Journal of Hard Tissue Biology 27[3] (2018)237-242
2018 The Hard Tissue Biology Network Association
Printed in Japan, All rights reserved.
CODEN-JHTBFF, ISSN 1341-7649

Original

The Effect of Cape on Steroid Induced Osteonecrosis of the Femoral Head in Rat Model

Vedat Uruc[1)], Ibrahim Gokhan Duman[1)], Serkan Davul[1)], Raif Özden[1)], Ramazan Gonenci[2)], Hasan Gokce[3)], Recep Dokuyucu[4)] and Abdurrahman Akdag[5)]

[1)] *Department of Orthopedics and Traumatology, Medical Faculty, Mustafa Kemal University, Hatay, Turkey*
[2)] *Faculty of Veterinary Medicine, Mustafa Kemal University, Hatay, Turkey*
[3)] *Department of Pathology, Medical Faculty, Mustafa Kemal University, Hatay, Turkey*
[4)] *Department of Physiology, Medical Faculty, Mustafa Kemal University, Hatay, Turkey*
[5)] *Department of Chemistry, Science and Arts Faculty, Mustafa Kemal University, Hatay, Turkey*
(Accepted for publication, June 1, 2018)

Abstract: The aim of this experimental study was to examine the effect of Caffeic acid phenethyl ester (CAPE) on steroid-induced osteonecrosis of femoral head (ONFH) in rats. Thirty-one male Wistar albino rats were divided into 4 groups: control group (7 rats), methylprednisolone treatment group (MPS, 8 rats), CAPE treatment group (8 rats) and MPS+CAPE administered group (8 rats). The rats of group MPS and CAPE+MPS, On days 2, 3 and 4 were treated with 20 mg/kg/day methylprednisolone (MPS; Pfizer Pharmaceutical, Puurs, Belgium) intramuscularly. 10 µmol/kg/day CAPE was intraperitoneally injected to the rats of group CAPE from 13 weeks of age for 4 weeks. The control group was fed and housed under identical conditions without any treatment. All rats were sacrificed at 17 weeks of age by taking blood from the heart. Both proximal femoral parts were taken for histopathological and immunohistochemical analysis . Total oxidant status, total antioxidant status, and oxidative stress index (OSI), lipid parameters, coagulation parameters were assessed in blood specimens. Much lesser amount of osteonecrosis lesions were observed in the MPS+CAPE group compared to MPS group. In immunhisochemical analysis, oxidative stress was found significantly decreased in CAPE+MPS group compared to MPS group. OSI levels were significantly decreased in CAPE+MPS group compared to MPS group ($p<0.001$). In CAPE+MPS group lipid and coagulation parameters were found positively affected compared to MPS group. In conclusion, CAPE has strong protective effect against the steroid induced femoral head osteonecrosis in rats.

Key words: CAPE, Femoral head necrosis, Glucocorticoid, Rat

Introduction

Osteonecrosis of femoral head (ONFH) continues to be a major problem. It occurs in two forms, post-traumatic and non-traumatic. ONFH is a disease which is characterized with subchondral bone fractures, breakdown of the femoral head surface and resulting in osteoarthritis[1,2)]. Annually, 50,000 new cases of ONFH is reported in USA[3,4)]. Unfortunately, there is not an effective treatment of ONFH, but generally definitive treatment with total hip arthroplasty is provided[5)]. Steroid-induced ONFH, constitutes an important part of non-traumatic osteonecrosis[4,6)]. Glucocorticoids are used at high dose and for long periods in organ transplantations, connective tissue diseases, systemic lupus erythematosus, rheumatoid arthritis and asthma[4,7-11)]. A significant proportion of these patients are young and elderly people. The pathophysiology of osteonecrosis of glucocorticoids is not yet fully elucidated. However, there are some studies suggesting that degradation of lipid metabolism, oxidative stress and deterioration of the coagulation system may play a role[12-14)].

Caffeic acid phenethyl ester (CAPE) is an important component derived from propolis, a component of beehive[15)]. There are more than 300 substances in propolis. Phenolic acid, phenolic acid esters, amino acids, steroids, flavonoids are some of them[16,17)]. The antioxidant, anticoagulant, antilipidemic, anticancer, anti-inflammatory, antiviral, anti-metastatic, immunomodulatory effects of CAPE are well known[18,19,15,16,20,21)].

We hypothesize that CAPE is intended to prevent the steroid-induced ONFH. Our aim in the present study was to investigate the effects of CAPE in a rat model of steroid-induced ONFH.

