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Protective Effects of Caffeic Acid Phenethyl Ester Against Carbon Tetrachloride-induced Testicular Damage in Rats: A Histological Study

Sıçanlarda Karbon Tetraklorür ile Oluşturulan Testis Hasarına Karşı Kafeik Asit Fenetil Esterin Koruyucu Etkileri: Histolojik Çalışma

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ABSTRACT

Aim: Carbon tetrachloride (CCl_4) is a volatile organic chemical agent that can cause damage to many tissues. Caffeic acid phenethyl ester (CAPE), which is structurally similar to flavonoids, is an active component of honeybee propolis. CAPE is known for its antitoxic, antioxidant, and anti-inflammatory effects. In this study, we aimed to investigate the effects of CAPE against testicular damage caused by CCl_4.

Materials and Methods: Twenty-eight Wistar albino rats were divided into 4 groups (n=7) as, Group 1: control (5% ethanol, 1 mL/day/ip), Group 2: olive oil (0.5 mL/day over/ip), Group 3: CCl_4 (0.5 mL/kg over/ip), Group 4: CCl_4+CAPE (10 µmol/kg/day/ip). Tissue samples collected at the end of the experiment were detected in 10% formaldehyde and embedded in paraffin. Five-micron-thick sections taken from paraffin blocks were stained with hematoxylin-eosin. To evaluate testicular damage, 100 tubules from each section were randomly examined at 20x magnification under a light microscope and classified as intact, atrophic, and degenerated tubules. Sections were examined by using Leica DFC 280 light microscope and Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd. Cambridge, UK).

Results: The testicular sections of the control group and the olive oil group had a normal histological appearance. In the CCl_4 group, 55.00±4.22% of the seminiferous tubules were intact, 25.00±2.67% were atrophic and 20.00±1.88% were degenerative. In addition, multinucleated giant cells were found in the lumen of some seminiferous tubules. In the CCl_4+CAPE group, 72.14±3.91% of the tubules were intact, 16.42±2.10% were atrophic, and 11.42±2.36% were degenerative. While the number of affected tubules significantly increased in the CCl_4 group compared to the control group ($p<0.05$), the number of affected seminiferous tubules decreased significantly in the CCl_4+CAPE group compared to the CCl 4 group ($p<0.05$).

Conclusion: We think that CAPE may be useful in reducing the damaging effects of CCl_4 on the testicle.

Keywords: CAPE, carbon tetrachloride, rat, testis

ÖZ

Amaç: Karbon tetraklorür (CCl_4) birçok dokuda hasara yol açabilen, uçucu organik bir kimyasal ajandır. Yapıca flavonoidlere benzeyen kafeik asit fenetil ester (CAPE), bal arısı propolisinin aktif bir bileşenidir. CAPE'nin antitoksik, antioksidan, anti-enflamatuvar etkileri olduğu bilinmektedir. Bu çalışmada CCl_4'ün neden olduğu testis hasarına karşı CAPE'in etkilerini incelemeyi amaçladık.

Gereç ve Yöntem: Yirmi sekiz adet Wistar-albino sıçan 4 gruba ayrıldı (n=7). Grup 1: Kontrol (%5 etanol, 1 mL/gün/ip), Grup 2: Zeytinyağı (0,5 mL/gün aşırı/ip), Grup 3: CCl_4 (0,5 mL/kg gün aşırı/ip), Grup 4: CCl_4+CAPE (10 µmol/kg/gün/ip). Deney sonunda alınan doku örnekleri %10'luk formaldehitte tespit edilerek parafine gömüldü. Parafin bloklardan alınan 5 µm kalınlığındaki kesitler hematoksilen-eozin ile boyandı. Testiküler hasarı değerlendirmek için ışık mikroskobunda her kesitten x20 büyütmede rastgele 100 tübül incelenerek sağlam, atrofik ve dejenere tübüller olarak sınıflandırıldı. Kesitler, Leica DFC 280 ışık mikroskobu ve Leica Q Win Görüntü Analiz sistemi (Leica Micros Imaging Solutions Ltd. Cambridge, UK) kullanılarak incelendi.

