Arrival Date: 08.05.2023 | **Published Date:** 30.07.2023 | **Vol:** 8, **Issue:** 3 | **pp:** 504-512 | **Doi Number:** http://doi.org/10.5281/zenodo.8205274

THE EFFECTS ON POMEGRANATE SEED OIL IN LIPOPOLYSACCHARIDE - INDUCED UVEITIS IN RATS

SIÇANLARDA LİPOPOLİSAKKARİT İLE OLUŞTURULAN ÜVEİT ÜZERİNE NAR ÇEKİRDEĞİ YAĞININ ETKİLERİ

Tugba Nurcan YUKSEL[1], Muhammed YAYLA[2], Erdem TOKTAY[3], Duygu KOSE[4], Zekai HALICI[5]

[1] Tekirdağ Namık Kemal University, Faculty of Medicine, Tekirdağ, Turkey
[2] Kafkas University, Faculty of Medicine, Kars, Turkey
[3] Kafkas University, Medical Faculty, Kars, Turkey
[4] Sutcu İmam University, Faculty of Medicine, Kahramanmaraş, Turkey
[5] Ataturk University, Faculty of Medicine, Erzurum, Turkey

ABSTRACT

Purpose: Pomegranate seed oil (PSO) contains antioxidant and anti-inflammatory phenolic compounds such as punicalagin. We aimed to determine the protective effects of PSO on lipopolysaccharide-induced uveitis in rats.

Method: 50 Wistar Albino rats were randomly divided into five groups. Each group had two subgroups: the 3rd and 24th hour (n=5). Group healthy received intraperitoneal normal saline, group uveitis received subcutaneous 200 µg/kg lipopolysaccharide (LPS), group dexamethasone received 200µg/kg LPS plus 1mg/kg dexamethasone intraperitoneal, group PSO 0.5 received 200µg/kg LPS plus 0.5 mg/kg PSO, and Group PSO 1 received 200µg/kg LPS plus 1 mg/kg PSO. Each group had two subgroups: the 3rd and 24th hour. At the end of the experiment, eye tissues were removed for biochemically (Glutathione, Superoxide dismutase and Malondialdehyde levels) and histopathologically (staining with Harris Hematoxylin and Eosin Y) investigation.

Results: PSO administration significantly ameliorated modifications in uveitis-induced oxidative stress factors. PSO administration eliminated histopathological changes in the eye tissues, including inflammatory findings, in comparison to the uveitis group. However, all these results were not as strong as those of the healthy group.

Conclusion: Future studies may be conducted with different doses of pomegranate seed oil to determine its protective effects against uveitis. Perhaps, PSO, with its antioxidant and anti-inflammatory effects, in addition to its natural content, may play an important role in the production of natural preventive drugs for uveitis.

Keywords: Anti-Inflammatory, Anti-Oxidant, Pomegranate Seed Oil, Punicalagin, Uveitis

ÖZET

Amaç: Nar çekirdeği yağı (NÇY), punikalagin gibi antioksidan ve antiinflamatuar özelliklere sahip fenolik bileşikler içerir. Bu çalışmada deneysel lipopolisakkarit (LPS) kaynaklı üveit modelinde NÇY'nin koruyucu etkilerini değerlendirmeyi amaçladık.

Yöntem: 50 Wistar Albino sıçan rastgele beş gruba ayrıldı. Her grup 3. ve 24. saat olmak üzere iki alt gruba ayrıldı (n=5). Sağlıklı grup intraperitoneal normal salin, üveit grubu subkutan 200 µg/kg LPS, dexamethasone grubu 200µg/kg LPS ile birlikte intraperitoneal 1mg/kg deksametazon, NÇY 0.5 grubu 200µg/kg LPS ile birlikte 0,5 mg/kg NÇY ve NÇY 1 grubu 200µg/kg LPS ile birlikte 1 mg/kg NÇY aldı.. Deneyin sonunda göz dokuları toplandı ve biyokimyasal (Glutatyon, Süperoksit distmütaz ve Malondialdehit seviyeleri) ve histopatolojik (Harris Hematoksilen ve Eosin Y ile boyama) olarak incelendi.

Bulgular: NÇY uygulaması, üveitin sebep olduğu oksidatif stres faktörlerindeki değişiklikleri önemli ölçüde iyileştirdi. NÇY uygulaması üveit grubuna kıyasla göz dokularında inflamasyon bulguları dahil olmak üzere histopatolojik değişiklikleri ortadan kaldırdı. Ancak, tüm bu sonuçlar sağlıklı grubunki kadar güçlü değildi.

Sonuç: Üveite karşı koruyucu etkilerini belirlemek için gelecekte nar çekirdeği yağının farklı dozları ile çalışmalar yapılabilir. Belki de NÇY, doğal içeriğinin yanısıra antioksidan ve antienflamatuar etkileri ile üveit için doğal koruyucu ilaçların üretilmesinde önemli bir rol oynayabilir.

