CAFFEIC ACID PHENETHYL ESTER (CAPE) MEDIATED DECREASE IN METASTASIS OF COLON CANCER CELLS: AN *IN VITRO* AND *IN VIVO* STUDY

Weiqiang Wu[#]*, Feng Gao[#], Xi Heng, Feng Song, Yong Zhao, Ming Xu, Xin Zhang, Zengqiang Yang

Department of Colorectal surgery, Lanzhou General Hospital of Lanzhou Command. PLA, Lanzhou 730050, China. [#]These two authors contribute to this work equally.

Corresponding Author E-mail: weiqiangwu136@hotmail.com

Abstract

Background: Caffeic acid phenethyl ester (CAPE) is a phytochemically active component obtained from honeybee hive propolis. CAPE has been reported to show antimitogenic, anticancer, and other beneficial medicinal properties. Many of its activities have been reported to be mediated by inhibiting levels of matrix metalloproteinase, that is, MMP-2 and MMP-9. We hypothesize the effect of CAPE on the metastasis of colon cancer cells in both *in vitro* and *in vivo*.

Methods: Cell migration, motility, invasion were evaluated also expression of protein and matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 were measured in SW-480 cancer cells *in vitro*. The cells were exposed to Phorbol 12-myristate 13-acetate (PMA) and were treated with various concentration of CAPE.

Results: The treatment of CAPE caused significant decrease (P<0.05) in both cell motility and invasion. The treatment of CAPE inhibited activity of MMP-2 and MMP-9 and their protein with increasing dose in SW-480 cancerous cells. Anti-metastatic activity was evaluated *in vivo* in BALB/c mice by injecting them with CT-26 mouse colon cancer cells via tail vein and were treated with CAPE (20 mg/kg) orally for 21 days. The CAPE treatment significantly (P<0.05) reduced count of pulmonary nodules. The mice showed decreased plasma MMP-2 and MMP-9 activity after 21 days treatment with CAPE.

Conclusion: The study suggested beneficial role of CAPE in preventing invasion of colon cancer and metastasis via MMP-2 and MMP-9 mediated pathway.

Keywords: CAPE, colon cancer, SW-480, CT-26, anti-metastatic

Introduction

Colorectal cancer is carcinoma of colonic mucosal lining ranked third highest occurring cancer worldwide. Colon cancer occurs due to series of mutations of genes both suppressor and oncogenic. Mutations lead to generation of polyps leading to adenocarcinoma (Shao-Rong et al., 2013). Occurrence of metastatic colon cancer has been found to be one of the primary cause of death in patients (Wang et al., 2009). The attack of tumor metastasis commences with encroachment of tumor cells in colon. The adherence of cancerous tumor cells on the membrane leads to endothelial contraction followed by release of destructive proteolytic enzymes such as matrix metalloproteinase also called as MMPs and serine proteinase causing destruction of extracellular matrix (ECM) (Okegawa et al., 2004; Pepper et al., 1996; Pepper, 2001). Over expression of MMPs has been correlated to invasion of tumor cells on ECM (Rujirek et al., 2013; Stetler-Stevenson et al., 2001; Vince et al., 1999). Matrix metalloproteinase-2 (MMP-2) are found to be one of the main factors in destruction of ECM mediated by invasion of tumor cells due to breakdown of collagen type IV and laminin. Over expression of MMP-2 also suggests advancement and level of malignancy of gliomas (Forsyth et al., 1999; Wang et al., 2003).

Caffeic acid phenethyl ester also known as CAPE is reported to be among the major active medicinal moiety of propolis which is produced by honeybees of American and European countries. Propolis is reported to contain variety of chemical constituents such as flavonoids, esters of phenolic acids, terpenoids and many aminoacids (Burdock, 1998).

