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# The protective effects of pomegranate on liver and remote organs caused by experimental obstructive jaundice model

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**Abstract. – OBJECTIVE: We aimed to investigate the protective potential of pomegranate extract on the liver and remote organs in rats with obstructive jaundice.**

**MATERIALS AND METHODS: The rats were split into 4 groups. In Group 1 (G1) (sham group) rats, the common bile duct was mobilized without any ligation. Group 2 (G2) received a combination of the sham operation and synchronous treatment with pomegranate. Group 3 (G3) received common bile duct ligation (CBDL). Group 4 (G4) were subjected to CBDL and treatment with pomegranate. After 8 days, we measured total oxidative status (TOS) and antioxidant capacity in the rats' liver tissue and remote organs, and evaluated blood levels of malondialdehyde and total antioxidant capacity (TAC).**

**RESULTS: G3 rats showed significantly raised malondialdehyde level as compared to G1 rats ($p < 0.001$). Following the pomegranate therapy, a decrease in malondialdehyde was observed ($p = 0.015$). TAC levels were significantly raised in the G3 rats compared to the G1 rats ($p = 0.004$). TAC levels dropped after pomegranate therapy ($p = 0.011$). CBDL caused elevated TOS levels in the liver and remote organs, with a statistically significant increase in the lung tissue ($p = 0.002$). TOS levels in the CBDL groups decreased after pomegranate treatment ($p < 0.001$).**

**CONCLUSIONS: This study reveals the marked protective effect of pomegranate on the liver and remote organs in obstructive jaundice.**

*Key Words:*
Pomegranate, Obstructive jaundice, Remote organ, Oxidative stress.

## Introduction

Obstructive jaundice (OJ) is a common health problem caused by interrupted bile drainage. OJ has high morbidity and mortality[1]. In spite of improved surgical techniques and the advent of new potent antibiotics, sepsis after surgery is still a significant cause of mortality in OJ[1,2].

Rats with OJ have been reported to display an overproduction of free oxygen radicals (FOR) in the liver and blood[1]. Both toxic bile products and neutrophil migration contribute to the synthesis of FOR in the liver parenchyma[3].

It has shown that FOR has an important role in the pathogenesis of tissue damage associated with OJ[4]. Parenchymal damage is aggravated by FORs. Decreased antioxidant enzyme activity and an increased oxidized:reduced glutathione ratio are observed in the liver in OJ[5].

Multiple organ dysfunctions can occur due to oxidative stress and damage induced by increased FOR. The pathogenesis of OJ-induced tissue damage has not yet been fully understood, although an abundance of contentious ideas aims to explain this phenomenon[4].

The body defends itself against oxidative stress by antioxidant enzymes acting as mediators which constantly remove free radical intermediates. Other mechanisms are of non-enzymatic nature and include many natural or synthetic antioxidants (*e.g.*, glutathione, α-tocopherol) which prevent oxidative damage by reacting with free radicals. A diet made up of low-molecular-weight antioxidants contributes to the antioxidant defense system[5].

The flower of *Punica granatum* makes a popular beverage that is consumed in Turkey and worldwide. It is known to have antioxidant, anti-inflammatory, antibacterial, antitumor, antidiarrheal, antifungal and antiulcer properties.

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Different parts of this plant are used to prepare various extracts. Pomegranate flavonoids can display anti-inflammatory effects such as inhibiting low-stimuli activation of the inflammatory processes. Moreover, *Punica granatum* has gained importance because of its antioxidant potential[6].

Pomegranate juice, seed oil and peel are known to have strong antioxidant effects. The rich phenolic contents of pomegranate include anthocyanins and hydrolysable tannins[7]. Maternal dietary supplementation with pomegranate juice has been shown to have a neuroprotective impact on neonatal mouse brains with hypoxic-ischemic injury. Moreover, pomegranate juice is known to have a protective influence against systemic oxidative stress in mice. Studies show that pomegranate extract exerts a gastroprotective activity via antioxidant mechanisms, as well. A review study reported that pomegranate has beneficial effects on oxidative stress and inflammation[5,7-10].