Anahtar Kelimeler: Anti-inflamatuvar, Antioksidant, Nar Çekirdeği Yağı, Punikalagin, Üveit

Sorumlu Yazar / Corresponding Author: Tugba Nurcan YUKSEL, Asst. Prof., Namık Kemal University, Faculty of Medicine, Department of Pharmacology, Tekirdağ, Turkey **E-mail:** tnyuksel@nku.edu.tr

Bu makaleye atıf yapmak için / Cite this article: Yuksel, T.N., Yayla, M., Toktay, E., Kose, D., & Halıcı, Z. (2023). The Effects on Pomegranate Seed Oil in Lipopolysaccharide - Induced Uveitis in Rats. *Gevher Nesibe Journal of Medical & Health Sciences*, 8(3), 504-512. http://doi.org/10.5281/zenodo.8205274

** This study was presented orally at the 6. International Gevher Nesibe Health Sciences Conference 13-15 November, 2020 congress.*

INTRODUCTION

Uveitis, the most common form of intraocular inflammation (Seve et al., 2017), is characterized as inflammation of the eye's vascular uveal tract (Jabs et al., 2005). Uveitis is prevalent in worldwide and can result in total blindness (Smith et al., 2009). Uveitis is believed to be a result of infections, autoimmune disorders, toxins, and a variety of other unidentified variables, but the etiologic and pathological mechanism of uveitis is still exactly not illuminated (Yadav and Ramana, 2019). However, multiple inflammatory genes' expression patterns change, and intracellular signaling cascades are activated due to significantly increased levels of cytokines and chemokines in ocular tissues (Curnow and Murray, 2006; Kim and Moudgil, 2008; Sijssens et al., 2008). Moreover, oxidative stress mechanisms are considered to play an important role in uveitis (Turk et al., 2014) Corticosteroids and immunosuppressive drugs are among the standard treatment strategies, but they cause unwanted side effects in long-term use (Imrie and Dick, 2007; Jaffe et al., 2016; Sen et al., 2014). For these reasons, it is necessary to investigate new and effective agents to prevent the occurrence of uveitis.

Lipopolysaccharide-induced uveitis (LIU), an acute model of inflammation and oxidative stress (Smith et al., 1998), is induced by the injection of the Lipopolysaccharide (LPS) of a Gram-negative bacterial cell wall (Shoeb et al., 2018). The existence of studies showing that increased expression of cytokines such as TNF-α is concurrent with maximum LIU (Devos et al., 1994; Planck et al., 1994) suggests that cytokine levels can be used as markers to monitor inflammation and the course of the disease, and to elucidate the insoluble mechanism of LIU (Zhang et al., 2017).

Pomegranate is part of the Punicaceae family and the Punica genus (Da Silva et al., 2013). Pomegranate originating in the Middle East has been traditionally used as a medicinal fruit (Adams et al., 2006). Pomegranate consists of the edible part 78% juice and 22% seed (Seeram et al., 2005). Pomegranate seeds account for approximately 3% of the total weight of the fruit and contain approximately 12-20% seed oil (Lansky and Newman, 2007). Pomegranate seed oil (PSO) is rich in phenolic compounds including punicalagins and punicic acid and significant fatty acids, including elagic acid, gallic acid and linoleic acid (Gil et al., 2000; Xu et al., 2005). The high amount of punicic acid and punicalagins in PSO ensures many useful biological impacts, including antioxidant anti-inflammatory, anticancer, anti-apoptotic, and so on (Aviram and Rosenblat, 2012; Viladomiu et al., 2013). Also, there are several studies showing that PSO has high levels of strong anti-inflammatory (Boussetta et al., 2009; Harzallah et al., 2016) and antioxidants properties (Kaseke et al., 2020; Shrivas et al., 2023). However, there is no study in the literature about PSO and protective effects on uveitis.

Based on all this information, in this study, we aim to investigate the protective effects of PSO on LIU in rats with biochemical and histopathological methods.

MATERIALS AND METHODS

Animals

For this study, 50 female Wistar Albino rats (4-6 months / 300-330gr) were provided by the Atatürk University Medical Experimental Research Center. Animals weighing 300 to 330 g were randomly divided into 5 groups. The experiments were performed under normal temperature conditions (22 °C) and according to international guidelines for animal experiments. This study and all its protocols were approved by Atatürk University Animal Experiments Local Ethics Committee on 28.02.2020 with document number 75296309-050.01.04-E.2000069533. The rats were placed in plastic cages with sawdust bedding as standard. During the experiment, the animals were kept under controlled light conditions (12 h light/dark cycle) with air-conditioning. Standard rat chow and tap water were provided ad libitum.

Chemicals

LPS (E. coli O111:B4), which is derived from the cell wall of most Gram-negative bacteria, was purchased from Sigma-Aldrich, USA. Thiopental sodium was purchased from Ibrahim Ethem ULAGAY AS (Istanbul, Turkey) and all other chemicals for the laboratory experiments were purchased from Sigma and Merck (Germany).

Preparation of pomegranate seed oil extract

Plant essential oils were extracted using a Clevenger (Wisd-Wise Therm) device and water vapor distillation. The fruits were dried for this purpose, after which the seeds were separated. A total of 166 g of the plant were crushed in the shredder. 1600 ml of distilled water was added to the sample in a glass

balloon before it was put in a Clevenger apparatus and put to use. It was permitted to stand for three hours after the evaporation began. The hydrosol that had accumulated in the Clevengerin collection tube was taken during this time and placed in a clean, separate bottle. The remaining volatile oil was kept in the dark bottles and refrigerated at +4 °C until it was used in the experiment after the last hydrosol was collected in the collection tube.

Experimental Design

Fifty rats were randomly split into five groups. Each group had two subgroups: the 3rd and 24th hour (n=5).