Correspondence to: Dr. Vedat Uruç, Department of Orthopedics and Traumatology, Medical Faculty, Mustafa Kemal University, Hatay 31100, Turkey; Tel: +90 5533212045; Fax: +90 326 224556; E-mail: urucvedat@gmail.com

Materials and Methods

The experimental protocol applied in the study was confirmed by the Institutional Animal Care and Ethics Committee of the Mustafa Kemal University. Thirty-one male Wistar albino rats at the 13 weeks of age weighing 200–240g were used in the study. All rats were kept in a room maintained at 24 ± 2 ° C and 55 ± 2% moisture in biomedical research center. We divided all rats into four groups: control group (7 rats), methylprednisolone treatment group (MPS, 8 rats), CAPE treatment group (8 rats) and MPS+CAPE administered group (8 rats). The rats of group MPS and CAPE+MPS, On days 2, 3 and 4 were treated with 20 mg/kg/day methylprednisolone (MPS; Pfizer Pharmaceutical, Puurs, Belgium) intramuscularly[22)]. 10 µmol/kg/day CAPE was intraperitoneally injected to the rats of group CAPE from 13 weeks of age for 4 weeks[23)]. The control group was fed and housed under identical conditions without any treatment. All rats were sacrificed at 17 weeks of age by taking blood from the heart.

Hematological evaluation

Blood samples were taken from the heart and both femoral heads removed. The serum level of total cholesterol (T-Cho), high density lipoprotein (HDL) low density lipoprotein (LDL) and triglycerides (TG) was evaluated to investigate the steroid induced hyperlipidemia. In addition to examine the anticoagulant activity of CAPE, serum levels of prothrombin time (PT), active partial thromboplastin time (aPTT) and

Table 1. The scores of histopathology and immunohistochemistry

	Control	MPS	CAPE	MPS + CAPE
Fatty degeneration	0.00 ± 0.00	1.00 ± 0.00[a]	0.00 ± 0.00	0.20 ± 0.40[b]
Myeloid necrosis	0.00 ± 0.00	0.37 ± 0.48[a]	0.00 ± 0.00	0.00 ± 0.00[b]
Osteocyte necrosis	0.00 ± 0.00	0.12 ± 0.33[a]	0.00 ± 0.00	0.00 ± 0.00[b]
New bone formation	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Osteonecrosis	0.00 ± 0.00	1.50 ± 0.70[a]	0.00 ± 0.00	0.20 ± 0.40[b]
4-HNE	1.00 ± 0.00	1.37 ± 0.51[c]	1.00 ± 0.00	1.00 ± 0.00[d]
8-OHdG	1.25 ± 0.46	2.12 ± 0.35[c]	1.33 ± 0.50	1.42 ± 0.53[d]

CAPE: caffeic acid phenethyl ester; MPS:methylprednisolone; 4 –HNE: 4-hydroxy-2-nonenal; 8-OHdG: anti-8-hydroxy-2′-deoxyguanosine.
$P < 0.001$: [a]MPS vs control, [b]MPS+CAPE vs MPS.
$P < 0.05$: [c]MPS vs control, [d]MPS+CAPE vs MPS.

Table 2. Levels of oxidative status in groups

	Control (n=7)	MPS (n=8)	CAPE (n=8)	MPS+CAPE (n=8)
TAS (mmol/l)	1,42 ± 0,11	1,44 ± 0,23	1,23 ± 0,13	1,24 ± 0,19
TOS (mmol/l)	48,76 ± 16,80	197,4 ± 93,46[a]	44,81 ± 17,22[b]	44,40 ± 12,37[c]
OSI (=TOS/TAS)	33,74 ± 9,40	139,2 ± 72,35[a]	36,92 ± 16,17[b]	36,03 ± 10,19[c]

MPS: Methylprednisolone; CAPE: caffeic acid phenethyl ester; TAS: total antioxidant status; TOS: total oxidative status; OSI: oxidative stress index.
$P < 0.0001$: [a]MPS vs control; [b]CAPE vs MPS; [c]MPS+CAPE vs MPS.

international normalized rate (INR) were evaluated.

Measurement of total antioxidant status (TAS)

Serum TAS levels were measured by an automated method developed by Erel[24]. In this method, most powerful hydroxyl radicals are firstly generated. Reagents containing ferrous ions solution are mixed with the second reagent including hydrogen peroxide in the assay. The brown dianisidinil radical cation produced by the hydroxyl radicals is a strong radical. Non- potent radical reactions initiated by the hydroxyl radicals produced because of antioxidative effect of working examples was also measured. The assay has excellent precision (< 3%), the results are expressed as millimolar Trolox equivalent per liter (mmol Trolox Equiv./l).

Measurement of total oxidant status (TOS)

Serum TOS levels were measured by an automated method developed by Erel[25]. Oxidants present in the samples oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by abundant glycerol molecules in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay was calibrated with hydrogen peroxide. The results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/l).

Oxidative stress index

The percentage of TOS level to TAS level was accepted as OSI value [OSI = TOS (μmol H_2O_2 Equiv./l)/TAS(mmol Trolox Equiv./l)].

