Protective Effect of Micronized Purified Flavonoid Fraction on Ischemia/Reperfusion Injury of Rat Liver

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ABSTRACT

Background. Flavonoids have been subjected to considerable investigation because of its antioxidant and anti-inflammatory properties. However, there is no previously reported study about its effect on hepatic ischemia/reperfusion (I/R). We investigated the effects of micronized purified flavonoid fraction (MPFF) on hepatic I/R injury in rats.

Methods. Thirty rats were recruited in the study as follows: group A, sham operation (n = 10); group B, I/R (n = 10); and group C, I/R+MPFF (n = 10). In group C, rats received (80 mg/kg/day) MPFF by gavage for 3 days before surgery, 30 minutes before ischemia and just before the reperfusion. Blood samples were taken, and serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were measured to assess liver functions. Liver tissues were taken for histological evaluation and to determine the total antioxidant capacity (TAC), catalase (CAT), total oxidant status (TOS), oxidative stress index (OSI), and myeloperoxidase (MPO).

Results. The present data showed a decrease in AST, ALT, and LDH levels in the MPFF-treated rats when compared with I/R group rats ($P < .001$ for all). In the MPFF-treated rats, tissue levels of TOS, OSI, and MPO were significantly lower than those in the I/R group ($P < .01$, $P < .001$, and $P < .05$, respectively). Increases in TAC and CAT levels were statistically significant in the MPFF-treated rats compared with the I/R group ($P = .01$ for both). On the other hand, MPFF attenuated histological alterations that were induced by I/R.

Conclusions. The present study demonstrates that MPFF ameliorates I/R-induced liver damage, probably through antioxidant and anti-inflammatory properties.

ISCHEMIA/REPERFUSION (I/R) injury causes up to 10% of early liver failures and can lead to a higher incidence of acute and chronic rejection after liver transplantation [1]. Minimizing the adverse effects of I/R injury could significantly decrease the incidence of liver failure after transplantation.

During the reperfusion process, a large amount of molecular oxygen is supplied to the tissues and abundant amounts of reactive oxygen species (ROS), which are responsible for reperfusion injury, are produced [2]. Oxidative stress occurs when ROS are formed and when oxygen is reintroduced to ischemic tissues [3]. Many experimental studies on I/R injury in animals suggest a preventive effect of antioxidants [4,5].

The micronized purified flavonoid fraction (MPFF), consisting of 90% micronized diosmin (a flavone glycoside) and 10% flavonoids expressed as hesperidin (a flavanone glycoside), has been shown to have protective effects in some conditions such as I/R [5], oxidative stress [6], inflammation [5], or venous hypertension [7]. Although MPFF has antioxidant features, to date there is no study regarding the protective effect of MPFF on liver damage in rats subjected

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Table 1. Clinical Parameters in Sham, I/R, and I/R+MPFF Rats

	Sham (n = 10)	I/R (n = 10)	I/R+MPFF (n = 10)	*P* Value
AST (U/L)	132 ± 22	952 ± 251[*,¶]	260 ± 156[†,‡,‖]	.001
ALT (U/L)	86 ± 17	695 ± 206[*,¶]	149 ± 78[‡,‖]	.001
LDH (U/L)	534 ± 181	4334 ± 760[*,¶]	147 ± 1140[‡,‖]	.002
TAC (nmol/L Trolox Eqv./mg protein)	2.96 ± 0.4	2.17 ± 0.6[*,**]	3.33 ± 0.4[†,‡,§,¶]	.008
TOS (nmol/L H_2O_2 Eqv./mg protein)	10.4 ± 2.2	15.9 ± 2.0[*,¶]	11.6 ± 2.1[†,‡,§,¶]	.006
OSI (Arbitrary Units)	3.54 ± 0.7	7.76 ± 1.9[*,‖]	3.48 ± 0.6[†,‡,§,‖]	.002
MPO (U/gr protein)	9.4 ± 1.8	13.2 ± 1.7[*,¶]	10.7 ± 1.1[†,‡,§,**]	.007
CAT (U/mg protein)	18.4 ± 3.9	10.1 ± 1.9[*,¶]	17.8 ± 2.9[†,‡,§,¶]	.005