Table 1. Group Names and Experimental Design to Investigate Effects of Pomegranate Seed Oil on Lipopolysaccharide-Induced Uveitis in Rats

Group	Group Code (n=5 for each)	Administration	Time to end the experiment
1	Healthy-3rd h	Only intraperitoneally normal saline	3 h after LPS administration
2	Uveit-3rd h	200 µg/kg LPS	
3	DEX-3rd h	200 µg/kg LPS+1 mg/kg DEX	
4	PSO 0.5-3rd h	200 µg/kg LPS+0.5mg/kg PSO	
5	PSO 1-3rd h	200 µg/kg LPS+1mg/kg PSO	
6	Healthy-24th h	Only ip normal saline	
7	Uveit-24th h	200 µg/kg LPS	24 h after LPS administration
8	DEX-24th h	200 µg/kg LPS+1 mg/kg DEX	
9	PSO 0.5-24th h	200 µg/kg LPS+0.5 mg/kg PSO	
10	PSO 1-24th h	200 µg/kg LPS+1 mg/kg PSO	

Dex: Dexamethasone, LPS: Lipopolysaccharide, PSO: Pomegranate seed oil

Lipopolysaccharide - induced uveitis and treatments

LIU model was established by a single subcutaneous (sc) injection of 200 µg/kg LPS (Keles et al., 2014; Yadav et al., 2009). 1 mg/kg Dexamethasone (DEX) was administered intraperitoneally half an hour before the LPS injection, simultaneously as the LPS injection, and 30 min after the LPS injection (Keles et al., 2014). Two different PSO doses (0.5mg/kg and 1 mg/kg) (Bachagol et al., 2018; Yayla et al., 2018) were each administrated to its respective group orally for 7 days before LPS injection. The maximum inflammatory response in the LIU model is achieved 24th h after the LPS injection (Chang et al., 2008; Rosenbaum et al., 1980). The eye tissues were collected at two time points, 3 and 24 h after LPS injection. Eye tissues stored at -80 °C for biochemical analysis and in a 10% formalin solution for histopathological analysis (Keles et al., 2014).

Biochemical Analysis

100 mg of all specimens reserved for biochemical investigations was treated with 1 ml of PBS, ground in liquid nitrogen with a Tissue Lyser II (Qiagen), and centrifuged. After the grinding process, all eye samples were centrifuged at the relevant speed according to the measurement protocol in the kits and the supernatant portions were taken. Supernatant portion of each tissue was studied in 3 replicates and the level of oxidant-antioxidant parameters were determined by Elisa-Plate Reader Bio Tek EL x 800 and Elisa-plate washer Bio Tek EL x 508 according to kit protocols. Glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) levels were measured manually and expressed as nmol/mg protein for GSH, U/mg protein for SOD and MDA similar to the previous studies (Ugan and Un, 2020). The Lowry method was used to manually calculate total protein amounts (Lowry et al., 1951). Each sample's mean absorbances and the standard curve were computed. All data was presented as mean±standard deviation (SD) for each mg of protein.

Histopathological Analysis

Pretreatment procedures such as solution preparation, tissue tracking, section preparation, and staining were carried out in accordance with our previous research (Palabiyik et al., 2016; Tatar et al., 2016).

Hematoxylin and Eosin Staining Process: Staining with Hematoxylin and Eosin: After the staining pretreatment, three minutes were spent staining with Harris hematoxylin paint. At the end of

the painting process, right eye tissue samples were washed in running water to remove excess paint for 5 minutes. Right eye tissue samples were kept in Eosin Y solution for 2 minutes for counter staining. For counterstaining, right eye tissue samples were immersed in Eosin Y solution for 2 minutes. Following five minutes in 96% alcohol to remove excess paint, the slides were immersed in 3% alcohol and three series of xylene for two minutes each. Entellan bonding balm was then used to cover tissue surfaces with lamella. Light microscope was used to examine stromal lamellar detachments, edema and dilatation areas, polymorphonuclear leukocyte aggregations and infiltrations in eye tissues for histopathological examinations. At least 5 areas in each tissue slide were evaluated at 100 enlargement.

Statistical Analysis

Data are expressed as means∓standard deviation (SD). The findings obtained were evaluated statistically using the APSV 19.00 package program. Statistical differences between groups were determined one-way analysis of variance test (ANOVA). $p<0.05$ was accepted as statistically significant.

RESULTS

Biochemical Results

In the present study, in order to evaluate the effect of PSO in the LIU model in rats, the antioxidant (the levels of GSH and SOD) and oxidant (the levels of MDA) parameters among different doses of PSO were measured at the 3rd and 24th hours (Fig. 2-4). Groups of each watch were compared among themselves. '' * '' was used to compare with uveit group. ''+'' was used to compare with healthy group. * : p<0,05 , ** : p<0,01 *** : p<0,001 , + : p<0,05 , ++ : p<0,01 , +++ : p<0,001

As showed in Fig 1, there were significantly decreased GSH and SOD level (p<0,01) , whereas increased MDA level in the uveit model group with LPS alone applicated compared to the healthy group (p<0,001). These results show us that our model has been formed. On the other hand, the alteration in antioxidant and oxidant parameters stimulated by uveitis were fixed with application of PSO in a dose-dependent manner. The application of PSO at dose 0.5 and 1 mg/kg were raised in GSH and SOD levels, and decreased in MDA level compared to the uveit model group with LPS alone applicated. However, it was seen that changes in GSH and SOD levels after application of PSO were not close to those of the healthy group (p>0,05), while MDA levels were similar to the healthy group (p<0,001).