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Bulgular: Kontrol ve zeytinyağı gruplarına ait testis kesitleri normal histolojik görünümdeydi. CCl_4 grubunda seminifer tubullerin %55,00±4,22'si sağlam, %25,00±2,67'si atrofik ve %20,00±1,88'i dejeneratif olarak gözlendi. Ayrıca, bazı seminer tübüllerin lümeninde multinükleer dev hücrelere rastlandı. CCl_4+CAPE grubunda ise tübüllerin %72,14±3,91'i sağlam, %16,42±2,10'u atrofik ve %11,42±2,36'sı dejeneratifti. CCl_4 grubunda etkilenen tübüllerin sayısı kontrol grubuna göre istatistiksel olarak anlamlı şekilde artarken ($p<0,05$), CCl_4+CAPE grubunda etkilenen seminifer tübüllerin sayısının, CCl_4 grubuna göre istatistiksel olarak anlamlı şekilde azaldığı tespit edildi ($p<0,05$).

Sonuç: CAPE'nin, CCl_4'ün testis üzerindeki hasar verici etkilerinin azaltılmasında faydalı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: CAPE, karbon tetraklorür, sıçan, testis

INTRODUCTION

Xenobiotic carbon tetrachloride (CCl_4) is a colorless liquid with a sweet odor[1]. CCl_4, which is a highly toxic substance with potential carcinogenic effects in humans and animals, is now used extensively in the manufacture of chlorofluorocarbon refrigerants in industry and thus in air conditioning and refrigeration systems. CCl_4 has toxic effects on the kidney, heart, lung, brain, and testicular tissues, especially on the liver[2,3]. Studies have shown that CCl_4 causes spermatogenic cell damage, basal membrane separation, seminiferous tubular atrophy, expansion in the interstitial area, and a decrease in sperm count[4,5].

Caffeic acid phenethyl ester (CAPE), which is structurally similar to flavonoids, is an active component of honeybee propolis[6]. CAPE is known for its antitoxic, antioxidant, anti-inflammatory, antiviral, immunomodulatory, neuroprotective, and cytostatic effects[7,8]. Since it strongly modulates the arachidonic acid cascade compared to other propolis components, its anti-inflammatory effect is more pronounced[6]. It has been shown to block all reactive oxygen species formed by the xanthine dehydrogenase/xanthine oxidase system at a concentration of 10 µmol/L[9].

In this study, we aimed to investigate the histological changes in the testicular tissue of rats with CCl_4-induced and the curative effect of CAPE on these changes by histochemical methods.

MATERIALS AND METHODS

Experimental Animals

The 28 3-month-old male Wistar albino rats weighing 200-250 g, which were used in our study, were obtained from İnönü University Experimental Animals Production and Research Center. Approval was obtained from the Experimental Animals Ethics Committee of İnönü University Faculty of Medicine (protocol no: 2012/A-49, date: 21.03.2012). The rats were housed in rooms where the room temperature was between 24 and 27 °C, ventilation conditions were met, and the lighting was 12 hours light and 12 hours dark. The rats were fed standard pellet feed *ad libitum* throughout the study.

Experimental Groups

Randomly selected subjects were divided into 4 different groups.

1. Control group: Rats in this group were administered 5% ethanol intraperitoneally (i.p) 1 mL/day for 10 days.

2. Olive oil group: 0.5 mL/day extra olive oil was administered i.p to the rats in this group for 10 days.

3. CCl_4 group: 0.5 mL/kg CCl_4 was administered i.p to the rats in this group for 10 days.

4. CCl_4+CAPE group: 0.5 mL/kg prepared by dissolving in olive oil was administered i.p to rats in this group for 10 days by dissolving in CCl_4 followed by dissolving in 10 µmol/kg CAPE (Sigma, St. Louis, MO).

The study's 11th 5 mg/kg xylazine and 50 mg/kg ketamine i.p. were administered to the rats, and their abdomens were opened under general anesthesia with a midline incision. The testicular tissue samples were collected for histological examination.