Values are mean ± SD.
Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.
*Difference between sham and I/R groups.
†Difference between sham and I/R+MPFF groups.
‡Difference between I/R and I/R+MPFF groups.
§Nonsignificant.
‖$P < .001$.
¶$P < .01$.
**$P < .05$.

to hepatic I/R. Therefore, this study was designed to investigate the effects of MPFF on post-ischemic liver injury by measurement of biochemical parameters such as total antioxidant capacity (TAC), catalase (CAT), total oxidant status (TOS), oxidative stress index (OSI), and myeloperoxidase (MPO) in the liver tissue and serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels in blood. We also examined histopathological changes in liver parenchyma.

MATERIALS AND METHODS

Experiments were performed at the Harran University Experimental Research Center. All surgical procedures were performed while the rats were under intraperitoneal ketamine (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia. Animals were divided into 3 groups: group A, I/R (n = 10); group B, I/R plus MPFF (n = 10); and group C, sham (n = 10).

Only laparatomy was performed in the sham group. Total hepatic ischemia was induced for 60 minutes by clamping the hepatic artery, the portal vein, and the bile duct, with the use of a vascular clamp. Thereafter, the clamp was removed and blood was reperfused for 60 minutes. In the I/R period, all rats survived; after reperfusion, all were euthanized. Animals in the I/R+MPFF group were treated with 80 mg/kg MPFF (Daflon, 500 mg; Servier, Turkey) by gavage once a day throughout 3 days before surgery, 30 minutes before ischemia, and immediately before the reperfusion period. The blood samples and a part of the liver were taken.

TAC of supernatant fractions was determined through the use of a novel automated measurement method, developed by Erel [8]. TOS of supernatant fractions was determined through the use of a novel automated measurement method developed by Erel [9]. OSI value was calculated according to the following formula [10]: OSI (Arbitrary Unit) = TOS (nmol/L H_2O_2 Equiv./mg protein.)/TAC (nmol/L Trolox Equiv./mg protein). The MPO (EC 1.11.1.7) activity was determined with the use of a 4-aminoantipyrine/phenol solution [11]. Liver catalase activities were determined by means of Goth's colorimetric method [12]. For histopathological evaluation, 4-mm slides were stained with hematoxylin and eosin.

For statistical analyses, nonparametric independent group comparisons were made. For multiple comparisons, the Kruskal-Wallis test was used for comparisons between groups, and the Mann-Whitney test was used if any statistical significance was found.

RESULTS

Plasma AST, ALT, and LDH levels were significantly higher in the I/R group than in those in the I/R+MPFF and sham groups ($P < .001$ for all and $P < .01$ for all, respectively). AST was significantly higher in the I/R+MPFF group than in the sham group ($P < .05$). Besides this, ALT was higher and LDH was lower in the I/R+MPFF group than in the sham group; however, the results were not statistically significant ($P > .05$). The results are summarized in Table 1.

In this study, we found that there were significant differences between the I/R group and the I/R+MPFF group with respect to oxidant and antioxidant parameters. The tissue TAC and CAT levels were significantly lower in the I/R group than those in the sham and I/R+MPFF groups ($P < .05$, $P < .01$ and $P < .01$, $P < .01$, respectively). However, TOS, OSI, and MPO levels were significantly higher in the I/R group rats than those in the sham and I/R+MPFF groups ($P < .01$, $P < .001$, $P < .01$ and $P < .01$, $P < .001$, $P < .05$, respectively). In the I/R+MPFF group, TOS, OSI, and MPO levels were significantly lower than those in the I/R group ($P < .01$, $P < .001$, and $P < .05$, respectively). There were no statistically significant differences between the I/R+MPFF and sham groups regarding the oxidant and antioxidant parameters. The results are summarized in Table 1.

In histopathological evaluation, there were no pathological changes in liver tissue of the sham group (Fig 1A). Liver specimens from rats after ischemia-reperfusion exhibited focal necrosis and infiltration of leukocytes (Fig 1B). MPFF treatment significantly decreased these pathological changes (Fig 1C). Histological tissue damage