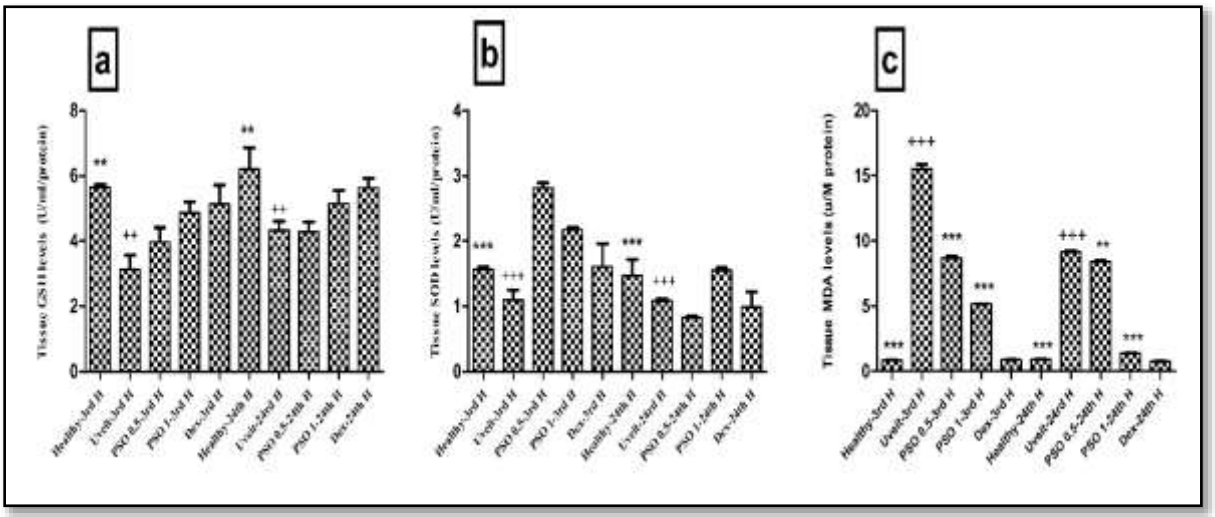

Figure 1. The biochemical results of the effects of PSO on LIU
a. MDA levels (nmol/mg protein.); **b.** GSH levels (nmol/mg protein) * comparison with the uveitis group, $^{+}$: comparison with the healthy group. Dex: Dexamethasone; GSH: Glutathione; LIU: Lipopolysaccharide-induced uveitis; MDA: Malondialdehyde; PSO: Pomegranate seed oil; SOD: Superoxide dismutase.

Histopathology findings

In this study, right eye tissues of rats were stained with Hematoxylin and eosin staining by applying two different doses of PSO in order to evaluate the effect of PSO in the LIU model in rats and were investigated histopathological findings of corneal stromal lamella separation, edema, polymorphonuclear leukocyte aggregation and infiltration.

In healthy group, normal appearing iris structure were observed. There was no sign of inflammation or edema in the connective tissue **(Fig 1-A).** When the cornea was examined, neatly ordered and tightly packed collagen fibril lamellae were observed in the stroma layer **(Fig 1-B).** Any pathological findings was not found in the histopathological appearance of this group.

In the uveit model group with LPS alone applicated, significant dilatations in vascular structures within the connective tissue, polymorphonuclear leukocytes (PMNL) aggregations in the vessel, and lymphocyte and PMNL infiltrations entering into the tissue were observed in the iris **(Fig 1-C).** The structures of the collagen fibril lamellae in the stroma showed advanced separations when the cornea was examined **(Fig 1-D).**

In the Dex group, in the iris, small vascular dilatations and PMNL aggregation were seen. **(Fig 1-E).** Also, when the cornea was examined, in the stroma, separations between collagen fibril lamellae were rarely seen. This group's histopathological appearance was comparable to that of the groups that were healthy **(Fig 1-F).**

In the PSO-0.5 group, while vascular dilatations and PMNL aggregation in the vessel were observed in the iris, these findings were less than in the uveit group **(Fig 1-G).** Separations in the collagen fibril lamellae structures in the stroma were also seen when the cornea was examined, though they were less obvious and less certain than in the uveit group **(Fig 1-H).** In the PSO-1 group, while vascular dilatations and PMNL aggregation in the vessel were observed in the iris **(Fig 1-I).** In the corneal stroma, separations were observed in the collagen fibril lamellae structures **(Fig 1-J).**

