were expressed as "nmol/g protein" and "KU/g", respectively. GPx activity was determined using cellular assay kit (Calbiochem®, Darmstadt, Germany and Cat. No.: 354104). The principle of this kit is the following; it consists of a spectrophotometric assay kit where glutathione peroxidase activity is quantitated by measuring the change in absorbance at 340 nm caused by the oxidation of NADPH. GPx was expressed as mU/ml.

### Statistical analysis

The results were expressed as the mean ± standard error of seven animals per group. One way analysis of variance (ANOVA) and Tukey test were used for the analysis and comparison of data within and between groups (SPSS 11.0 for windows). Differences were considered significant at $p < 0.05$.

## Results

The results of this study of the protective effect of HPE at the dose of 50 mg/kg bw against hepatic ischemia–reperfusion injury in rats are presented in Tables 1 and 2.

### Changes in serum ALT, AST and LDH levels

As shown in Table 1, in ischemia–reperfusion groups, treatment with HPE at the dose of 50 mg/kg bw significantly decreased the ALT, AST and LDH levels

**Table 1** The ALT, AST and LDH activities in serum of rats treated with *H. perforatum* L. or saline

| Experimental groups[c] | ALT (U/L) | AST (U/L) | LDH (U/L) |
|---|---|---|---|
| NC | 46.38 ± 5.92 | 65.38 ± 4.02 | 157.03 ± 27.44 |
| I/R-C or I/R + saline | 868.12 ± 39.22[a] | 522.55 ± 53,96[a] | 2648.25 ± 245.95[a] |
| I/R + $HPE_{50}$ | 402.66 ± 24.48[a,b] | 302.62 ± 18.59[a,b] | 1454.48 ± 202.44[a,b] |

Date are mean ± SE values (n = 7)

$p < 0.05$, significantly different from the [a] NC group and [b] I/R-C group by Tukey's multiple range tests

[c] For details, see "Materials and methods" section

**Table 2** The CAT, GPx activities and MDA levels in liver tissue of rats treated with *H. perforatum* L. or saline

| Experimental groups[c] | MDA (nmol/g protein) | CAT (KU/g) | GPx (mU/ml) |
|---|---|---|---|
| NC | 12.48 ± 2.08 | 7.02 ± 1.26 | 30.62 ± 4.56 |
| I/R-C or I/R + saline | 34.44 ± 2.92[a] | 3.38 ± 1.19[a] | 17.99 ± 4.18[a] |
| I/R + $HPE_{50}$ | 22.32 ± 2.75[a,b] | 6.68 ± 1.32[b] | 28.02 ± 4.47[b] |

Date are mean ± SE values (n = 7)

$p < 0.05$, significantly different from the [a] NC group and [b] I/R-C group by Tukey's multiple range tests

[c] For details, see "Materials and methods" section

in the serum ($p < 0.05$). Although administration of HPE significantly reduced the serum ALT (53.61 %), AST (42.09 %) and LDH (45.08 %) levels, the values were not restored to the same levels as those of the NC group.

Changes in liver tissue MDA levels

Liver tissue MDA level in the I/R + $HPE_{50}$ group was significantly lower than I/R-C group ($p < 0.05$). Although administration of HPE at the dose 50 mg/kg bw significantly reduced the tissue MDA levels (35.20 %), the values were not restored to the same levels as those of the NC group (Table 2).

Changes in liver tissue CAT and GPx activities

In ischemia–reperfusion groups, HPE at the dose of 50 mg/kg bw significantly increased ($p < 0.05$) CAT and GPx activities in the liver. Not only administration of HPE at the dose of 50 mg/kg bw significantly increased the tissue CAT (97.63 %) and GPx (55.75 %) activities in the liver, but also the values were restored to the same levels as those of the NC group (Table 2).

## Discussion

Free radicals are generated naturally and are believed to be critically involved in several disease states, including drug-associated toxicity, ischemic disease and complications resulting from transplantation. In particular, oxygen-derived free radicals induced by ischemia–reperfusion have been studied as a contributing cause of cellular injury in the lung, intestine, liver etc. (Iwamoto et al. 2002). Also, oxidative stress means an alteration in the delicate balance between free radicals and the scavenging capacity of antioxidant enzymes in favour of free radicals in the body systems (Avci et al. 2012).

