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# Antioxidant effect of *Rosa pimpinellifolia* L. fruit extract on cholestatic liver injury: an experimental study

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## SUMMARY

**BACKGROUND:** Antioxidants have been considered a rational curative strategy to prevent and cure liver diseases involving oxidative stress. An acute obstructive jaundice rat model was established to investigate the ***in vivo*** hepatoprotective efficacy of *Rosa pimpinellifolia* L.

**METHODS:** The experimental jaundice model was performed by binding the main bile duct in 25 male Sprague-Dawley rats. All rats were randomly divided into five groups: first group: laparotomy-sham-only, second group: biliary tract binding (control), and third, fourth, and fifth groups: treatment groups with 250, 500, and 750 mg/kg fruit extracts daily, respectively.

**RESULTS:** Considering dosage, although there was no significant therapeutic effect in the 250 mg/kg of *Rosa pimpinellifolia* L. group, the best results were found in the 500 mg/kg dose group, while results in the 750 mg/kg dose group showed consistent correlation with proinflammatory response. With regard to biochemical parameters, lipid hydroperoxide level in the rat serum and liver tissue was significantly decreased in all treatment groups. Amadori products, which are one of the early markers of glycol-oxidative stress, showed statistical significance in the treatment.

**CONCLUSION:** It was revealed that the antioxidant effect of *Rosa pimpinellifolia* L. was more prominent in the early stages of hepatic injury secondary to oxidative stress.

**KEYWORDS:** Cholestasis. Liver. Antioxidants. *Rosaceae*.

## INTRODUCTION

Antioxidants are chemicals that help organisms to lessen or eliminate oxidative stress caused by free radicals. Many wild fruits are usually considered a good source of antioxidants and have been used as natural therapeutic agents due to their bioactive phenolic compounds[1]. One of the most important wild fruit groups is the *Rosaceae* family which includes thousands of species, and some of the vital phytochemicals and antioxidants in the fruits of this family have potential health benefits[2].

*Rosa pimpinellifolia* L. fruit that belongs to the Rosaceae family is an endemic member of the Rosaceae family growing in the (Bayburt Province) East of Turkey[3].

Recently, Ergen et al., showed the antioxidant activity of *R. pimpinellifolia* L. *in vitro*[4]. However, *in vivo* antioxidant activity has not yet been confirmed. Therefore, this study was designed to investigate the potential antioxidant activity of *R. pimpinellifolia* L. fruit extract on an experimental model of liver injury.

## METHODS

### Animals and preparations

A total of 25 adult male Sprague-Dawley rats (age, 6–8 weeks; weight, 290±30 g) were housed at a constant temperature (22±1°C), with 50% relative humidity and a 12-h light/dark cycle. The rats had access to food including 21% protein and autoclaved water *ad libitum*. All animal procedures were approved by the Animal Experiments Ethics Committee.

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Procedures were performed under general anesthesia. Anesthesia was provided with ketamine and xylazine (10 mg/kg xylazine and 50 mg/kg ketamine) administered intraperitoneally.

Bile duct surgery was performed as previously reported by Criado et al.[5]. The common bile duct (CBD) was tied with 5/0 silk thread from two places to create obstructive jaundice by clearing it from surrounding tissues.

### Groups and treatments

The rats were randomly divided into five groups, with five animals in each group:

**Group 1:** Animals were performed only laparotomy and treated orally with normal saline (0.9%) for 10 days.

**Group 2:** Control animals were performed laparotomy and CBD ligation and treated orally with normal saline (0.9%) for 10 days.

**Group 3:** After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 250 mg/kg for 10 days.

**Group 4:** After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 500 mg/kg for 10 days.

**Group 5:** After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 750 mg/kg for 10 days.

*Rosa pimpinellifolia* L. fruits were collected from Bayburt (Eastern Turkey). The collected seeds were dried in an oven and pounded in a porcelain mortar. Notably, 20 g of sample taken from the powdered sample was added to the 100 mL ethanol (EtOH)-water (50/50 mL) mixture. The mixture was extracted in a water bath under a constant temperature of 40°C for 48 h. Then, the extract was filtered and, to remove EtOH, it was subjected to evaporation at 40°C using an evaporator. The extract was frozen at -80°C and dried in a lyophilizer, and *R. pimpinellifolia* L. 50% EtOH and water extract was obtained and stored at -20°C.

The experiment was completed. Before sacrification, a circulating blood sample (7 mL) was obtained by cardiac puncture. A portion of the left liver lobe was taken for histochemical and biochemical analyses.