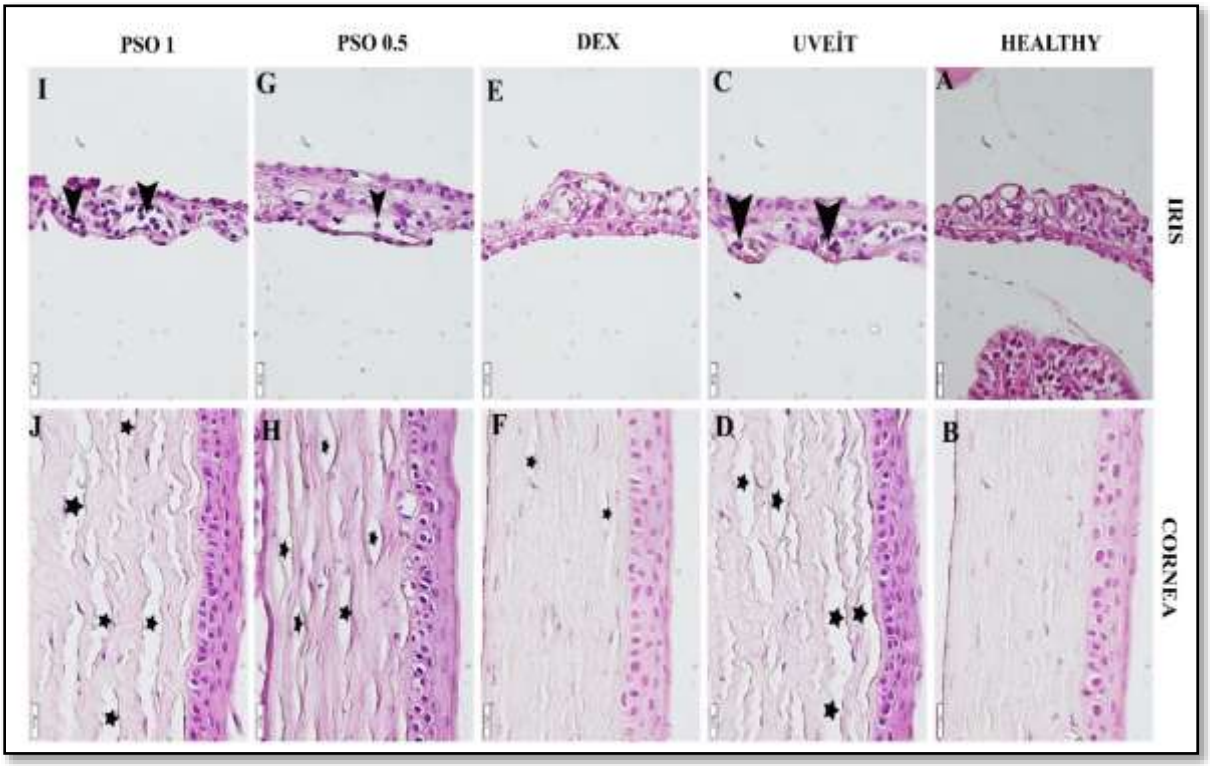

Figure 2. Pathologic changes: Hematoxylin-eosin staining findings of the effects of PSO in eye tissue on LIU Dex: Dexamethasone; LIU: Lipopolysaccharide-induced uveitis; PSO: Pomegranate seed oil; (Star: Stromal lamellae separations, Arrow heads: Dilatation and polymorphonuclear leukocytes)

DISCUSSION

Uveitis that usually causes destructive effects on ocular tissues and visual functions is a complex disease (Dunn, 2015). Due to its complex etiology and multiple disease recurrences, uveitis also causes many ocular comorbidities including cataracts, cystoid macular edema and glaucomatous optic neuropathy (Dick et al., 2016; Patel et al., 2016). The main purpose in the treatment of uveitis is to control inflammation and preserve the visual function of the eye. Preserving the visual function of the eye and controlling inflammation are the main treatment strategies for uveitis (Durrani et al., 2004; Yanai et al., 2014). Pomegranate seed oil (PSO) has a wide range of applications, including as a food ingredient, lubricant, fuel, and additive for paint formulations. Nevertheless, it is frequently regarded as a waste

product. Because of their high concentration of hydrophilic and lipophilic bioactive components, they have a wide range of nutritional, pharmaceutical, and cosmetic applications, seed oils in general have attracted growing interest in recent years (Straccia et al., 2012). Researchers are interested in natural products as a potential source of medicinal compounds because of their popularity, ease of use, low risk of side effects, and safety (Boroushaki et al., 2016). With this information in mind, the effects of PSO in the LIU model were evaluated biochemically and histopathologically.

Oxidative stress is a factor in the development of uveitis complications (Yadav et al., 2011). Previous studies demonstrate that oxidative stress, one of the indicators that is crucial in the uveitis pathogenesis, rises in the LIU model (Satici et al., 2003). Therefore, modifications in oxidative stress indicators are crucial for inflammatory eye conditions and for the ongoing monitoring of the illness. GSH plays a critical role in the endogenous defense system against oxygen free radicals. SOD is an essential antioxidant enzyme and an oxygen free radical scavenger in the body, and MDA is a crucial product of lipid peroxidation (Aboutaleb et al., 2019). Based on this knowledge, the effects of PSO in LIU model were investigated oxidative stress parameters including GSH, SOD and MDA. The current findings showed that the uveitis group's MDA level increased while GSH and SOD levels decreased. Previous studies have shown that after uveitis, GSH (Zhang et al., 2013) and SOD (Chesnokova et al., 2014) level is decreased and MDA level is increased (Turk et al., 2014) in uveitis. Merida et al. demonstrated that markers of oxidative stress significantly changed in a rat model of LIU (Merida et al., 2018). In fact, in our previous studies, we showed that oxidant parameters increased and antioxidant parameters decreased due to uveite (Yuksel et al., 2023). Although not as effective a corrected as that of the healthy group, GSH and SOD levels important raised in the uveitis plus PSO groups. More importantly, in the uveitis plus PSO groups, MDA levels important decreased. The final byproduct of membrane proteins degrading under oxidative stress is MDA. MDA has been used as a gold standard in experimental studies as an indicator of oxidative damage. Yayla et al. demonstrated that PSO improves oxidative stress markers including GSH and MDA in rats with ovarian ischemia/reperfusion injury (Yayla et al., 2018). Białek et al. reported that PSO modulated MDA levels in the cardio-oncological rat model research (Bialek et al., 2021). Furthermore, earlier research showed that taking PSO significantly reduced oxidative stress (Hosseini et al., 2022; Shaban et al., 2022). Consistent with other findings reported in the literature, our result suggests that PSO treatment reduces oxidant parameter generation while increasing anti-oxidant parameter generation. These PSO effects appeared to be linked to oxidative stress inhibition and, to a lesser extent, inflammatory responses.

Uveitis has been linked to serious histological changes in the eye tissue. Previous research found that uveitis caused histopathological damage to the eye tissue, including the cornea (Shi et al., 2019), iris (Lu et al., 2022). The eye tissues of rats after LPS and PSO treatment were examined histopathologically in the current study to determine the potential medicinal importance of PSO in uveitis. The uveitis group's eye tissues had widespread histopathological injury areas. Although not as effective a corrected as that of the healthy group, PSO administration fixed histopathological injury resulting from LIU in eye tissues.

CONCLUSION

PSO with its antioxidant and anti-inflammatory effects, in addition to its natural content, may play an important role in the production of natural preventive drugs for uveitis. However, 0.5mg/kg and 1mg/kg doses of PSO did not have a protective effect against LIU in the rats. Future studies may be conducted with different doses of pomegranate seed oil to determine its protective effects against uveitis.

Acknowledgement

Thank you to Tekirdag Namik Kemal University, Faculty of Medicine, Dean's Office.