Histological Analyses

Detection and follow-up of tissues were started for histopathological evaluation. Then, 10% formaldehyde was added to ensure good fixation of the tissues. The specimens were then divided into smaller pieces of 3-4 mm, placed in plastic tissue follow-up cassettes, and fixed in formaldehyde for 24 hours. After the fixation process was completed, the parts were rinsed in running tap water for 24 hours. They were then dehydrated in graduated alcohols, made transparent in, and embedded in paraffin. Five-micron sections were taken from paraffin blocks using a Leica RM2145 microtome. The sections were stained with hematoxylin and eosin to observe the general histological structure. To evaluate testicular damage, 100 tubules from each section were randomly examined under a light microscope at 20x magnification and classified as intact, atrophic, and degenerated tubules. Sections were examined by using Leica DFC 280 light microscope and Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd. Cambridge, UK).

Statistical Analysis

Statistical analyses were conducted via the Statistical Package for the Social Sciences (SPSS) program (SPSS for Windows version 13) and MedCalc (2007, Belgium) statistical software. All results are expressed as arithmetic mean ± standard error.

The measurable variables in all groups did not have a normal distribution according to the Shapiro-Wilk normality test (p>0.05). For this reason, the Kruskal-Wallis analysis of variance, one of the non-parametric tests, was used for the general comparison of the groups in terms of all variables, whereas the Connover test was used for the pairwise comparison of the groups. The results were considered significant at p<0.05.

RESULTS

Histopathological Evaluation

When the testicular sections of the control and olive oil groups were examined, the seminiferous tubules had a normal histological appearance. The tubules consisted of a seminiferous epithelium sitting on a distinct basal lamina. Sertoli cells and spermatogenic serial cells in the seminiferous epithelium were clearly distinguishable. While spermatogonia were located just above the basal membrane and had a round or oval shape, spermatids were observed in the lumen (Figure 1A, 1B).

In the CCl_4 group, degenerative cells with eosinophilic cytoplasm were found in some tubules, which arrested spermatocytes in different stage of division (Figure 1C). In addition, multinucleated giant cells were observed in the lumen of some seminiferous tubules (Figure 1D). In this group, 55.00±4.22% of the seminiferous tubules were intact, 25.00±2.67% were atrophic and 20.00±1.88% were degenerative. In the CCl_4+CAPE group, 72.14±3.91% of the tubules were intact, 16.42±2.10% were atrophic, and 11.42±2.36% were degenerative (Figure 1E, 1F). While the number of affected tubules significantly increased in the CCl_4 group compared to the control group (p<0.05), the number of affected seminiferous tubules significantly decreased in the CCl_4+CAPE group compared to the CCl_4 group (p<0.05) (Table 1).

DISCUSSION

Exposure to toxic substances in the environment and/or workplace is considered to be one of the main factors responsible for sperm quality decline[10]. The persistence of toxic substances in our environment and all their effects on reproductive health, especially general health, make them a public health concern.

CCl_4 is a colorless, volatile, and toxic industrial substance that is rapidly absorbed by humans and animals after being released into the air, water, and soil through toxic emissions. Studies have shown that CCl_4 causes liver[3], lung[11], kidney[12-14] and testicular damage[15] in experimental animals. Studies have reported that CCl_4 administration damages the reproductive system by causing oxidative toxicity in male rats[4,14,16].

Given the changes caused by the adverse effects of many drugs and chemical agents, the possible effects of healing agents on different tissues of the body need to be investigated. Propolis is a sticky substance with very strong antiviral, antibacterial, and antifungal effects consisting of a mixture of various oils, pollens, special resins, and waxy substances collected by honeybees from the cones and barks of trees and from buds and sprouts of plants[17]. CAPE is an active component of propolis and has been shown to be a pharmacologically safe compound with anti-inflammatory, antimitogenic, anticarcinogenic, antioxidant, and immunomodulatory effects[18-22]. This study was designed to histologically evaluate the therapeutic effects of CAPE on testicular injury induced by CCl_4.

