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Comparison of the effects of topical corneal inhibitory agents on TTL and PON1 in rats.

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ABSTRACT

The aim of this study was to compare the effects of topical corneal inhibitory agents on total thiol (TTL) and paraoxonase 1 (PON1) levels in rats with experimentally acquired keratoconjunctivitis. Thirty-five rats were divided into five groups. Twenty-four hours prior to the experiment, keratoconjunctivitis was established in the right eye of the rats using sodium hydroxide. The treatments of the five groups were as follows: group I: (control) isotonic saline (0.9%), group II: topical 0.05% cyclosporine A, group III: topical 1% diluted propolis, group IV: topical 3% diluted propolis, and group V: 0.1% dexamethasone. At the end of the 10th day, one rat in each group, except the cyclosporine group (group II), had died. The treatment was applied to all groups three times a day for 10 days. Subsequently, blood samples were obtained and used for determining the levels of TTL and PON1 (Architect C16000). All statistical analyses were performed using IBM SPSS for Windows Version 20.0 software. Descriptive statistics were calculated from the values obtained from this study and shown as arithmetic mean and standard deviation. Kruskal Wallis variance analysis was conducted. p values found to be under 0.05 were accepted as statistically significant. The study was performed after the approval [By The Animal Research Ethics Committee, Bolu Abant Izzet Baysal University, Number: 13.30.2.ABU.0.05.05-050.01.04-1, January.8.2016]. The TTL results were as follows (µmol/L): group I: 253.24, group II: 238.70, group III: 281.39, group IV: 284.80 and group V: 260.65. No marked differences were observed between the control group and the other groups (P>0.05). The PON1 results were as follows (U/L): group I: 521.49, group II: 472.30, group III: 362.37, group IV: 327.48 and group V: 440.31. No marked difference was observed between the control group and the other groups (P > 0.05). However, there was a marked difference in the PON1 results between the 1% and 3% propolis groups. CNV inhibitor agents' effects on TTL and PON1 values were similar.

Keywords: Total thiol, Paraoxonase 1, Corneal neovascularisation, Corneal inhibitory, Propolis

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INTRODUCTION

Numerous studies have been conducted and various agents have been applied in efforts to prevent corneal neovascularisation (CNV). However, no particularly efficient treatment has been established. For the treatment of CNV, the main strategy involves inhibiting angiogenesis. Propolis, collected of or relating to plant sources by honey bee, has been used as a popular natural herbal remedies in folk medicine all the way through the world. [1]. Propolis is a brownish resinous material of waxy consistency collected by bees from the buds of trees and used as a cement in repairing and maintaining the hive.

In recent years, numerous in vivo and in vitro studies have been conducted on propolis. In one experiment, it was shown that the ethanolic extract of propolis had antioxidant, anti-inflammatory and antibacterial effects in cases of keratitis. In addition, an increasing number of studies on the effects of propolis on the cornea have been conducted in the field of complementary medicine [2]. However, the effects of propolis still require further investigation [3, 4].

Another agent used in the treatment of CNV is cyclosporine A. Cyclosporine A isolated from the fungus Tolypocladium inflatum is an 11-amino-acid polypeptide. Recently, it has been increasingly used for treating CNV that develops following penetrating keratoplasty and chemical burns [5, 6]. However, the systemic adverse effects of topical cyclosporine and the effects of topical cyclosporine on total thiol (TTL) and paraoxonase 1 (PON1) levels have not yet been examined.

Corticosteroids are accepted as standard medication in the treatment of CNV and inflammation of the cornea [7,8]. Dexamethasone is an analogue of synthetic corticosteroids and is a strong anti-inflammatory drug. It is used in the treatment of many inflammatory, immunologic and trauma-derived ocular diseases. Steroids act as antiangiogenic agents by inhibiting the synthesis of prostaglandin [9].

Serum PON activity shows wide variations between different population groups and within individuals. The PON enzyme is a glycoprotein with a molecular weight of 44 kDa that combines with certain proteins, such as apo A-I and apo J, found in high-density lipoproteins. In recent years, studies have revealed the correlation of PON1 with many chronic disorders [10].

TTL is becoming increasingly used as an index of oxidative stress. Automated approaches are now generally applied for measuring TTL [11]. This is particularly beneficial given that research has uncovered the correlations of TTL level with cardiac syndrome X, oxidative stress and inflammation [12]. These findings, among others, support the use of TTL in a clinical context, given the suggestion that oxidative stress and inflammation could be closely related to a decrease in TTL level [12].

The effects of topical agents used in the treatment of CNV on TTL and PON1 levels may be associated with various complications. Examining these effects should contribute to clinical practice in the field of ophthalmology and the modification of treatment protocols. Therefore, in this study, TTL and PON1 levels were analysed in rats with keratoconjunctivitis subjected to various treatment protocols. To the best of our knowledge, this research is the first of its kind.