We aimed to investigate the ameliorating effects of pomegranate extract on the liver and remote organs in OJ.

## Materials and Methods

### Study Sample

All procedures were in accordance with the guidelines of the Care and Use of Laboratory Animals. Forty albino rats (200 ± 20 g) were included in our experimental study. Our study was started after gaining the approval of the Committee of Experimental Animals at Dicle University. The rats were housed in cages under standard conditions in an air-conditioned room with 12:12-h light:dark cycle at a room with controlled temperature (22 ± 2°C). They were allowed free access to standard rat chow and water until the tests. The animals were fasted the day before surgery, but had free access to water.

### Experimental Design

The rats were split into 4 groups in a randomized fashion, with 10 rats in each group. The groups were designed as follows:

**Group 1** – Sham-operated group.
**Group 2** – Drug control group (The same in Sham group plus Pomella®, Verdure Sciences, Noblesville, IN, USA; 250 mg/kg/day).
**Group 3** – Common bile duct ligation (CBDL) group.
**Group 4** – Treatment group (CBDL plus Pomella®, Verdure Sciences, Noblesville, IN, USA; 250 mg/kg/day).

All surgeries were performed on rats under anesthesia with ketamine 50 mg/kg IP (Ketalar, Parke-Davis, Istanbul, Turkey) and xylazine HCl 10 mg/kg (Rompun®; Bayer AG, Leverkusen, Germany). The operations were carried out in sterile settings. After the midline abdominal incision, the common bile duct was identified and mobilized. For CBDL groups (groups 3 and 4), the common bile duct was then doubly ligated using 5-0 silk and divided. Sham-operated rats (groups 1 and 2) underwent a similar incision followed by mobilization of the common bile duct, without any ligation or sectioning. Thereafter, the abdominal wall was closed with 2-0 silk sutures using a continuous suture pattern. Relaparotomy was performed on the 8$^{th}$ day.

For groups 2 and 4, pomegranate was delivered for 8 days as a single dose per day via temporary orogastric intubation. At the end of the 8-day period, no mortality was observed in any of the groups. Eight days after the development of OJ, the animals received laparotomy under sterile conditions.

### Biochemical Analyses

Total antioxidant capacity (TAC) and malondialdehyde (MDA) analyses were performed on blood samples. Total oxidative status (TOS) and TAC analyses were performed on tissue samples obtained from liver, lung and kidney. OSI was measured in tissue samples as well.

### Homogenization of Tissues

Tissues stored at –80 °C were removed from the deep freezer and brought to the laboratory in dry ice. Tissue pieces of about 0.30 to 0.50 grams were transferred into test tubes and 2 ml of Tris-HCl buffer was added. Tissues in the tubes were placed into an ice-filled plastic container and processed in the phosphate buffer (PBS) (50 mM, pH 7.0) for 1-3 minutes at 14,000 rpm by a homogenizer (Ultra Turrax Type T8, IKA Labortechnic, Germany). The homogenate was centrifuged for 30 minutes at +40 °C. The Total oxidant activity (TOA) and TAC analyses were carried out with samples obtained from this supernatant.

### Measurement of the Malondialdehyde Activity

The double heating method of Draper and Hadley was used to measure malondialdehyde (MDA) levels[11].

### Measurement of the Total Antioxidant Capacity, Total Oxidant Status

Total antioxidant status (TAS) and total oxidant status (TOS) of serum were determined using novel automated measurement methods, developed by Erel[12,13].

### Measurement of the Oxidative Stress Index

Oxidative Stress Index (OSI) was defined as the ratio of TOS level to TAC level. OSI value was calculated based on the following formula[14]: OSI (Arbitrary Unit) = TOS (nmol $H_2O_2$ Equiv./mg protein)/TAC (nmol Trolox Equiv./mg protein). The results were expressed as Arbitrary Unit.

### Statistical Analysis

SPSS 16 package program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were presented as mean ± standard deviation. The differences between the groups were evaluated by Kruskal-Wallis test. For binary comparisons, Mann-Whitney U-test was used. $p < 0.05$ was recognized as statistically significant.