Author Contributions

Plan, design: TNY, MY and ZH; **Material, methods and data collection**: TNY, MY, ET and DK; **Data analysis and comments:** TNY, MY and ZH; **Writing and corrections:** TNY, MY, ET, DK and ZH.

Conflict of interest
We declare that there is no conflict of interest in this study.

Funding
This study was not financially supported.

REFERENCES

Aboutaleb, N., Jamali, H., Abolhasani, M., & Pazoki Toroudi, H. (2019). Lavender oil (Lavandula angustifolia) attenuates renal ischemia/reperfusion injury in rats through suppression of inflammation, oxidative stress and apoptosis. Biomed Pharmacother, 110, 9-19. doi:10.1016/j.biopha.2018.11.045

Adams, L. S., Seeram, N. P., Aggarwal, B. B., Takada, Y., Sand, D., & Heber, D. (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J Agric Food Chem, 54(3), 980-985. doi:10.1021/jf052005r

Aviram, M., & Rosenblat, M. (2012). Pomegranate Protection against Cardiovascular Diseases. Evid Based Complement Alternat Med, 2012, 382763. doi:10.1155/2012/382763

Bachagol, D., Joseph, G. S., Ellur, G., Patel, K., Aruna, P., Mittal, M., . . . Sharan, K. (2018). Stimulation of liver IGF-1 expression promotes peak bone mass achievement in growing rats: a study with pomegranate seed oil. Journal of Nutritional Biochemistry, 52, 18-26. doi:10.1016/j.jnutbio.2017.09.023

Bialek, A., Bialek, M., Lepionka, T., Pachniewicz, P., & Czauderna, M. (2021). Oxysterols and lipidomic profile of myocardium of rats supplemented with pomegranate seed oil and/or bitter melon aqueous extract - Cardio-oncological animal model research. Chem Phys Lipids, 235, 105057. doi:10.1016/j.chemphyslip.2021.105057

Boroushaki, M. T., Mollazadeh, H., & Afshari, A. R. (2016). Pomegranate seed oil: A comprehensive review on its therapeutic effects. Int J Pharm Sci Res, 7(2), 430.

Boussetta, T., Raad, H., Letteron, P., Gougerot-Pocidalo, M. A., Marie, J. C., Driss, F., & El-Benna, J. (2009). Punicic acid a conjugated linolenic acid inhibits TNFalpha-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. PLoS One, 4(7), e6458. doi:10.1371/journal.pone.0006458

Chang, Y. H., Horng, C. T., Chen, Y. H., Chen, P. L., Chen, C. L., Liang, C. M., . . . Chen, J. T. (2008). Inhibitory effects of glucosamine on endotoxin-induced uveitis in Lewis rats. Invest Ophthalmol Vis Sci, 49(12), 5441-5449. doi:10.1167/iovs.08-1784

Chesnokova, N. B., Neroev, V. V., Beznos, O. V., Beishenova, G. A., Nikol'skaia, II, Kost, O. A., . . . Shekhter, A. B. (2014). [Oxidative stress in uveitis and its correction with superoxide dismutase antioxidative enzyme (experimental study)]. Vestn Oftalmol, 130(5), 30-34. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25711059

Curnow, S. J., & Murray, P. I. (2006). Inflammatory mediators of uveitis: cytokines and chemokines. Current Opinion in Ophthalmology, 17(6), 532-537. doi:DOI 10.1097/ICU.0b013e32801094b5

Da Silva, J. A. T., Rana, T. S., Narzary, D., Verma, N., Meshram, D. T., & Ranade, S. A. (2013). Pomegranate biology and biotechnology: A review. Scientia Horticulturae, 160, 85-107. doi:10.1016/j.scienta.2013.05.017

Devos, A. F., Vanharen, M. A. C., Verhagen, C., Hoekzema, R., & Kijlstra, A. (1994). Kinetics of Intraocular Tumor-Necrosis-Factor and Interleukin-6 in Endotoxin-Induced Uveitis in the Rat. Investigative Ophthalmology & Visual Science, 35(3), 1100-1106. Retrieved from <Go to ISI>://WOS:A1994MZ26100042

Dick, A. D., Tundia, N., Sorg, R., Zhao, C., Chao, J., Joshi, A., & Skup, M. (2016). Risk of Ocular Complications in Patients with Noninfectious Intermediate Uveitis, Posterior Uveitis, or Panuveitis. Ophthalmology, 123(3), 655-662. doi:10.1016/j.ophtha.2015.10.028

Dunn, J. P. (2015). Uveitis. Prim Care, 42(3), 305-323. doi:10.1016/j.pop.2015.05.003

Durrani, O. M., Tehrani, N. N., Marr, J. E., Moradi, P., Stavrou, P., & Murray, P. I. (2004). Degree, duration, and causes of visual loss in uveitis. Br J Ophthalmol, 88(9), 1159-1162. doi:10.1136/bjo.2003.037226

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry, 48(10), 4581-4589. doi:10.1021/jf000404a

Harzallah, A., Hammami, M., Kepczynska, M. A., Hislop, D. C., Arch, J. R., Cawthorne, M. A., & Zaibi, M. S. (2016). Comparison of potential preventive effects of pomegranate flower, peel and seed oil on insulin resistance and inflammation in high-fat and high-sucrose diet-induced obesity mice model. Arch Physiol Biochem, 122(2), 75-87. doi:10.3109/13813455.2016.1148053

Hosseini, A., Rajabian, A., Forouzanfar, F., Farzadnia, M., & Boroushaki, M. T. (2022). Pomegranate seed oil protects against tacrolimus-induced toxicity in the heart and kidney by modulation of oxidative stress in rats. Avicenna J Phytomed, 12(4), 439-448. doi:10.22038/AJP.2022.19703