Tissue preparation

Both the proximal parts of the femur were fixed in 10% neutral buffered of formalin for 24 hours, then were decalcified by using 20% diluted formic acid solution for 48 hours and embedded in paraffin at fixed reaction time and temperature. Paraffin blocks were sliced in 5 micrometers thickness with microtome. Four sections were obtained through teres ligament and stained with hematoxylin and eosin to assess the general histomorphological architecture and osteonecrosis of femoral heads.

For immunohistochemical analysis, 20 sections were obtained by randomly selecting 5 femoral heads, from separate rats, from each of the 4 groups.

Assessment of osteonecrosis

Histopathological changes were evaluated by light microscopy. We evaluated the osteonecrosis based on methodology of Ficat *et al*[26] including the presence or lack of fatty degeneration, myelocyte necrosis, osteocyte necrosis and appositional bone formation[27].

Immunohistochemistry

For the detection of oxidative stress, immunohistochemical analysis was performed using the avidin-biotin immunoperoxidase technique for 8-hydroxy-2′-deoxyguanosine (anti-8-OHdG) (Biorbyt, Cambridge, UK, orb10011, 1:500 dilution) and 4-hydroxy-2-nonenal (anti-4 –HNE) (Biorbyt, Cambridge, UK, orb100588, 1:500 dilution) polyclonal antibodies. Use of the anti-8-OHdG polyclonal antibody is based on the fact that when oxidative stress, due to active oxygen species, is increased, 8-OHdG is formed in DNA, and detected in the nucleus. For 8-OHdG, findings were scored on a 3-point scale: (1) areas of observation were unstained; (2) some myelocytes were stained; and (3) all bone marrow cells were positively stained[28]. Anti-4-HNE polyclonal antibody detects 4-HNE, which is a secondary product of oxidation of w-6 polyunsaturated fatty acids that is found particularly around the adipose cells. For 4-HNE, findings were scored employing a 3-point scale: (1) areas of observation were unstained; (2) only adipose cell borders were stained; and (3) bone marrow cells were also positively stained[28]. The intensity of staining was also taken into account when assessing the staining for 4HNE and 8-OHdG. Also, to evaluate the findings objectively, we assessed the staining of the cells and the peripheral structures such as blood vessels, adipocytes, bone marrow cells, and trabeculae using a three-point scale staining (negative, positive and intensely positive) and calculated the average total score of each group by a "3-point method[28].

Table 3. Laboratory data and blood coagulation

	Control (n=7)	MPS (n=8)	CAPE (n=8)	MPS+CAPE (n=8)
T-Cho (mg/dl)	60,83 ± 5,14	175,6 ± 35,88[a]	60,60 ± 10,28[b]	38,06 ± 4,74[c]
TG (mg/dl)	48,25 ± 17,33	88,02 ± 34,08[d]	70,00 ± 43,73	34,20 ± 5,945[e]
LDL (mg/dl)	30,91 ± 5,60	107,0 ± 21,20[a]	28,56 ± 11,97[b]	19,07 ± 4,08[c]
HDL (mg/dl)	20,27 ± 2,23	48,48 ± 9,11[a]	18,04 ± 4,12[b]	12,15 ± 1,69[c]
PT	14,49 ± 0,45	14,56 ± 1,68	14,37 ± 0,99[f]	19,41 ± 1,57[cg]
aPTT	36,64 ± 6,62	40,90 ± 13,89	34,19 ± 8,20[h]	51,09 ± 5,17[i]
INR	1,12 ± 0,04	1,13 ± 0,17	1,11 ± 0,10[f]	1,63 ± 0,18[cg]

MPS, Methylprednisolone acetate; CAPE, caffeic acid phenethyl ester; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PT, prothrombin time; aPTT, activated partial thromboplastin time; INR, International normalized ratio, T-Cho, total cholesterol; TG, trigliserid
P < 0.0001: [a]MPS vs control; [b]CAPE vs MPS; [c]MPS+CAPE vs MPS; [f]CAPE vs MPS+CAPE;
[g]MPS+CAPE vs Control
P = 0.015:[d]MPS vs control
P = 0.023: [e]MPS+CAPE vs MPS
P < 0.001: [h]CAPE vs MPS+CAPE; [i]MPS+CAPE vs Control