I/R frequently is encountered during liver transplantation and hepatectomies performed under vascular exclusion. Restoration of blood flow after a period of liver ischemia is associated with a series of events that aggravate the ischemic injury (Smyrniotis et al. 2005).

The implicated factors include free oxygen radicals, leukocyte migration and activation, microcirculatory abnormalities, sinusoidal endothelial cell damage, activation of the coagulation cascade, Kupffer cell activation due to the release of inflammatory cytokines, and proteolytic enzymes (Kucuk et al. 2009).

During the ischemic period, adenosine triphosphate is catabolized to hypoxanthine, which then accumulates in the tissues. Xanthine dehydrogenase, which is highly concentrated in the venous endothelium and tissue, is converted to xanthine oxidase (XO) via a protease. Due to the large amount of oxygen influx during reperfusion, XO catalyzes the conversion of hypoxanthine to xanthine, and simultaneously generates superoxide (Iwamoto et al. 2002).

To control the detrimental effects of ROS (especially superoxide), besides inhibiting its production, organisms have developed a variety of antioxidant defence systems, especially the endogenous antioxidant enzymes (Seth et al. 2000; Wu et al. 2011; Wang et al. 2011), such as SOD (which dismutes superoxide to hydrogen peroxide, which is the first step of the antioxidant pathway), heme-containing CAT and/or the selenoenzyme GPx (which catalyses hydrogen peroxide conversion to water, the second step of the antioxidant pathway) (el Jihen et al. 2009; Kim et al. 2009). In general, while GPx is more important than CAT in removing hydrogen peroxide, CAT has a predominant role at least in peroxisomes where it is

concentrated (Kim et al. 2009). Also, overexpression of GPx has been shown to be protective against oxidative stress in cultured cells and whole animals (Day 2009).

In I/R injury, ROS exerts a central role in the injury of cellular membranes leading to lipid peroxidation in ischemic organs (Daglar et al. 2009). Endothelial cells, as well as Kupffer cells, primarily generate oxygen radicals by NADPH oxidase after hepatic I/R. MDA, which is one of the stable end-products of lipid peroxides, is produced from cell membrane destruction by oxygen radicals. Therefore, MDA is considered as a marker of oxygen radicals and lipid peroxidation of endothelial cells (Nakano et al. 2009) or a sensitive index to assess lipid peroxidation (Daglar et al. 2009). In the present study, ischemia–reperfusion resulted in a significantly elevated MDA level and markedly decreased CAT and GPx activity. These results support the hypothesis that the lipid peroxidation process causes liver cell damage during I/R. Treatment with HPE at the dose 50 mg/kg bw significantly ameliorated the rise in tissue MDA, the decrease in CAT and GPx activity (Table 2). Improvement of liver functions indicates the suppression of oxygen radicals by treatment of HPE effectively inhibited the lipid peroxidation of endothelial cells and thereby preserved liver function.

Enzymes such as ALT, AST, and LDH are used as markers of cellular damage following hepatic I/R injury. In various studies, it has been shown that the serum concentrations of these enzymes increase in proportion with the duration of ischemia (Kucuk et al. 2009). In the present study following I/R injury the serum ALT, AST and LDH levels of the I/R-$HPE_{50}$ were significantly lower than those of the I/R-C group (Table 1). This suggests that HPE protects liver tissue against ischemia/reperfusion damage. This effect of HPE after I/R injury may be due to protection of sinusoidal endothelial cells, which are the first target, and due to improvement of sinusoidal blood flow by HPE.

In conclusion, oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants, and/or a depletion of antioxidants (De Paola et al. 2005). In oxidative stress generated by hepatic ischemia–reperfusion, *H. perforatum* L. as an antioxidant agent contributes an alteration in the delicate balance between the scavenging capacity of antioxidant defence systems and free radicals in favour of the antioxidant defence systems in the body.

