Uveitis is a chronic inflammatory eye disease which may be accompanied by some systemic diseases, often considered as idiopathic. LPS-induced inflammation is an experimental animal models of acute uveitis. Propolis is a natural substance collected by honeybees from buds and exudates of certain trees which has antioxidant, antibacterial, antiviral and anti-inflammatory effects [1,2]. The aim of this study is to investigate the effects of propolis on endotoxin induced uveitis (EIU) by immunohistochemical, ultrastructural and biochemical methods.

24 male Wistar albino rats were divided into four groups (n=6). A single dose of LPS (150 µg/kg/ip) was administered to 12 animals intraperitonally (ip). Water extract of propolis (WEP) from Adapazarı region of Turkey (50mg/kg/ip) was administered to 6 of them after LPS injection. Control group was injected with saline ip. After 24 hours, the aqueous humor of both eyes of animals were collected under anesthesia for biochemical analysis of inflammatory markers, namely TNF-α ve HIF-1α levels. The right eyeballs were fixed with formalin and paraffin-embedded for immunohistochemical staining of Nf-kb p65 and left eyeballs were fixed with 4% glutaraldehyde and araldite-embedded for ultrastructural analysis.

Immunoreactivity against anti-NF-κB p65 at corpus ciliare was significantly decreased in EIU group treated with Turkish propolis extract (Figure1-2). Moreover, ELISA analysis of HIF-1α and TNF-α levels in aqueous humour was significantly decreased in same group (Figure 3). Ultrastructural analysis of retinal regions showed that EIU group treated with Turkish propolis extract has less vacuoles and mitochondria degeneration at retinal pigment epithelium (RPE) than EIU group (Figure 4). The intercellular spaces of the inner nuclear layer and outer limiting membrane were compatible with control group; no polymorphonuclear cells were seen in both intravascular and extravascular spaces and no stasis symptoms were detected at the capillary lumen (Figure 4).

Our report is the first study that established anti-inflammatory effect of Adapazarı/Turkish propolis on LPS-induced uveitis rat model. As a marker of inflammation, NF-κB reactivity was increased significantly in corpus ciliare and coroid of eyes of LPS-induced rats, accompanied by TNF-α induction of HIF-1α, whereas the levels were markedly decreased in Propolis treated and LPS-induced group. We think that Adapazarı/Turkish propolis might be a new class of bioavailable dietary supplement for the treatment of inflammatory ophthalmic diseases such as uveitis.

References
Fig. 1: Immunohistochemical staining of Nf-κB p65 in rats. A. Negative control, B. Control group injected with saline, C. 50mg/kg/ip Turkish propolis injected group D. 150 µg/kg/ip LPS group without propolis-treatment, E. LPS group treated with propolis.

Fig. 2: Statistical analysis of H-SCORE results of Nf-κB immunostainings. One-way ANOVA was performed with Tukey-Kramer Multiple Comparisons Test between all groups and *P < 0.0001 is considered extremely significant.

Fig. 3: Statistical analysis of biochemical assay of HIF-1α and TNF-α in aqueous humor of rats. All groups are compared with Tukey-Kramer Multiple Comparisons Test. *P < 0.05 is considered significant compared to control group. #P < 0.01 is considered significant compared to Propolis group.

Fig. 4: Electron micrographs of retina layers. Control group: 1) x3000, 2) x6000, 3) x25000. LPS group: 4) x6000, 5) x5000, 6) x5000. LPS+Propolis group: 7) x5000, 8) x12000. Propolis: 9) x7500, 10) x6000.