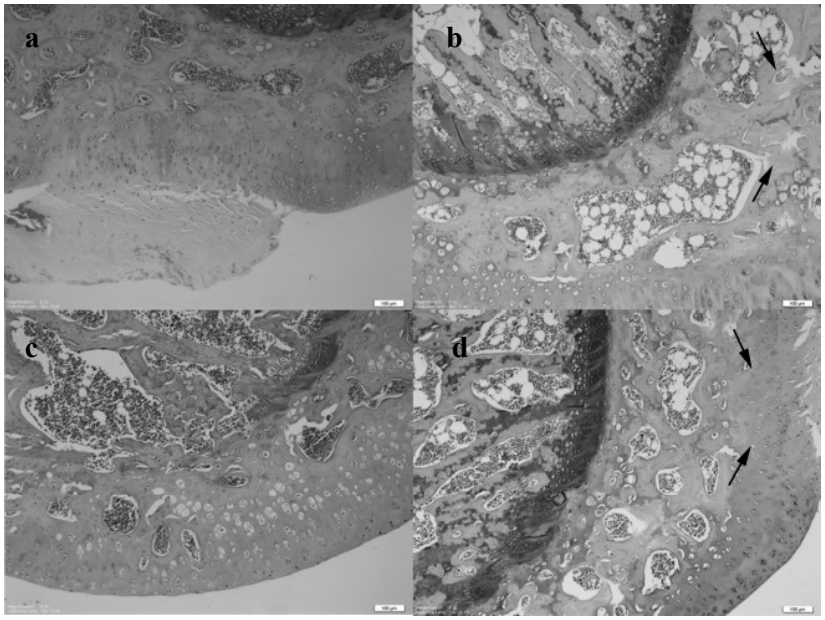


Figure 1. Histopathologic results for all groups.
The lacunae of some osteocytes are empty and focal osteonecrosis is present (arrows) (Hematoxyline and Eosin, scale bar = 100 μm).
a: Control, b: MPS, c: CAPE, d: MPS+CAPE.

Statistical analysis

All statistical data was performed by using SAS software version 9.1.3 (SAS Institute Inc., Cary, USA). Data were expressed as mean (SD). To determine the difference in incidence of ONFH between the groups, generalized estimating equations (GEEs) was used. A common linear modeling method (ANOVA) was used for comparisons of means among multiple groups. Tukey's multiple comparison test was used for continuous variables.

Results

Histopathological staining

A significant increase in terms of fatty degeneration, myeloid necrosis and necrosis of osteocytes was determined in the MPS group compared to the control group (P < 0.001). There was a significant reduction in the above-mentioned parameters in the treatment group (MPS+CAPE) compared to MPS group (P < 0.001) (Table 1).

Much lesser amount of osteonecrosis was observed in the MPS+CAPE group compared to MPS group (P < 0.05) (Table 1, Fig. 1).

Immunohistochemical Staining

When oxidative stress was compared using different staining such as anti-4-HNE and anti-8-OHdG polyclonal antibodies, similar tendencies were observed. In group MPS, the adipose cell and myelocytes were strongly stained and some osteocytes were also stained. The staining in the adipose cells was not diffuse, but localized around the membrane. In the MPS+CAPE group, staining of the adipose cell membrane was weaker than that in the MPS group (P < 0.05). In the control and CAPE groups, little or no staining for oxidative stress in the adipose cell membrane was observed (P > 0.05) (Table 1, Figs. 2 and 3).

TAS, TOS and OSI results

TAS levels significantly decreased in the CAPE group compared to both control and MPS groups (p<0.01) (Table 2). The TOS levels significantly increased in the MPS group compared to other groups (p<0.0001) and OSI levels significantly decreased in the MPS+CAPE group compared to MPS group (p<0.0001) (Table 2).