CAPE have been investigated for activities which include anti-bacterial, anti-fungal, anti-cancer, anti-inflammatory and anti-viral (Son and Lewis, 2002; Koltuksuz et al., 2001; Michaluart et al., 1999; Borrelli et al., 2002; Frenkel et al., 1993; Huang et al., 1996; Yang, 2005). LEE et al. (2008) while investigating the chemopreventive role of CAPE, found that the molecule exerted anti-metastatic effect via inhibiting levels of both metalloproteinase i.e (MMP-2, MMP-9). Since CAPE inhibits MMPs which contributes to be a factor in degradation of ECM the aim of work was to measure the effect of CAPE on advancement of gliomas using In vitro and In vivo approaches.

Materials and methods
Materials and reagents

CAPE was procured from Sigma-Aldrich Co. LLC. Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco USA and Ham's F12 nutrient mix from BRL USA, streptomycin and penicillin were procured from Sigma-Aldrich USA. Antibodies required for evaluation of MMPs for study were obtained from Sigma-Aldrich USA. Bovine serum albumin, transferrin, and other reagents were obtained from Sigma-Aldrich (USA).

The cancerous cell lines were procured from American type culture collection (USA), mouse colon cancer cells CT-25 were purchased from Korean Cell line Bank (Korea). All the animal protocols were sanctioned and were in accordance to animal ethical committee guidelines of Lanzhou General Hospital, Lanzhou, the approval number was LGH/415B/16/2016.

Cancerous Cell lines (SW480 and CT25)

For the study human colon cancer cells, that is, SW480 were selected and were procured from American Type Culture Collection (USA), mouse colon cancer cells CT-25 were purchased from Korean Cell line Bank (Korea). Both the cell lines, that is, SW480 and CT-25 were cultured in mixture of DMEM and F12 medium added with fetal bovine serum (FBS) (100 ml/L), penicillin 100000 U/L, Streptomycin (100 mg/L). After which the medium was subjected to replacement after 2-3 days regularly. For evaluating anti proliferative activity of CAPE on cancerous cells in vitro the SW480 were subjected to plating at with 2.5×10^4 cells /ml in a plate with 24 wells filled with mixture of DMEM, FBS (10%) and F12. The cells were submitted to 48 hr, during which the monolayers were serum starved, the medium was supplemented with bovine serum albumin 1 mg/ml, transferrin 5μg/ml and selenium 5ng/ml, for 24 hours. Obtained monolayers due to serum starvation were subjected to incubation in a serum free medium (SFM) along with various concentrations of CAPE (0,6,8 and 10 μmol/L) (Chih-Pin et al., 2012). Count of viable cell was done at interval of 24,48 and 96 hour opting assay of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Kim et al., 2003).

Cell Migration studies by boyden chamber assay

Cell migration studies were done by assessing the motility using boyden chamber (Lee et al., 2008). For the study PVPF filters were used having pore size of 8 μm, the filters coated with solution of 0.1% gelatin and were kept at 16 hours at room temperature conditions. The cancerous SW480 cells were suspended in SFM and Phorbol 12-myristate 13-acetate (PMA) (100 μg/L) and various concentrations of CAPE (0,6,8 and 10 μmol/L) in the chambers above, while the chambers below were filled up with FBS (10%). Boyden chamber was subjected to incubation of 16 hours at 37°C with 5% CO_2. The chamber was coated with 10 g/ml collagen (type 1) undersurface of filter. The cells were accorded to migrate for 24 hours followed by fixing them and subjecting to Hema-3 staining. The cancerous cells present on the lower side were fixed on glass slide. Counting was done by selecting five random microscopic areas at 400x.

Cell invasion assay

Cell invasion studies were done using invasion chambers which were pre-coated with Matrigel, the assay was done in accordance to previously described (Lee et al. 2008). The procedure started by filling Matrigel chambers (BD BioSci USA), with SFM at room temperature conditions. SW480 cells at concentration of 1×106 cell/mL were again suspended in SFM in presence and absence of PMA (100 μg/ L) and exposing them to various dilutions of CAPE as defined previously (0,6,8 and 10 μmol/L). Precisely instilled to upper portion and the lower side were loaded with 10% FBS for attracting cells. The chambers at room temperature were incubated for 16 hours in presence of CO_2 (5%). After 16 hours of incubation the cells present on the upper side were withdrawn by whipping the filters. The filters were then submitted to Diff-Quick staining followed by fixing of cells on lower surface of filters on a glass slide. Microscopic counting was done by selecting five randomly identified fields at 400x.