### Laboratory analysis

Serum biochemical parameter levels such as aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin were analyzed with the colorimetric method.

The liver tissues were homogenized in a buffer [phosphate buffer (pH 7.4)+0.1% digitonin] with a ball-bearing homogenizer and 10% homogenates were prepared.

Myeloperoxidase activity and nitrotyrosine level were calculated by ELISA kit; lipid hydroperoxide (LOOH) levels were calculated by extinction coefficient; malondialdehyde (MDA) total thiol levels and Amadori products were calculated by extinction coefficient; advanced glycation end products (AGE) were calculated by the spectrofluorimetric method; and ferric ion-reducing antioxidant power (FRAP) was calculated with the calibration graph.

### Histopathological assessment

The liver specimen was fixed in 10% formalin solution and embedded into paraffin blocks. The blocks were cut using microtome to 5 µm thickness. Thereafter, the sections were stained by hematoxylin-eosin (H&E) as well as Masson trichrome. Tissue slides were visualized under a light microscope. The same pathologist with at least 10 years of experience made all evaluations in liver pathology.

### Statistical analysis

Statistical analyses were performed using the SPSS software version 25.0 (IBM SPSS Statistics for Windows, Version 25.0. IBM Corp. Released 2017. Armonk, NY: Chicago, IL, USA).

Quantitative data were expressed as the mean±standard deviation. After determining whether the parameters were compatible with the normal distribution or not using the Shapiro-Wilk test, one-way analysis of variance (ANOVA) was applied for parametric tests with normal distribution using the Bonferroni test for *post hoc* comparisons when significance was determined by ANOVA. The Kruskal-Wallis test was used with nonparametric distribution. Values with $p<0.05$ were considered statistically significant. A *post hoc* test was used for paired group comparisons in parameters that were significant.

## RESULTS

### Biochemical results

#### *Rat serum liver function tests (aspartate transaminase/alanine transaminase/total bilirubin/gamma-glutamyltransferase/alkaline phosphatase)*

By examining liver function tests from rat serums, a comparative analysis between the control and treatment groups (Groups 2 and 3–5) and the sham group (Group 1) resulted in a significant difference ($p<0.05$) which was compatible with the cholestatic liver injury. However, in treatment groups compared with the control group, the best results—similar AST levels to the sham surgery group—considering AST were found in Group 4 (500 mg/kg). Although the alterations in ALT levels were unremarkable, better results were observed in Groups 4 and 5.

### *Rat serum oxidative damage parameters*

#### Lipid peroxidation and glycoxidative stress parameters

Lipid peroxidation (LOOH, MDA) and glycoxidative stress (AGE, Amadori) parameters were statistically significant in the control group (Group 2) (Table 1). When the extracted groups were compared with the control group, lipid peroxidation and glycoxidative stress parameters were found to be statistically significant and low.

#### *Other antioxidant parameters*

Considering the efficacy of treatment in terms of parameters other than FRAP in rat serum, no significant difference was found.

## Rat liver homogenate oxidative stress parameters

### *Protein oxidation parameters*

As indicated by the PCO, DT, and KYN (protein carbonyl groups, DT: dytyrosine, KYN: kynurenine) values in rat liver tissue, there were findings of oxidative damage in both control and therapeutic groups, and no positive effect was found between the groups in terms of different dosages.

### *Lipid peroxidation and glycoxidative stress parameters*

LOOH level in rat liver tissue increased significantly in the control group (Group 2), and a significant decrease was observed in the groups that were given the extract (p=0.014). There was no significant change in MDA levels in terms of treatment efficacy.

A statistical significance was achieved with the increase of Amadori, one of the early markers of glycoxidative stress, in the control group (Group 2), while approaching the level of the sham group in the groups that were given the extract (p<0.001). On the contrary, no significant difference was found in the AGE level, which indicates the advanced stage of glycoxidative stress (Table 2).

### *Other antioxidant parameters*

Among other antioxidant parameters studied from rat liver tissue, T-SH levels were not affected. In addition, although there is a regression in FRAP levels in the extracted groups compared with the sham group, there is an increase in the antioxidant parameters when evaluated compared with the control group. Cu and Zn SOD levels increased in the groups that were given the extract compared with the control group. The effect of the increase in antioxidant levels in the liver tissue among the groups that were given the extract was highest in the group (Group 4), in which the extract was administered at a dose of 500 mg/kg (Table 3).