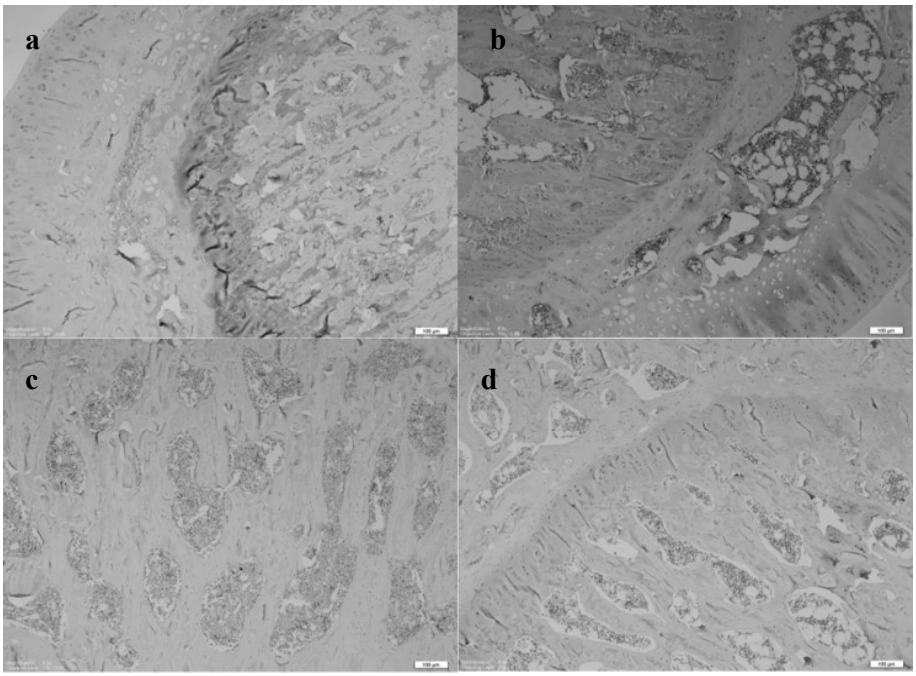


Figure 2. Immunohistochemical staining of 8-hydroxy-2′-deoxyguanosine polyclonal antibody (anti-8-OHdG, scale bar = 100 μm).
a: Control, b: MPS, c: CAPE, d: MPS+CAPE.

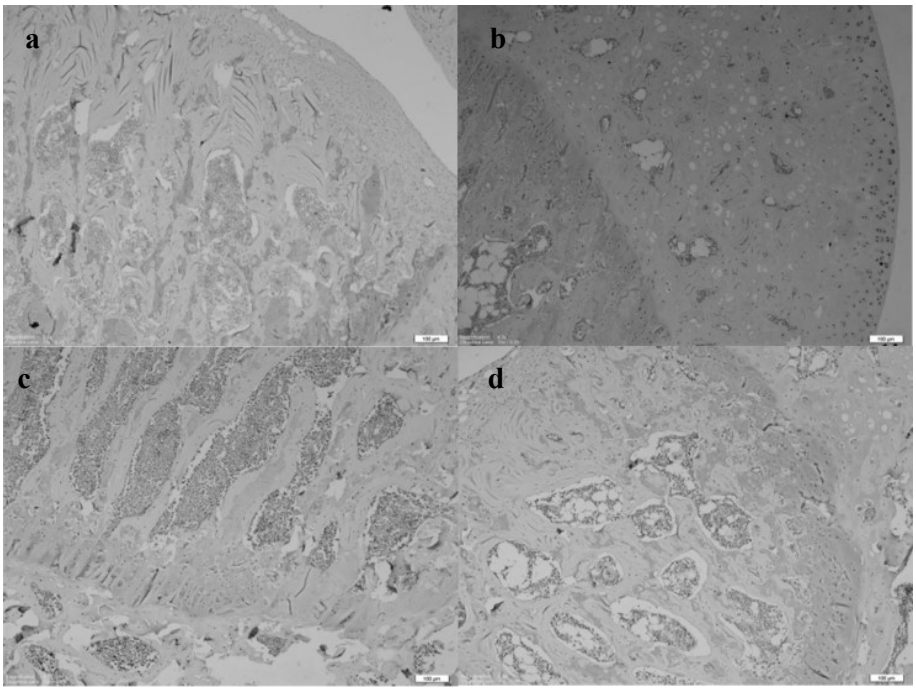


Figure 3. Immunohistochemical staining of 4-hydroxy-2-nonenal polyclonal antibody (anti-4-HNE, scale bar = 100 μm).
a: Control, b: MPS, c: CAPE, d: MPS+CAPE.

T-Cho, TG, HDL and LDL levels

In the present study, T-Cho, LDL and HDL levels increased significantly in the MPS group compared to the control group (P <0.0001), however, we observed reduced serum concentrations of T-Cho, LDL and HDL in MPS+CAPE group compared to the MPS group (P < 0.0001) (Table 3).

TG levels were significantly increased in the MPS group compared to the levels in control group (P = 0.015), while we observed significantly decreased serum level of triglyceride in the MPS+CAPE group compared to the MPS group (P = 0.023).

PT, aPTT and INR Results

PT and INR levels were significantly increased in the MPS+CAPE group compared to the MPS group (P <0.0001). Also aPTT levels were significantly increased in the MPS+CAPE group compared to the control group (P< 0.001) (Table 3).

Discussion

The results of this study has shown that CAPE has effectively reduced the steroid-induced ONFH in rat model.

Although the pathogenesis of steroid-induced ONFH was not clarified fully, hypercoagulability, oxidative stress, hyperlipidemia, intravascular fat embolism, extravascular adipogenesis and intravascular thrombotic occlusion have been suggested to play role in the etiology of steroid-induced ONFH[29-32].

Many drugs; pravastatin, sodium ferulate ,warfarin, low-molecular-weight heparin, statins, vitamin E, naringin and salidroside have been tested, resulting in prevention or suppression of steroid-induced ONFH[3,33-38]. However, to the best of our knowledge, there is no study reported in the literature, so far evaluating the effect of CAPE on steroid induced ONFH.