Evaluation of Matrix metalloproteinase (MMP) activity opting gelatin zymography

For evaluating MMP activity cancerous cells (1×10^6 cells/mL) were subjected to seeding in plates with 6 wells and were incubated in FBS medium (10%) for 48h. After seeding the cancerous cells were incubated to various concentrations of CAPE (0,6,8 and 10 µmol/L) for 12h. After 12 hours the collected supernatants were concentrated using Millipore filters. The concentrated supernatants were evaluated for MMP activity opting gelatin zymography by mixing each sample two times of its volume with buffer. The gel zymography was done using polyacrylamide gel (10%) and gelatin (1%). Coomassie blue staining was done to visualize MMP activity.

Western blot analysis

Expression of MMP activity in cultured cancerous cells was done by western blot method (Lee et al. 2008). The media of cells previously conditioned was obtained and processed similarly as described for gel zymography. The proteins were isolated on gel electrophoresis composing of SDS polyacrylamide by gradient mode and were shifted to membrane of immobilon-P (Millipore). The immobilon-P membranes obtained were blocked for 1 hour at room temperature conditions using milk (5%) in TBST composing Tween-20 0.1%, Tris-HCl 20mmol and NaCl 137 mmol/L and opting pH 7.4 and subjecting to incubation for 3 hours along with anti antibodies of MMP-2 and MMP-9 (1:200). Followed by incubation the membranes were washed at room temperature with TBST for 30 min. The membranes were incubated along with anti-mouse IgG horseradish peroxidase and mixtre of 5% milk and TBST in dilution ratio of 1:1000. The bound antibodies were observed by chemiluminescence. Intensity of immunoreactive bands was found by densitometry.

In-Vivo experimental metastasis in BALB/c mice's

In vivo studies for experimental metastasis were done as per procedure described previously (Soel et al., 2007). For the study mature male BALB/c mice of age 8 weeks averaging 22-25 g in weight were chosen. The animals were obtained from Yangzhou Uni. Comparative Medical Center (Yangzhou, China) and were housed in standard laboratory conditions supplied with free access to standard food and water ad libitum. The mice were divided into 5 groups randomly. CAPE was administered orally at a dose of 20 mg/kg (Parke et al. 2004). The animals were maintained throughout in accordance to animal ethical committee guidelines of Lanzhou General Hospital, Lanzhou. Lung metastasis was induced by injecting animals with suspension of CT-26 cells (5×10^5 cells/mL.) administered intravenously by tail. After injecting the cancerous cells the mice were treated with CAPE (20 mg/kg of body weight) orally for period of 3 weeks, the animals were then sacrificed for removing lungs. The lung tissues were processed for sections and subjected to staining with Bouin's solution followed by counting for presence of colonies (metastatic). For evaluating the activity of MMP in plasma 2 µL of plasma was collected and mixed along with 4 µL of buffer followed by gel zymography of 10% polyacrylamide and gelatin (1%). The activity of MMPs was seen by subjecting the slides with Coomassie blue staining.

Statistical evaluation

Statistical evaluation of data was done by using Graph pad prism. The results were obtained as mean with standard deviations. Significance of results was established for $P<0.05$.