**Conflict of interest** The authors declare that there is no conflict of interest.

## References


Arokiyaraj S, Balamurugan R, Augustian P (2011) Antihyperglycemic effect of *Hypericum perforatum* ethyl acetate extract on streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 1:386–390. doi:10.1016/S2221-1691(11)60085-3

Avci G, Kadioglu H, Sehirli AO, Bozkurt S, Guclu O, Arslan E, Muratli SK (2012) Curcumin protects against ischemia/reperfusion injury in rat skeletal muscle. J Surg Res 172:39–46. doi:10.1016/j.jss.2011.08.021

Castro FC, Magre A, Cherpinski R, Zelante PM, Neves LM, Esquisatto MA, Mendonça FA, Santos GM (2012) Effects of microcurrent application alone or in combination with topical *Hypericum perforatum* L. and *Arnica montana* L. on surgically induced wound healing in Wistar rats. Homeopathy 101:147–153. doi:10.1016/j

Coskun I, Tayfun Uzbay I, Ozturk N, Ozturk Y (2006) Attenuation of ethanol withdrawal syndrome by extract of *Hypericum perforatum* in wistar rats. Fundam Clin Pharmacol 20:481–488. doi:10.1111/j.1472-8206.2006.00432

Daglar G, Karaca T, Yuksek YN, Gozalan U, Akbiyik F, Sokmensuer C, Gurel B, Kama NA (2009) Effect of montelukast and MK-886 on hepatic ischemia–reperfusion injury in rats. J Surg Res 153:31–38. doi:10.1016/j.jss.2008.02.052

Day BJ (2009) Catalase and glutathione peroxidase mimics. Biochem Pharmacol 77:285–296. doi:10.1016/j.bcp.2008.09.029

De Paola R, Muià C, Mazzon E, Genovese T, Crisafulli C, Menegazzi M, Caputi AP, Suzuki H, Cuzzocrea S (2005) Effects of *Hypericum perforatum* extract in a rat model of ischemia and reperfusion injury. Shock 24:255–263

Donmez AA (2000) *Hypericum* L. In: Guner A, Ozhatay N, Ekim T, Baser KHC (eds) Flora of Turkey and the East Aegean Islands, vol 11. Edinburgh University Press, Edinburgh, pp 71–72

Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR (2010) Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*. Pak J Pharm Sci 23:29–34

el Jihen H, Imed M, Fatima H, Abdelhamid K (2009) Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver of the rat: effects on the oxidative stress. Ecotoxicol Environ Saf 72:1559–1564. doi:10.1016/j.ecoenv.2008.12.006

Góth L (1991) A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 196:143–151

Iwamoto A, Egashira T, Takayama F, Yamanaka Y, Noguchi T (2002) Change in free radical related substances in plasma following ischemia–reperfusion in rat liver. Pathophysiology 8:167–174. doi:10.1016/S0928-4680(02)00010-x

Jin G, Prabhakaran MP, Kai D, Annamalai SK, Arunachalam KD, Ramakrishna S (2013) Tissue engineered plant extracts as nanofibrous wound dressing. Biomaterials 34:724–734

Kim SJ, Lee JW, Jung YS, Kwon do Y, Park HK, Ryu CS, Kim SK, Oh GT, Kim YC (2009) Ethanol-induced liver injury

and changes in sulfur amino acid metabolomics in glutathione peroxidase and catalase double knockout mice. J Hepatol 50:1184–1191. doi:10.1016/j.jhep.2009.01.030

Korkmaz A, Kolankaya D (2010) Protective effect of rutin on the ischemia/reperfusion induced damage in rat kidney. J Surg Res 164:309–315. doi:10.1016/j.jss.2009.03.022

Kucuk C, Akcan A, Akyıldız H, Akgun H, Muhtaroglu S, Sozuer E (2009) Effects of amrinone in an experimental model of hepatic ischemia–reperfusion injury. J Surg Res 151:74–79. doi:10.1016/j.jss.2008.02.008

Mukherjee PK, Verpoorte R, Suresh B (2000) Evaluation of in vivo wound healing activity of *Hypericum patulum* (Family: Hypericaceae) leaf extract on different wound model in rats. J Ethnopharmacol 70:315–321. doi:10.1016/S0378-8741(99)00172-5

Nakano Y, Kondo T, Matsuo R, Murata S, Fukunaga K, Ohkohchi N (2009) Prevention of leukocyte activation by the neutrophil elastase inhibitor, sivelestat, in the hepatic microcirculation after ischemia–reperfusion. J Surg Res 155:311–317. doi:10.1016/j.jss.2008.07.025

Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A (2010) Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis 29:81–88. doi:10.1007/s10096-009-0824-3

Ozturk Y, Aydın S, Başer KHC, Kırımer N, Kurtar-Ozturk N (1992) Hepatoprotective activity of *Hypericum perforatum* L. alcoholic extract in rodent. Phytother Res 6:44–46. doi:10.1002/ptr.2650060111

Ozturk N, Korkmaz S, Ozturk Y (2007) Wound-healing activity of St. John's Wort (*Hypericum perforatum* L.) on chicken embryonic fibroblasts. J Ethnopharmacol 111:33–39. doi:10.1016/j.jep.2006.10.029

Ozturk N, Tunçel M, Potoğlu-Erkara İ (2009) Phenolic compounds and antioxidant activities of some *Hypericum* ssp.: a comparative study with *H. perforatum*. Pharm Biol 47:120–127

Padma V, Sowmya P, Arun FT, Baskaran R, Poornima P (2011) Protective effect of gallic acid against lindane induced toxicity in experimental rats. Food Chem Toxicol 49:991–998. doi:10.1016/j.fct.2011.01.005

Robson NKB (1967) *Hypericum* L. In: Davis PH (ed) Flora of Turkey and the East Aegean Islands, vol 2. Edinburgh University Press, Edinburgh, pp 355–401

Robson NKB (1988) *Hypericum* L. In: Davis PH, Mill RR, Tan K (eds) Flora of Turkey and the East Aegean Islands, vol 10. Edinburgh University Press, Edinburgh, pp 96–103

Sagratini G, Ricciutelli M, Vittori S, Ozturk N, Ozturk Y, Maggi F (2008) Phytochemical and antioxidant analysis of eight *Hypericum* taxa from Central Italy. Fitoterapia 79:210–213. doi:10.1016/j.fitote.2007.11.011

Sener G, Tosun O, Sehirli AO, Kaçmaz A, Arbak S, Ersoy Y, Ayanoğlu-Dulger G (2003) Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. Life Sci 72:2707–2718. doi:10.1016/S0024-3205(03)00187-5

Seth P, Kumari R, Madhavan S, Singh AK, Mani H, Banaudha KK, Sharma SC, Kulshreshtha DK, Maheshwari RK (2000) Prevention of renal ischemia–reperfusion-induced injury in rats by picroliv. Biochem Pharmacol 59:1315–1322. doi:10.1016/S0006-2952(00)00268-9

Smyrniotis V, Arkadopoulos N, Kostopanagiotou G, Theodoropoulos T, Theodoraki K, Farantos C, Kairi E, Paphiti A (2005) Attenuation of ischemic injury by N-acetylcysteine preconditioning of the liver. J Surg Res 129:31–37. doi:10.1016/j.jss.2005.07.028

Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278

Uzbay IT, Coskun I, Kayir H, Ozturk N, Ozturk Y (2007) Extract of *Hypericum perforatum* blocks caffeine-induced locomotor activity in mice: a possible role of nitric oxide. Phytother Res 21:415–419. doi:10.1002/ptr.2085

Wang J, Kan Q, Li J, Zhang X, Qi Y (2011) Effect of neferine on liver ischemia–reperfusion injury in rats. Transplant Proc 43:2536–2539. doi:10.1016/j.transproceed.2011.04.013

Wu C, Wang P, Rao J, Wang Z, Zhang C, Lu L, Zhang F (2011) Triptolide alleviates hepatic ischemia/reperfusion injury by attenuating oxidative stress and inhibiting NF-κB activity in mice. J Surg Res 166:205–213. doi:10.1016/j.jss.2010.10.005

Yaylak F, Canbaz H, Caglikulekci M, Dirlik M, Tamer L, Ogetman Z, Polat Y, Kanik A, Aydin S (2008) Liver tissue inducible nitric oxide synthase (iNOS) expression and lipid peroxidation in experimental hepatic ischemia reperfusion injury stimulated with lipopolysaccharide: the role of aminoguanidine. J Surg Res 148:214–223. doi:10.1016/j.jss.2007.10.008