CAPE is a natural bioactive compound which has various pharmacological and biological activities such as antioxidant, anticancer, anti-inflammatory, antiviral, anti-metastatic, immunomodulatory, antiplatelet and antilipidemic effect[21,18,19,15,16,20,39]

An animal study revealed that high serum levels of low-density lipoprotein cholesterol (LDL-C) was significantly associated with the incidence of ONFH[40]. In another study total serum cholesterol and triglyceride levels were found significantly higher in animals with ONFH compared to controls[35]. Pritchett showed that statins reduce the incidence of osteonecrosis in 284 patients treated with high-dose steroid[37]. Lovastatin has significantly decreased the incidence of ONFH in steroid-treated rabbits[41]. There are also some studies that reported protective effects of lanosterol, pentosan, and pravastatin against ONFH[28,36,42]. CAPE has anti-hyperlipidemic effect due to the fact that it suppresses fatty acid synthase and adipocyte fatty acid binding protein (aP2)[19,15]. In our study, CAPE had significantly decreased serum triglyceride and cholesterol levels and this may contribute to the protective effect of CAPE against the ONFH

Another theory is that hyperlipidemia is associated with abnormal coagulation and this contributes to the progression of ONFH. There are few studies reported in the literature about the antithrombotic effect of cape[43]. Chen TG *et al*. reported that CAPE reveals antiplatelet effect by effecting the cyclic GMP pathway[44]. Motomura et al. compared the combined anticoagulant and anti-lipid treatment with the single treatment groups in animal models and concluded that combined treatment has the lowest rate of ONFH[45]. Similar results were reported in another rabbit steroid induced osteonecrosis model[46]. In our study CAPE revealed significant anticoagulant and anti-lipid effects and this may suggest superiority over other agents.

Another well-known property of CAPE is the strong antioxidant effect. Many studies have reported that suppression of steroid induced oxidative stress can prevent vascular endothelial injury and furthermore the progression of ONFH[47]. Vitamin E is a well-known antioxidant agent and some studies, conducted on animal models of steroid-induced ONFH, concluded significant reduction in oxidative stress and rate of osteonecrosis[33,48]. Our Immunohistochemical, TAS, TOS and OSI results provide further evidence that CAPE has significantly reduced the steroid induced oxidative stress, both locally and systemically.

The limitations of this study are the number of rat were relatively low and wistar albino rat was used instead of hypertensive stroke-prone (SHRSP)/Izm male rats which closely resembles human ONFH, not only histologically but also physiologically. Because this rat species are not available in our country we used wistar albino rats instead and obtained satisfactory ONFH at the end of the study.

In conclusion, CAPE has strong protective effect against the steroid induced ONFH in rats. The results of this study also suggest that CAPE performs the protective effect via its antioxidative, antilipidemic and antiplatelet effects. However further molecular studies are needed to elucidate the underlying molecular mechanism.

Conflict of Interest

All the authors declare that they have no conflict of interest

References

1. Wang L, Zhang L, Pan H, Peng S, Zhao X and Lu W. Abnormal subchondral bone microstructure following steroid administration is involved in the early pathogenesis of steroid-induced osteonecrosis. Osteoporos Int 27: 153-159, 2016
2. Kubo T, Ueshima K, Saito M, Ishida M, Arai Y and Fujiwara H. Clinical and basic research on steroid-induced osteonecrosis of the femoral head in Japan. J Orthop Sci 21: 407-413, 2016
3. Beckmann R, Shaheen H, Kweider N, Ghassemi A, Fragoulis A, Hermanns-Sachweh B, Pufe T, Kadyrov M and Drescher W. Enoxaparin prevents steroid-related avascular necrosis of the femoral head. ScientificWorld Journal 2014, 2014
4. Mont MA and Hungerford DS. Non-traumatic avascular necrosis of the femoral head. JBJS 77: 459-474, 1995
5. Kaushik AP, Das A and Cui Q. Osteonecrosis of the femoral head: An update in year 2012. World J Orthop 3: 49-57, 2012
6. Lavernia CJ, Sierra RJ and Grieco FR. Osteonecrosis of the femoral head. J Am Acad Orthop Surg 7: 250-261, 1999
7. Buttgereit F, Wehling M and Burmester GR. A new hypothesis of modular glucocorticoid actions: steroid treatment of rheumatic diseases revisited. Arthritis Rheumatol 41: 761-767, 1998
8. Maillefert J, Tavernier C, Toubeau M and Brunotte F. Non-traumatic avascular necrosis of the femoral head. J Bone Joint Surg Am 78: 473, 1996
9. Busse WW, Pedersen S, Pauwels RA, Tan WC, Chen Y-Z, Lamm CJ, O'byrne PM and Group SI. The inhaled steroid treatment as regular therapy in early asthma (START) study 5-year follow-up: effectiveness of early intervention with budesonide in mild persistent asthma. J Allergy Clin Immunol 121: 1167-1174, 2008
10. Donadio Jr JV, Holley KE, Ferguson RH and Ilstrup DM. Treatment of diffuse proliferative lupus nephritis with prednisone and combined prednisone and cyclophosphamide. N Engl J Med 299: 1151-1155, 1978
11. De Nijs R, Jacobs J, Algra A, Lems W and Bijlsma J. Prevention and treatment of glucocorticoid-induced osteoporosis with active vitamin D3 analogues: a review with meta-analysis of randomized controlled trials including organ transplantation studies. Osteoporos int 15: 589-602, 2004
12. Ichiseki T, Matsumoto T, Nishino M, Kaneuji A and Katsuda S. Oxidative stress and vascular permeability in steroid-induced osteonecrosis model. J Orthop Sci 9: 509-515, 2004
13. Ichiseki T, Ueda Y, Katsuda S, Kitamura K, Kaneuji A and Matsumoto T. Oxidative stress by glutathione depletion induces osteonecrosis in rats. Rheumatology 45: 287-290, 2005
14. Wang G-J, Cui Q and Balian G. The pathogenesis and prevention of steroid induced osteonecrosis. Clin Orthop Relat Res 370: 295-310, 2000
15. Murtaza G, Karim S, Akram MR, Khan SA, Azhar S, Mumtaz A and Bin Asad MH. Caffeic acid phenethyl ester and therapeutic potentials. Biomed Res Int 2014: 145342, 2014
16. Pan D and Li D. Protective effect of caffeic acid phenethyl ester on myocardial injury due to anti-oxidant action. Anadolu kardiyoloji dergisi : AKD = the Anatolian journal of cardiology 14: 583-584, 2014