Previous experimental studies have reported that CCl_4 exposure causes structural and functional damage in the male reproductive system[23]. Moreover, similar previous studies have shown that histopathological changes occur in testicular tissues due to CCl_4 toxicity[24]. In the CCl_4 study conducted by Türk et al.[5] with rats, it was reported that they observed degeneration in germ cells, edema, and congestion in the interstitial area. Similarly, in the study conducted by Khan and Ahmed[4] with rats, degeneration, basal membrane separation, seminiferous tubule atrophy, and expansion in the interstitial area were reported in the spermatogenic serial cells of CCl_4. In their study with rats, Horn et al.[16] reported that they observed only Sertoli cells in the seminiferous tubules as a result of loss of germ cells, vacuolization in the germinal epithelium, and interruption of meiosis with the administration of CCl_4. In our study, we classified the tubules according to the presence of

Table 1. Histopathological score values of all groups

Groups	Intact tubule	Atrophic tubule	Degenerated tubule
1. Control (%)	83.57±2.10	10.71±0.71	5.71±1.70
2. Olive oil (%)	80.00±2.43	10.71±1.70	9.28±1.30
3. CCl_4 (%)	55.00±4.22[a]	25.00±2.67[a]	20.00±1.88[c]
4. CCl_4+CAPE (%)	72.14±3.91[b]	16.42±2.10[b]	11.42±2.36[d]

[a]Statistically significant difference with control (p=0.0008).
[b]Statistically significant difference with CCl_4 (p=0.0008).
[c]Statistically significant difference with control (p=0.0025).
[d]Statistically significant difference with CCl_4 (p=0.0025).
CCl_4: Carbon tetrachloride, CAPE: Caffeic acid phenethyl ester