Imrie, F. R., & Dick, A. D. (2007). Biologics in the treatment of uveitis. Curr Opin Ophthalmol, 18(6), 481-486. doi:10.1097/ICU.0b013e3282f03d42

Jabs, D. A., Nussenblatt, R. B., Rosenbaum, J. T., & Standardization of Uveitis Nomenclature Working, G. (2005). Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol, 140(3), 509-516. doi:10.1016/j.ajo.2005.03.057

Jaffe, G. J., Dick, A. D., Brezin, A. P., Nguyen, Q. D., Thorne, J. E., Kestelyn, P., . . . Suhler, E. B. (2016). Adalimumab in Patients with Active Noninfectious Uveitis. N Engl J Med, 375(10), 932-943. doi:10.1056/NEJMoa1509852

Kaseke, T., Opara, U. L., & Fawole, O. A. (2020). Effect of Microwave Pretreatment of Seeds on the Quality and Antioxidant Capacity of Pomegranate Seed Oil. Foods, 9(9). doi:10.3390/foods9091287

Keles, S., Halici, Z., Atmaca, H. T., Yayla, M., Yildirim, K., Ekinci, M., . . . Bayraktutan, Z. (2014). The ocular endothelin system: a novel target for the treatment of endotoxin-induced uveitis with bosentan. Invest Ophthalmol Vis Sci, 55(6), 3517-3524. doi:10.1167/iovs.14-14193

Kim, E. Y., & Moudgil, K. D. (2008). Regulation of autoimmune inflammation by pro-inflammatory cytokines. Immunol Lett, 120(1-2), 1-5. doi:10.1016/j.imlet.2008.07.008

Lansky, E. P., & Newman, R. A. (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnopharmacology, 109(2), 177-206. doi:10.1016/j.jep.2006.09.006

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem, 193(1), 265-275. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14907713

Lu, H., Chen, D. D., & Zhu, S. Q. (2022). Therapeutic potential of pupilloplasty combined with phacomulsification and intraocular lens implantation against uveitis-induced cataract. Int J Ophthalmol, 15(10), 1598-1603. doi:10.18240/ijo.2022.10.06

Merida, S., Sancho-Tello, M., Almansa, I., Desco, C., Peris, C., Moreno, M. L., . . . Bosch-Morell, F. (2018). Bevacizumab Diminishes Inflammation in an Acute Endotoxin-Induced Uveitis Model. Front Pharmacol, 9, 649. doi:10.3389/fphar.2018.00649

Palabiyik, S. S., Karakus, E., Akpinar, E., Halici, Z., Bayir, Y., Yayla, M., & Kose, D. (2016). The Role of Urotensin Receptors in the Paracetamol-Induced Hepatotoxicity Model in Mice: Ameliorative Potential of Urotensin II Antagonist. Basic Clin Pharmacol Toxicol, 118(2), 150-159. doi:10.1111/bcpt.12447

Patel, A. K., Newcomb, C. W., Liesegang, T. L., Pujari, S. S., Suhler, E. B., Thorne, J. E., . . . Systemic Immunosuppressive Therapy for Eye Diseases Research, G. (2016). Risk of Retinal Neovascularization in Cases of Uveitis. Ophthalmology, 123(3), 646-654. doi:10.1016/j.ophtha.2015.10.056

Planck, S. R., Huang, X. N., Robertson, J. E., & Rosenbaum, J. T. (1994). Cytokine Messenger-Rna Levels in Rat Ocular-Tissues after Systemic Endotoxin Treatment. Investigative Ophthalmology & Visual Science, 35(3), 924-930. Retrieved from <Go to ISI>://WOS:A1994MZ26100021

Rosenbaum, J. T., McDevitt, H. O., Guss, R. B., & Egbert, P. R. (1980). Endotoxin-induced uveitis in rats as a model for human disease. Nature, 286(5773), 611-613. doi:10.1038/286611a0

Satici, A., Guzey, M., Gurler, B., Vural, H., & Gurkan, T. (2003). Malondialdehyde and antioxidant enzyme levels in the aqueous humor of rabbits in endotoxin-induced uveitis. Eur J Ophthalmol, 13(9-10), 779-783. doi:10.1177/1120672103013009-1008

Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y. T., Zhang, Y. J., Nair, M. G., & Heber, D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. Journal of Nutritional Biochemistry, 16(6), 360-367. doi:10.1016/j.jnutbio.2005.01.006

Sen, H. N., Vitale, S., Gangaputra, S. S., Nussenblatt, R. B., Liesegang, T. L., Levy-Clarke, G. A., . . . Kempen, J. H. (2014). Periocular corticosteroid injections in uveitis: effects and complications. Ophthalmology, 121(11), 2275-2286. doi:10.1016/j.ophtha.2014.05.021

Seve, P., Cacoub, P., Bodaghi, B., Trad, S., Sellam, J., Bellocq, D., . . . Kodjikian, L. (2017). Uveitis: Diagnostic work-up. A literature review and recommendations from an expert committee. Autoimmun Rev, 16(12), 1254-1264. doi:10.1016/j.autrev.2017.10.010

Shaban, N. Z., Mohammed, A. S., Abu-Serie, M. M., Maher, A. M., & Habashy, N. H. (2022). Inhibition of oxidative stress, IL-13, and WNT/beta-catenin in ovalbumin-sensitized rats by a novel organogel of Punica granatum seed oil saponifiable fraction. Biomed Pharmacother, 154, 113667. doi:10.1016/j.biopha.2022.113667

Shi, L., Hager, T., Fries, F. N., Daas, L., Holbach, L., Hofmann-Rummelt, C., . . . Szentmary, N. (2019). Reactive uveitis, retinal vasculitis and scleritis as ocular end-stage of Acanthamoeba keratitis: a histological study. Int J Ophthalmol, 12(12), 1966-1971. doi:10.18240/ijo.2019.12.20