17. Kumazawa S, Ahn M-R, Fujimoto T and Kato M. Radical-scavenging activity and phenolic constituents of propolis from different regions of Argentina. Nat Prod Res 24: 804-812, 2010
18. Kinis V, Ozbay M, Akdag M, Alabalik U, Gul A, Yilmaz B, Ozkan H and Topcu I. Effects of caffeic acid phenethyl ester on wound healing of nasal mucosa in the rat: an experimental study. Am J Otolaryngol. 35: 482-486, 2014
19. Kinis V, Ozbay M, Alabalik U, Gul A, Yilmaz B, Ozkurt FE, Sengul E and Topcu I. Effect of caffeic acid phenethyl ester on myringosclerosis development in the tympanic membrane of rat. Eur Arch Otorhinolaryngol 272: 29-34, 2015
20. Ucan MC, Koparal M, Agacayak S, Gunay A, Ozgoz M, Atilgan S and Yaman F. Influence of caffeic acid phenethyl ester on bone healing in a rat model. J Int Med Res. 41: 1648-1654, 2013
21. Altuntas A, Yilmaz HR, Uz E, Demir M, Gokcimen A, Aksu O, Bayram DS and Sezer MT. Caffeic acid phenethyl ester protects against amphotericin B induced nephrotoxicity in rat model. Biomed Res Int 2014: 702981, 2014
22. Chen S, Li J, Peng H, Zhou J and Fang H. Administration of erythropoietin exerts protective effects against glucocorticoid-induced osteonecrosis of the femoral head in rats. Int J Mol Med. 33: 840-848, 2014
23. Trumbeckaite S, Pauziene N, Trumbeckas D, Jievaltas M and Baniene R. Caffeic Acid Phenethyl Ester Reduces Ischemia-Induced Kidney Mitochondrial Injury in Rats. Oxid Med Cell Longev 2017, 2017
24. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37: 277-285, 2004
25. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38: 1103-1111, 2005
26. Ficat P and Arlet J. [Pre-radiologic stage of femur head osteonecrosis: diagnostic and therapeutic possibilities]. Revue de chirurgie orthopedique et reparatrice de l'appareil moteur 59: Suppl 1:26-38, 1973
27. Nozaki Y, Kumagai K, Miyata N and Niwa M. Pravastatin reduces steroid-induced osteonecrosis of the femoral head in SHRSP rats. Acta Orthop 83: 87-92, 2012
28. Miyata N, Kumagai K, Osaki M, Murata M, Tomita M, Hozumi A, Nozaki Y and Niwa M. Pentosan reduces osteonecrosis of femoral head in SHRSP. Clinical and Experimental Hypertension 32: 511-516, 2011
29. Kerachian MA, Harvey EJ, Cournoyer D, Chow TY, Nahal A and Seguin C. A rat model of early stage osteonecrosis induced by glucocorticoids. J Orthop Surg Res 6: 62, 2011
30. Komurcu E, Oktay M, Kaymaz B, Hatay Golge U, Goksel F and Nusran G. Preventive effects of coenzyme Q10 (CoQ10) on steroid-induced osteonecrosis in rats. Acta Orthop Traumatol Turc 48: 217-222, 2014
31. Tian L, Wen Q, Dang X, You W, Fan L and Wang K. Immune response associated with Toll-like receptor 4 signaling pathway leads to steroid-induced femoral head osteonecrosis. BMC Musculoskelet Disord 15: 18, 2014
32. Janke LJ, Liu C, Vogel P, Kawedia J, Boyd KL, Funk AJ and Relling MV. Primary epiphyseal arteriopathy in a mouse model of steroid-induced osteonecrosis. Am J Pathol 183: 19-25, 2013
33. Mikami T, Ichiseki T, Kaneuji A, Ueda Y, Sugimori T, Fukui K and Matsumoto T. Prevention of steroid-induced osteonecrosis by intravenous administration of vitamin E in a rabbit model. J Orthop Sci 15: 674-677, 2010