## Results

All animals survived during the experiments. Table I shows the serum biochemical values which significantly varied between the study groups. MDA level, an indicator of lipid peroxidation, was significantly increased in the CBDL group ($p < 0.001$) as compared to the sham group, presumably due to oxidative stress after CBDL. MDA showed a decrease after the pomegranate treatment ($p = 0.015$). Although TAC serum levels demonstrated a significant increase ($p = 0.004$) after CBDL, they decreased ($p = 0.011$) with pomegranate treatment.

As shown in Table I, CBDL exerts a significant impact on the liver and remote organs. The sham group and the drug control groups revealed no difference with regard to the liver, lung, and kidney.

The CBDL group exhibited elevated TOS levels in the liver and remote organs, with a statistically significant rise in the lung tissue ($p= 0.002$). Similarly, OSI was significantly higher in the lung ($p < 0.001$) and kidney ($p < 0.001$) tissues, which was suggestive of increased oxidative stress. However, TAC levels after CBDL were significantly decreased in the kidney ($p = 0.002$) and lung ($p = 0.002$) tissue, as compared to the sham group.

Pomegranate treatment demonstrated a protective effect against oxidative stress (Table I) in the experimental OJ model. The raised TOS levels in liver and remote organs in the CBDL groups decreased after pomegranate treatment. Although the TOS reductions were not statistically significant, the decreases in OSI were statistically significant in the kidney ($p = 0.002$) and lung ($p < 0.001$) tissues as compared to the CBDL group. Moreover, pomegranate treatment was observed to improve the antioxidant capacity of the liver and remote organs. However, the differences were not statistically significant.

**Table I.** Biochemical parameters of remote organs.

| | Sham | Drug control | CBDL | Pomegranate | p |
|---|---|---|---|---|---|
| TAS-liver | 3.43 ± 0.23 | 3.29 ± 0.28 | 3.41 ± 0.57 | 3.80 ± 0.30 | 0.18 |
| TOS-liver | 73.6 ± 15.1 | 70.2 ± 10.8 | 88.1 ± 7.4 | 83.6 ± 9.0 | 0.006 |
| OSI-liver | 21.5 ± 4.3 | 21.4 ± 3.8 | 26.5 ± 5.4 | 22.2 ± 3.7 | 0.095 |
| TAS-kidney | 2.60 ± 0.41 | 2.65 ± 0.40 | 2.13 ± 0.13 a | 2.25 ± 0.14 | 0.001 |
| TOS-kidney | 105.8 ± 12.2 | 105.4 ± 8.8 | 124.2 ± 14.2 | 109.2 ± 9.4 | 0.010 |
| OSI-kidney | 41.4 ± 7.5 | 40.6 ± 7.2 | 58.4 ± 5.9 a | 48.9 ± 5.8 | < 0.001[b] |
| TAS-lung | 2.40 ± 0.20 | 2.43 ± 0.20 | 1.94 ± 0.30 a | 2.21 ± 0.13 | 0.001 |
| TOS-lung | 91.3 ± 11.6 | 92.8 ± 8.1 | 115.5 ± 16.9 a | 97.0 ± 10.8 | 0.003 |
| OSI-lung | 38.4 ± 6.4 | 38.3 ± 4.5 | 60.2 ± 10.2 a | 44.0 ± 5.7 | < 0.001[b] |

Data were given as mean ± SD. *p*: *p*-value of the Kruskal Wallis test. $p < 0.008$ was accepted statistically significant according to the Bonferonni correction after Mann Witney U test. [a]: Statistically significant when compared with sham group. [b]: Statistically significant when compared with CBDL group.

## Discussion

OJ is a complex clinical entity with systemic involvement and elevated surgical morbidity and mortality. There are several challenges in the diagnosis and management of patients with OJ[15]. Among the principal systemic problems encountered in OJ are prolonged wound healing, reticulo-endothelial system dysfunction, renal failure, cardiovascular disorders, and lung failure[16-18].

Recent studies on tissue damage have focused on leukocytes and leukocyte adhesion molecules, as well as their effects involving FORs[19], cytotoxic enzyme release[20], and increased cytokine release[21].