Shoeb, M., Zhang, M., Xiao, T. L., Syed, M. E., & Ansari, N. H. (2018). Amelioration of Endotoxin-Induced Inflammatory Toxic Response by a Metal Chelator in Rat Eyes. Investigative Ophthalmology & Visual Science, 59(1), 31-38. doi:10.1167/iovs.17-22172

Shrivas, M., Khunt, D., Shrivas, M., & Misra, M. (2023). Studies on pomegranate seed oil enriched galantamine hydrobromide microemulsion: formulation, in vitro antioxidant and neuroprotective potential. Pharm Dev Technol, 28(2), 153-163. doi:10.1080/10837450.2023.2171433

Sijssens, K. M., Rijkers, G. T., Rothova, A., Stilma, J. S., & de Boer, J. H. (2008). Distinct cytokine patterns in the aqueous humor of children, adolescents and adults with uveitis. Ocul Immunol Inflamm, 16(5), 211-216. doi:10.1080/09273940802409969

Smith, J. A., Mackensen, F., Sen, H. N., Leigh, J. F., Watkins, A. S., Pyatetsky, D., . . . Goldstein, D. A. (2009). Epidemiology and course of disease in childhood uveitis. Ophthalmology, 116(8), 1544-1551, 1551 e1541. doi:10.1016/j.ophtha.2009.05.002

Smith, J. R., Hart, P. H., & Williams, K. A. (1998). Basic pathogenic mechanisms operating in experimental models of acute anterior uveitis. Immunol Cell Biol, 76(6), 497-512. doi:10.1046/j.1440-1711.1998.00783.x

Straccia, M. C., Siano, F., Coppola, R., La Cara, F., & Volpe, M. (2012). Extraction and characterization of vegetable oils from cherry seed by different extraction processes. Chemical Engineerin g Transactions, 27, 391-396.

Tatar, A., Yayla, M., Kose, D., Halici, Z., Yoruk, O., & Polat, E. (2016). The role of endothelin-1 and endothelin receptor antagonists in allergic rhinitis inflammation: ovalbumin-induced rat model. Rhinology, 54(3), 266-272. doi:10.4193/Rhin15.059

Turk, A., Aykut, M., Akyol, N., Kola, M., Mentese, A., Sumer, A., . . . Erdol, H. (2014). Serum anti-carbonic anhydrase antibodies and oxidant-antioxidant balance in patients with acute anterior uveitis. Ocul Immunol Inflamm, 22(2), 127-132. doi:10.3109/09273948.2013.830753

Ugan, R. A., & Un, H. (2020). The Protective Roles of Butein on Indomethacin Induced Gastric Ulcer in Mice. Eurasian J Med, 52(3), 265-270. doi:10.5152/eurasianjmed.2020.20022

Viladomiu, M., Hontecillas, R., Lu, P., & Bassaganya-Riera, J. (2013). Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. Evid Based Complement Alternat Med, 2013, 789764. doi:10.1155/2013/789764

Xu, J., Guo, C. J., Yang, J. J., Wei, J. Y., Li, Y. F., Pang, W., . . . Cheng, S. (2005). [Intervention of antioxidant system function of aged rats by giving fruit juices with different antioxidant capacities]. Zhonghua Yu Fang Yi Xue Za Zhi, 39(2), 80-83. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15842822

Yadav, U. C., Kalariya, N. M., & Ramana, K. V. (2011). Emerging role of antioxidants in the protection of uveitis complications. Curr Med Chem, 18(6), 931-942. doi:10.2174/092986711794927694

Yadav, U. C., Subramanyam, S., & Ramana, K. V. (2009). Prevention of endotoxin-induced uveitis in rats by benfotiamine, a lipophilic analogue of vitamin B1. Invest Ophthalmol Vis Sci, 50(5), 2276-2282. doi:10.1167/iovs.08-2816

Yadav, U. C. S., & Ramana, K. V. (2019). Endotoxin-Induced Uveitis in Rodents. Methods Mol Biol, 1960, 161-168. doi:10.1007/978-1-4939-9167-9_14

Yanai, R., Takeda, A., Yoshimura, T., & Sonoda, K. H. (2014). [Pathophysiology and new treatment of uveitis]. Nihon Rinsho Meneki Gakkai Kaishi, 37(2), 74-82. doi:10.2177/jsci.37.74

Yayla, M., Cetin, D., Adali, Y., Kilicle, P. A., & Toktay, E. (2018). Potential therapeutic effect of pomegranate seed oil on ovarian ischemia/reperfusion injury in rats. Iran J Basic Med Sci, 21(12), 1262-1268. doi:10.22038/ijbms.2018.30149.7268

Yuksel, T. N., Yayla, M., Kose, D., Halici, Z., Bozkurt, E., & Toktay, T. (2023). Protective effects of melatonin receptor agonists on endotoxin-induced uveitis in rats. Iran J Basic Med Sci, 26(5), 540-548. doi:10.22038/IJBMS.2023.67297.14749

Zhang, J., Zhao, F. F., Bai, S., Zheng, J., Zheng, P., & Dai, G. D. (2013). [Study on inhibitory effect of lycium pigment on lipopolysaccharide-induced uveitis in rats and its mechanism]. Zhongguo Zhong Yao Za Zhi, 38(11), 1778-1782. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24010295

Zhang, N., Yu, S., Liu, X., & Lu, H. (2017). Low Dose of Lipopolysaccharide Pretreatment Preventing Subsequent Endotoxin-Induced Uveitis Is Associated with PI3K/AKT Pathway. J Immunol Res, 2017, 1273940. doi:10.1155/2017/1273940