34. Wada M, Kumagai K, Murata M, Yasuko S and Shindo H. Warfarin reduces the incidence of osteonecrosis of the femoral head in spontaneously hypertensive rats. J Orthop Sci 9: 585-590, 2004
35. Tian L, Dang X-q, Wang C-s, Yang P, Zhang C and Wang K-z. Effects of sodium ferulate on preventing steroid-induced femoral head osteonecrosis in rabbits. J Zhejiang Univ Sci B 14: 426-437, 2013
36. Jiang Y, Zhang Y, Zhang H, Zhu B, Li P, Lu C, Xu Y, Chen W and Lin N. Pravastatin prevents steroid-induced osteonecrosis in rats by suppressing PPARγ expression and activating Wnt signaling pathway. Exp Biol Med 239: 347-355, 2014
37. Pritchett JW. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. Clin Orthop Relat Res 386: 173-178, 2001
38. Huang D, Li Z, Chen B, Fang G, Sun X, Li F, Xu H, Chen Y and Ding W. Naringin protects against steroid-induced avascular necrosis of the femoral head through upregulation of PPARγ and activation of the Notch signaling pathway. Mol Med Rep 17: 3328-3335, 2018
39. Juman S, Yasui N, Okuda H, Ueda A, Negishi H, Miki T and Ikeda K. Caffeic acid phenethyl ester inhibits differentiation to adipocytes in 3T3-L1 mouse fibroblasts. Biol Pharm Bull 33: 1484-1488, 2010
40. Davarci I, Alp H, Ozgur T, Karcioglu M, Tuzcu K, Evliyaoglu O, Motor S and Durgun Yetim T. Ameliorating effects of CAPE on oxidative damage caused by pneumoperitoneum in rat lung tissue. J Clin Exp Med 7: 1698-1705, 2014
41. Pengde K, Fuxing P, Bin S, Jing Y and Jingqiu C. Lovastatin inhibits adipogenesis and prevents osteonecrosis in steroid-treated rabbits. Joint bone spine : 75: 696-701, 2008
42. Zhao G, Yamamoto T, Motomura G, Yamaguchi R, Ikemura S, Iwasaki K and Iwamoto Y. Cholesterol-and lanolin-rich diets may protect against steroid-induced osteonecrosis in rabbits. Act Orthop 84: 593-597, 2013
43. Zhou K, Li X, Du Q, Li D, Hu M, Yang X, Jiang Q and Li Z. A CAPE analogue as novel antiplatelet agent efficiently inhibits collagen-induced platelet aggregation. Die Pharmazie 69: 615-620, 2014
44. Chen TG, Lee JJ, Lin KH, Shen CH, Chou DS and Sheu JR. Antiplatelet activity of caffeic acid phenethyl ester is mediated through a cyclic GMP-dependent pathway in human platelets. Chin J Physiol 50: 121-126, 2007
45. Motomura G, Yamamoto T, Miyanishi K, Jingushi S and Iwamoto Y. Combined effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. Arthritis Rheum 50: 3387-3391, 2004
46. Kang P, Gao H, Pei F, Shen B, Yang J and Zhou Z. Effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. Int J Exp Pathol 91: 235-243, 2010
47. Yagi S, Aihara K, Ikeda Y, Sumitomo Y, Yoshida S, Ise T, Iwase T, Ishikawa K, Azuma H, Akaike M and Matsumoto T. Pitavastatin, an HMG-CoA reductase inhibitor, exerts eNOS-independent protective actions against angiotensin II induced cardiovascular remodeling and renal insufficiency. Circ Res 102: 68-76, 2008
48. Kuribayashi M, Fujioka M, Takahashi KA, Arai Y, Ishida M, Goto T and Kubo T. Vitamin E prevents steroid-induced osteonecrosis in rabbits. Acta Orthop 81: 154-160, 2010