ROS are naturally eliminated by enzymatic and non-enzymatic antioxidants in the body. When ROS are not removed by natural scavengers, damage occurs due to peroxidation of the polyunsaturated fatty acids within the phospholipid structure in the cell membrane. Lipid peroxidation reduces both the fluidity and the barrier function of membranes, leading to disturbances in structural organization, enzymatic inhibition, and even cell death. Moreover, lipid peroxides may inhibit protein synthesis, preclude macrophage function, and alter chemotactic activity[22]. In such cases, the use of antioxidants should bring about a potential benefit by neutralizing ROS[5].

Fruits and vegetables are known to have high antioxidant properties, based on their phenolic contents, which contribute to the removal of free radicals. Among them, pomegranate has a high phenolic content, including anthocyanins, tannin, and ellagitannin, with significant antioxidant and anti-inflammatory effects[23]. In a comparative study[24], anthocyanin, an ingredient of pomegranate fruit extract, has been shown to have a greater antioxidant effect than vitamin E ($\alpha$-tocopherol), ascorbic acid, or $\beta$-carotene.

The treatment of OJ should be started immediately after diagnosis, because even short delays will put the patient at significantly higher risk. Therefore, supportive medical treatment should be given before the surgery in order to preserve the hepatocellular function in OJ. Considering the morbidity and mortality of surgical procedures, pre-operative preparations should be deemed as important as the operation itself. The main goal of pre-operative preparations is to prepare the patient for surgery in optimal conditions, thus to prevent potential complications[15].

In the recent years, several investigators have attempted to disclose the mechanisms behind the beneficial effects of pomegranate. The main focuses of these studies have been the antioxidant, anti-inflammatory, and antibacterial effects of pomegranate[5].

Raised MDA levels are an indicator of lipid peroxidation and membrane disintegration due to oxidative damage[25-27]. In their study, Takayama et al[28] reported that MDA not only reflected the level of ROS, but also showed the severity of liver cell damage. Pomegranate has been shown to significantly decrease MDA levels[29]. In this study, as predicted, administration of pomegranate significantly decreased the MDA levels[5].

The assessment of either oxidant or antioxidant activity can provide information on oxidative stress. However, determination of both, may represent a more complete oxidative stress profile. In the present study, we used TAC for evaluating antioxidant status and TOA for evaluating oxidant status. Moreover, we used OSI to evaluate oxidative stress as an indicator of both TAC and TOA[30].

Although hepatic parenchymal injury has been demonstrated in OJ, the underlying mechanism of the hepatic sinusoidal endothelial cell injury remains unclear[31] Bile duct obstruction leads to significant increases in the lipid peroxidation, resulting in elevated ROS and reduced antioxidant enzyme activity. The culprit may be the enhanced production of reactive oxygen intermediates by way of augmenting lipid peroxidation in OJ, which has been implicated in hepatic injury[5].

Pomegranate juice consumption has been found to reduce hepatic oxidative stress in mice[17], and pomegranate seed extract is known to exert protective effects against cisplatin hepatotoxicity in rabbits[6]. Multiple organ failure can result from uncontrolled inflammatory response and raised FOR[17,32].

The activated inflammatory cells accumulate in the interstitial spaces of the lungs and the small airways, leading to raised FOR, development of acute pulmonary damage, and acute respiratory stress syndrome[33,34]. Pomegranate inhibits ROS-dependent luminol-amplified chemiluminescence in both resting and stimulated neutrophils, and markedly inhibits MPO activity, while PGE attenuates LPS-induced lung inflammation in mice[5].

Pomegranate prevents kidney damage by reducing oxidative stress[35]. Moreover, pomegranate protects the kidney from carbon tetrachloride-induced toxicity. Therefore, it may have a protective effect[36].

## Conclusions

The consumption of pomegranate extract ameliorated the effect of oxidative stress in tissues damaged by OJ in a rat model in our study. Pomegranate also showed beneficial effects with regard to prevention of remote organ damage after OJ. However, these protective effects of pomegranate should be further investigated in detail by future studies.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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