**Table 1.** Mean values of oxidative damage parameter tests (lipid peroxidation and glycoxidative) in serum samples of rats between groups.

| | LOOH* (µmol/mL) | MDA* (µmol/mL) | AGE** (FU/mg protein) | Amadori** (nmol/mg protein) |
|---|---|---|---|---|
| Group 1 | 1.26±0.02 | 2.01±0.09 | 1039±143 | 82199±11392 |
| Group 2 | 1.47±0.8 | 2.09±0.11 | 2581±360 | 204193±28509 |
| Group 3 | 1.29±0.06 | 2.20±0.11 | 1233±617 | 97547±48841 |
| Group 4 | 1.22±0.06 | 2.12±0.18 | 1411±686 | 111677±54325 |
| Group 5 | 1.2±0.07 | 1.93±0.76 | 1110±607 | 87816±48036 |
| p-value | ***<0.001*** | ***0.025*** | ***0.028*** | ***0.027*** |

*Parametric distribution; **non-parametric distribution. LOOH: lipid hydroperoxide; MDA: malondialdehyde; AGE: advanced glycation end products. Bold italics denote statistically significant p-value.

**Table 2.** Mean values of oxidative damage parameter tests (lipid peroxidation and glycoxidative) in liver tissue of rats between groups.

| | LOOH** (µmol/mg protein) | MDA** (µmol/mg protein) | AGE* (FU/mg protein) | Amadori* (nmol/mg protein) |
|---|---|---|---|---|
| Group 1 | 0.9±0.4 | 4±0.2 | 3156±567 | 47±19 |
| Group 2 | 2.5±0.2 | 5.7±1.1 | 3608±1508 | 94±13 |
| Group 3 | 0.8±0.1 | 4.6±1.01 | 3092±134 | 45±13 |
| Group 4 | 0.7±0.2 | 6±0.5 | 4490±848 | 40±16 |
| Group 5 | 0.7±0.1 | 5.5±0.9 | 3032±759 | 39±14 |
| p-value | ***0.014*** | 0.806 | 0.066 | ***<0.001*** |

*Parametric distribution; **non-parametric distribution. LOOH: lipid hydroperoxide; MDA: malondialdehyde; AGE: advanced glycation end products. Bold italics denote statistically significant p-value.

**Table 3.** Average values of other antioxidant levels in liver tissue of rats between groups.

| | T-SH* (nmol/mg protein) | FRAP** (µmol/mg protein) | Cu, Zn SOD* (U/mg protein) |
|---|---|---|---|
| Group 1 | 10±1.1 | 2303±15215 | 31.7±20 |
| Group 2 | 12.5±3.2 | 593±134 | 4.1±1.8 |
| Group 3 | 13.5±2.6 | 1627±805 | 24±52 |
| Group 4 | 14±2.4 | 1734±599 | 57±15 |
| Group 5 | 13.7±1.7 | 1240±420 | 33±12 |
| p-value | ***0.089*** | ***0.017*** | ***0.001*** |

*Parametric distribution; **non-parametric distribution. T-SH: total thiol groups; FRAP: ferric ion-reducing antioxidant power; Cu, Zn SOD: copper, zinc superoxide dismutase. Bold italics denote statistically significant p-value.

### Histopathological results

Except for the sham group, similar rates of ductular proliferation, inflammatory cell infiltration in the ducts, acidophilic necrosis, coagulation necrosis, and fibrosis were detected in all groups. However, when the groups were compared, none of the parameters were found to be statistically significant.

Although not statistically significant, increased mitotic activity was detected in the groups that were given the extract. Although it was not reflected in the statistical data, there was an increase in the number of mitoses correlated with the increase in the dose of the extract administered.

## DISCUSSION

The final results showed that the jaundice model created in rats was successful, and the best results considering antioxidant activity were achieved in the 500 mg/kg group. In the low dosage (250 mg/kg) group, a significant therapeutic effect was not observed, while the proinflammatory effect was more prominent in the high dosage (750 mg/kg) group.

The binding of the CBD in rats produces similar changes to those in human biliary cirrhosis[6]. In a study, it was determined that ALT, AST, total bilirubin, GGT, and ALP levels in plasma increased 10 days after the main bile duct was disconnected, causing significant cholestatic liver damage[7]. Likewise, we determined the study duration as 10 days. All values (AST, ALT, T.Bil, and GGT) except ALP obtained by liver function test were significantly higher in the study groups (p=0.014, 0.021, 0.014, and 0.009, respectively) compared with Group 1. In the evaluation of elevated transaminase levels, some diagnostic approaches, including the "De Ritis rate," have been determined. In 1957, Fernando De Ritis noted the importance of the ratio between serum AST and ALT levels. In the case of AST/ALT>1.5, intrahepatic cholestasis should be considered[8]. In this study, results were consistent with this rate. In Group 4, treated with 500 mg/kg *R. pimpinellifolia* L. fruit extract, AST, ALT, total bilirubin, and GGT levels were significantly reduced, and therefore, a therapeutic dose of 500 mg/kg could be more plausibly supposed to be optimal for preventing/reducing liver hepatocyte injury.