MATERIALS AND METHODS

Subjects

Thirty-five male Wistar rats were used in this study. They were 2 months old and 200–250 grams in weight. They were divided equally and randomly into five treatment groups as follows: group I: (control) isotonic saline (0.9%), group II: topical 0.05% cyclosporine A, group III: topical 1% propolis, group IV: topical 3% propolis and group V: 0.1% dexamethasone. Before any treatment was applied to the rats, they were put under anaesthesia by the intramuscular injection of ketamine HCl (25 mg/kg) and xylazine hydrochloride (5 mg/kg). Then, topical proparacaine HCl (0.5%) was dropped onto the right eye of each rat. Subsequently, sodium hydroxide-saturated paper, 3 mm in diameter, was applied to the central cornea of the right eye of the rats for 20 s. The cornea was then washed with 10 mL of isotonic saline solution. Twenty-four hours after washing, the treatments were applied to the rats with keratoconjunctivitis in the right eye, three times a day

for 10 days. By the end of the 10th day, one rat in each group except the cyclosporine group (group II) had died. Blood samples of the rats were subsequently obtained. The study was performed after the approval [By The Animal Research Ethics Committee, Bolu Abant Izzet Baysal University, Number: 13.30.2.ABU.0.05.05-050.01.04-1, January.8.2016].

Laboratory tests

All the samples were immediately centrifuged at 1500 rpm for 10 minutes to separate the serums. Then, the serums were stored at -80 °C until analysis. On the day of the analysis, the TTL and PON1 levels were examined using a fully automatic analyser (Architect C16000; Abbott Laboratories, Lake Bluff, IL, USA). Test results were determined as µmol/L for TTL and as U/L for PON1.

Statistical analysis

All statistical analyses were performed using IBM SPSS for Windows Version 20.0 software. Descriptive statistics were calculated from the values obtained from this study and shown as arithmetic mean and standard deviation. Kruskal Wallis variance analysis was conducted. p values found to be under 0.05 were accepted as statistically significant. The average values of TTL and PON1 were used to determine whether there were any pronounced differences between the control group and the other groups. The TTL and PON1 data were tested for normality by the Kolmogorov-Smirnov test with the Lilliefors correction applied. To assess the strength of the relationship between PON1 and TTL, Pearson's product-moment correlation coefficient was used. Because there were seven or fewer test subjects in each treatment group, to analyse the correlations among the values, Spearman's correlation analysis, which is a nonparametric test, was used.

RESULTS

The TTL results were as follows (µmol/L): group I: 253.24, group II: 238.70, group III: 281.39, group IV: 284.80, and group V: 260.65. No marked differences were observed between the control group and the other groups **(**P > 0.05). The PON1 results were as follows (U/L): group I: 521.49, group II: 472.30, group III: 362.37, group IV: 327.48, and group V: 440.31. No marked differences were observed between the control group and the other groups **(**P > 0.05). On the other hand, there was a striking difference in the PON1 results between the 1% and 3% propolis groups (Figure 1 & 2) (Table 1 & 2) . Regarding the results of Pearson's correlation analysis, a positive, moderate and significant correlation (r = 0.451, p = 0.011) was detected between the TTL and PON1 values, which were observed to exhibit normal distributions. The correlations between the TTL and PON1 values in each group were investigated using Spearman's correlation analysis. The results were as follows: group II: positive, moderate level, statistically insignificant (r = 0.393, p = 0.383) relationship; group III: positive, high level, statistically insignificant (r = 0.543, p = 0.266) relationship; group IV: negative, low level, statistically insignificant (r = −0.200, p = 0.704) relationship; and group V: positive, high level, statistically insignificant (r = 0.657, p = 0.156) relationship.

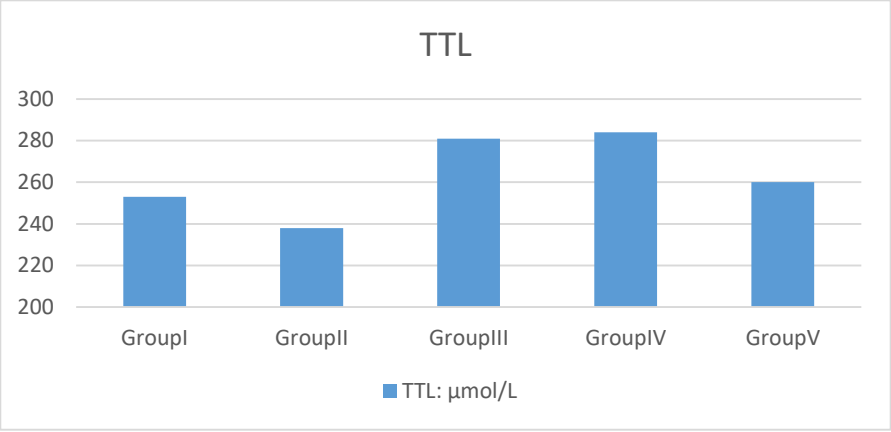

Figure 1. The average values of TTL

The TTL levels of groups III and IV were higher than those of the other groups.