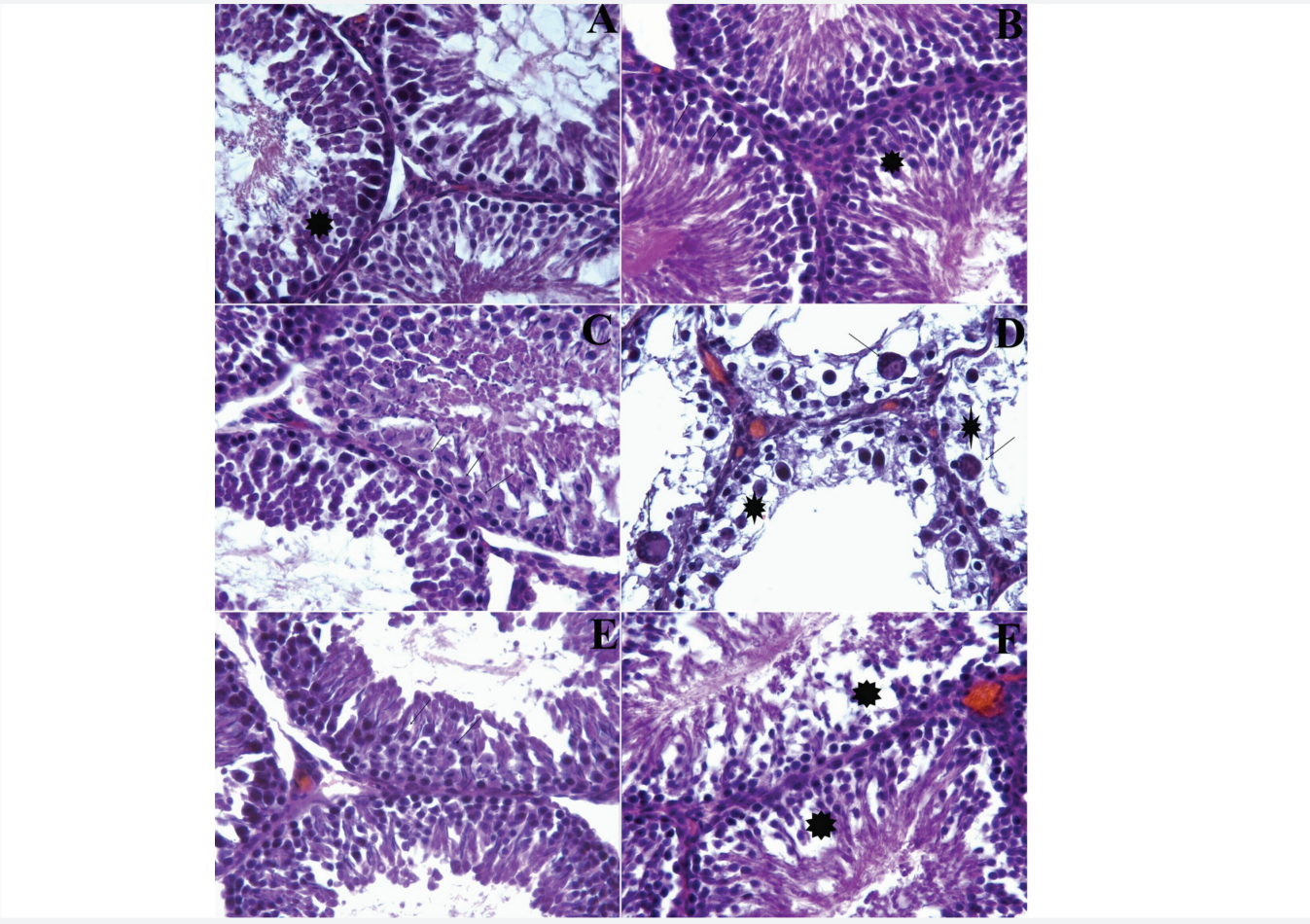

Figure 1. Control (A) and olive oil group (B), the view of Sertoli cells (arrows) and spermatogenic cells (star) in the seminiferous tubule epithelium. CCl_4 group (C), the appearance of arrested spermatocytes in different stage of division (arrows). (D) the view of germinal cell loss (asterisk) and multinuclear giant cells (arrows) CCl_4+CAPE group, (E) the view of arrested spermatocytes in different stage of division (arrows) (F) germinal cell loss (asterisk)

CCl_4: Carbon tetrachloride, CAPE: Caffeic acid phenethyl ester

degenerative cells and their atrophy and histopathologically showed the damage caused by CCl_4 to the testis. In our study, we found atrophic tubules in the testicular tissue, after CCl_4 administration, and degenerated cells with eosinophilic cytoplasm in some tubules, which paused at certain stages of meiosis and were observed in different ways. We also detected the presence of multinucleated giant cells in the lumen of some seminiferous tubules.

In our study, it was obeserved that the histological damage to the testis with the administration of CCl4 decreased with CAPE treatment. CAPE has these protective effects on the basis of antioxidant actions, but the exact mechanisms of anti-oxidant properties of CAPE are not known yet. However, it has been speculated that CAPE may affect transcription and/or translation of genes and gene products of anti-oxidant enzymes[25]. In addition, the protective effects of CAPE may be caused by its ability to block the bioactivation of CCl_4 by inhibiting CYP2E1 activity, in combination with its ability to scavenge free radicals[26]. In a study by Atik et al.[27], investigating the effects of CAPE against testicular damage caused by ischemia/reperfusion in rats, it was reported that the effect of histopathological damage decreased with CAPE application. Abdallah and El-Refaei[28] reported that the widespread inflammation, necrosis, and hemorrhage they observed in the testis when cadmium was administered to rats was alleviated by the administration of CAPE. In our study, we found that the number of affected seminiferous tubules decreased significantly in the CCl_4+CAPE group compared to the CCl_4 group.

Study Limitations

The most important limitation of our study is that it is not supported by functional recovery and biochemical parameters.

CONCLUSION

In conclusion, our study investigated the possible effect of CAPE on testicular damage by CCl_4. The results of our study show that CCl_4 causes testicular damage and that this testicular damage improves to some extent when CCl_4 and CAPE are given together. In view of these results, we think that the use of antioxidant and anti-inflammatory agents such as CAPE may have beneficial effects in the treatment of testicular damage.

Ethics

Ethics Committee Approval: Approval was obtained from the Experimental Animals Ethics Committee of İnönü University Faculty of Medicine (protocol no: 2012/A-49, date: 21.03.2012).

Informed Consent: Animal experiment.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: E.T., Design: E.T., Data Collection or Processing: B.G., N.V., A.T., H.E., Analysis or Interpretation: E.T., Literature Search: B.G., Writing: B.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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