Considering antioxidant features, statistical significance could not be achieved in therapeutic groups. However, most of the oxidative stress parameters were highest in Group 5. This was attributed to dose-related toxication risk, suggesting that the extract may have a pro-oxidant effect at higher doses.

Myeloperoxidase (MPO) plays an important role in tissue damage in both acute and chronic inflammation. It acts as a key enzyme in the generation of reactive oxygen species promoting inflammation and oxidative stress[9]. In our series, MPO and 3-nitrotyrosine were high in liver homogenate in therapeutic groups, though it was not reflected in the serum. A recent study investigating MPO levels in non-alcoholic steatohepatitis patients showed consistent findings with our results. There was no difference in serum MPO levels despite prominent hepatic inflammation and MPO-expressing cell counts in liver biopsies[10]. However, we anticipated that serum effects may be observed in a later phase requiring a longer study period considering all other parameters as well.

Some studies reported that oxidative damage of proteins occurs in liver injury caused by $CCl_4$[11]. While oxidative damage to DNA, protein, or lipid is extremely harmful, proteins are more susceptible because they often act as catalysts inside cells. The non-enzymatic modifications of proteins through Amadori reactions in the early phase and AGEs can accumulate as protein modifications[12]. In our study groups, glycoxidation products, such as Amadori products and AGE, were found to be significantly lower in serum levels of treatment groups. However, liver homogenate analysis revealed significantly higher Amadori levels which were indicative of early damage, while there was no significant difference in terms of AGE representing the advanced stage degeneration. In this case, it is thought that *R. pimpinellifolia* L. extract might be successful in protecting against liver injury, especially in the early stages of mild-moderate injury situations.

Lipid peroxidation has an important role in human health. The reaction of oxygen with unsaturated lipids produces different types of oxidation products[13].

We focused on main products such as LOOH which is formed due to fatty acid oxidation during the early stages of lipid peroxidation and MDA which is one of the secondary products of lipid peroxidation. A significant decrease in LOOH was observed in the treatment groups. This significant change in early lipid peroxidation parameters such as LOOH and no change in parameters in the later phase of lipid peroxidation may contribute to the idea of plausible efficacy of *R. pimpinellifolia* L. in the early stages of the damage.

This study has limitations. Although a sample size calculation before beginning the study was performed, it was smaller compared with similar studies concerning limited laboratory space and financial constraints in addition to animal welfare. We anticipated that, because this herbal product is new, the findings of this study may offer a potentially positive influence on this patient population.

## CONCLUSION

It is quite clear that phytotherapeutics can be very useful as a full or complementary therapy for many clinical conditions,

but it is necessary to provide more *in vivo* information[14]. These results showed for the first time that *R. pimpinellifolia* L. fruit extract enriched with flavonoids and polyphenolic compounds possesses significant *in vivo* anti-inflammatory activity manifested by combating particularly early-stage oxidative stress parameters involved in bile duct ligation-induced cholestatic liver injury. To determine the safety profile of *R. pimpinellifolia* L. fruit extract and to establish the "no observed adverse effect level" of the extract, adequately powered studies with larger sample size and with long periods of follow-up, including *in vitro* and *in vivo* toxicological assessments, should be performed.

## INFORMED CONSENT STATEMENT

All data from this research, which was planned as an animal study, will be made available upon request. The authors state that the information may be published in the data sharing statement. Data include Excel and/or SPSS version of results obtained for statistical analysis, hematoxylin-eosin stained preparations, immunohistochemistry stained preparations, and any other information obtained.

## AUTHORS' CONTRIBUTIONS

**MKD:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **PY:** Conceptualization, Investigation, Supervision, Writing – review & editing. **OC:** Data curation, Methodology, Resources, Validation. **KY:** Data curation, Formal Analysis, Methodology, Validation, Visualization. **MBYO:** Data curation. **ZGD:** Formal Analysis, Investigation, Software, Visualization, Writing – review & editing. **BC:** Funding acquisition, Resources, Supervision. **OBG:** Methodology, Supervision. **PA:** Resources, Supervision.

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