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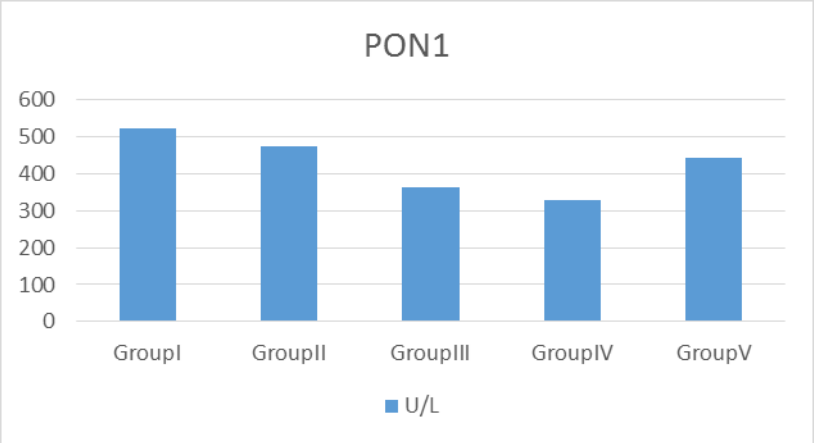

Figure 2. The average values of PON 1

PON1 values of all groups were found to be very close to that of the control group. A statistically significant difference was also observed between the 1% (group III) and 3% diluted propolis (group IV) groups.

Table 1. The average values of TTL

Group I (n=6)	Group II (n=7)	Group III (n=6)	Group IV (n=6)	Group V (n=6)	*P value*
253,24 ± 48,87	238,70 ± 56,96	281,39 ± 81,95	284,80 ± 35,31	260,65 ± 150,49	0,758
Data was shown as (arithmetic mean) ± (standard deviation) respectively					

*** p<0.05 was considered to indicate a statistically significant difference.**

Table 1: The p value, calculated using the arithmetic mean, standard deviation and Kruskal–Wallis one-way analysis of variance of the TTL values upon isotonic saline (0.9%, group I), topical 0.05% cyclosporine A (group II), 1% diluted propolis (group III), 3% diluted propolis (group IV) and 0.1% dexamethasone (group V) applications.

Table 2. The average values of PON 1

Group I (n=6)	Group II (n=7)	Group III (n=6)	Group IV (n=6)	Group V (n=6)	*P value*
521,49 ± 73,16	472,30 ± 101,2	362,37 ± 123,5	327,48 ± 79,44	440,31 ± 205,50	0,095
Data was shown as (arithmetic mean) ± (standard deviation) respectively					

*** p<0.05 was considered to indicate a statistically significant difference.**

Table 2: The p value, calculated using the arithmetic mean, standard deviation and Kruskal–Wallis one-way analysis of variance of the PON 1 values upon isotonic saline (0.9%, group I), topical 0.05% cyclosporine A (group II), 1% diluted propolis (group III), 3% diluted propolis (group IV) and 0.1% dexamethasone (group V) applications.

DISCUSSION

In a study by Altiparmak et al., it was suggested that a decrease in TTL level might be associated with oxidative stress and inflammation [12]. In our study, TTL was found to exhibit to greatest increase in the propolis group among the groups. This suggests the superiority of propolis compared with the other topical agents. In another study, conducted by Hepşen et al. and using rabbits, diluted propolis extract was compared with 1% dexamethasone in terms of angiogenesis inhibitory activity [13]. Although the animal chosen for analysis differed from that in our study, one eye of each animal was similarly damaged to generate keratoconjunctivitis. In that study, 1% topical dexamethasone was found to be less effective than propolis in the treatment of keratoconjunctivitis. Hepşen et al. explained this finding by the fact that propolis has inhibitory effects on cyclooxygenase and lipoxygenase. In another study, Keshavarz et al., demonstrated an association between propolis extract and the inhibition of CNV [14]. In that study, the topical application of

propolis was considered to be beneficial due to its blocking effect on the restoration process and CNV. In contrast, in our study, two different concentrations (1% and 3%) of propolis extract were examined in terms of their effects on PON1 and TTL levels in rats in which keratoconjunctivitis had been experimentally induced in one eye. In addition, in a study by Sonmez et al., recovery from CNV was observed in a case in which 0.05% topical cyclosporine had been applied [15]. This case was monitored for 6 months, with the complete recovery of CNV being observed by 2 months. However, the effectiveness of topical cyclosporine on PON1 and TTL had not been evaluated. Even though 0.05% topical cyclosporine was also used in this study, our study differs from this previous work in terms of the test parameters used. Furthermore, in a study by Nowak et al., PON1 and ceruloplasm activities were investigated in patients with diabetic retinopathy [16]. In that study, a significant decrease in the level of PON1 was identified, which was thought to be associated with microvascular complications of diabetes. In contrast, in our study, no significant difference was observed in the PON1 values upon use of the different topical agents, although a greater decrease in PON1 values was observed for 3% propolis than for 1% propolis.

CONCLUSION

Among the topical corneal inhibitor agents, propolis was found to have the greatest effect on TTL. Hence, it is concluded that the use of propolis extract would be preferable in terms of its effects on TTL level compared with other topical agents. Another notable finding of this study is that the different treatments showed similar efficacy regarding PON1, although the result that diluted propolis was more efficient at 1% than at 3% requires further investigation. Although the effects of CNV inhibitor agents, which were used in our study, on TTL and PON1 values were similar, there is need for further studies on this issue.

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