Results
CAPE exhibits anti-proliferative effect on SW480 cells

In the experiment to evaluate effect of CAPE on proliferation of cancerous SW-480 cells the cells present in the monolayer culture were subjected to incubation in SFM at various concentrations of CAPE (0,6,8 and 10 µmol/L) after which the number of viable cells were counted. The effect of CAPE on cell proliferation was time dependent, no significant changes were observed on incubation upto 24 h. the cell proliferation decreased significantly after 48 and was highest after 96 h at 10 µmol/L (Figure 1). In evaluating the anti-metastatic effect of CAPE on cancerous SW480 cells the results were independent of factors such as decreased cell proliferation, CAPE incubation time in all the experiments of metastasis, the time was never more than 48h
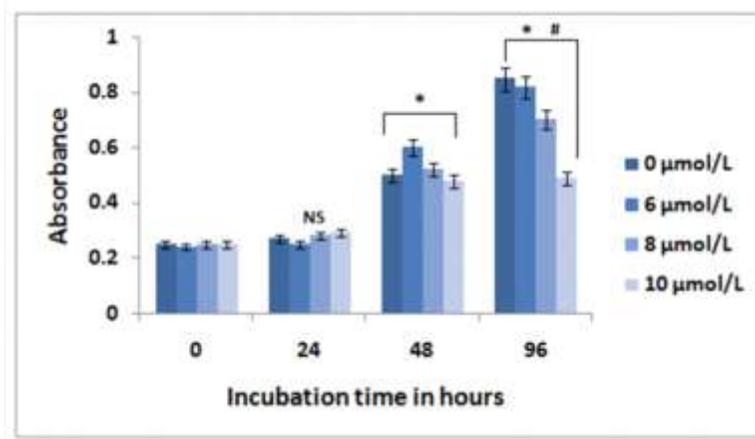
Figure 1: Effect of CAPE on proliferation of cancerous SW 480 cells. Vibale cell count was done by assay of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide. Results are presented as mean ± standard deviation calculated from 3 experiments independently. NS: Not significant, *P<0.05 compared to results of 0 hr. #P<0.05 compared to results of 48 hr.

Effect of CAPE on motility and invasion of cancerous SW480 cells

It has been previously established about role of cell migration in process of metastasis were the former playing an important role. PMA is established agent reported to cause tumor cell migration/invasion (Huang et al. 2004), in the current context we evaluated effect of CAPE on PMA mediated migration of cancerous SW480 cells. The study was carried using Boyden chamber and Matrigel invasion assay. The results (Fig 2 and Fig 3) showed incubation with CAPE significantly reduced (P<0.05) PMA induced motility and invasion of cancerous SW-480 cells.
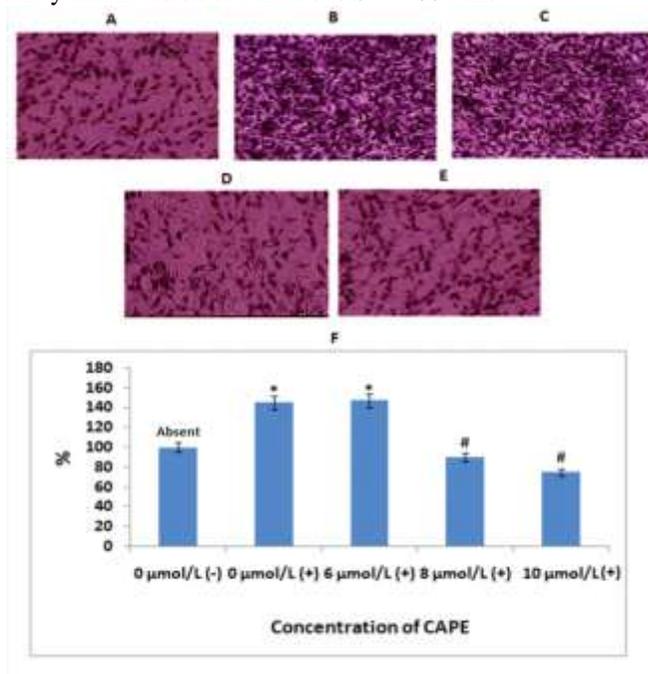

Figure 2: Effects of various concentrations of CAPE on PMA mediated motility in SW 480 cancerous cells. A: Untreated and not induced to PMA, B: PMA induced and untreated, C, D and E: PMA induced and treated with 6μmol/L, 8μmol/L and 10μmol/L of CAPE respectively. F: Results of cell motility assay.
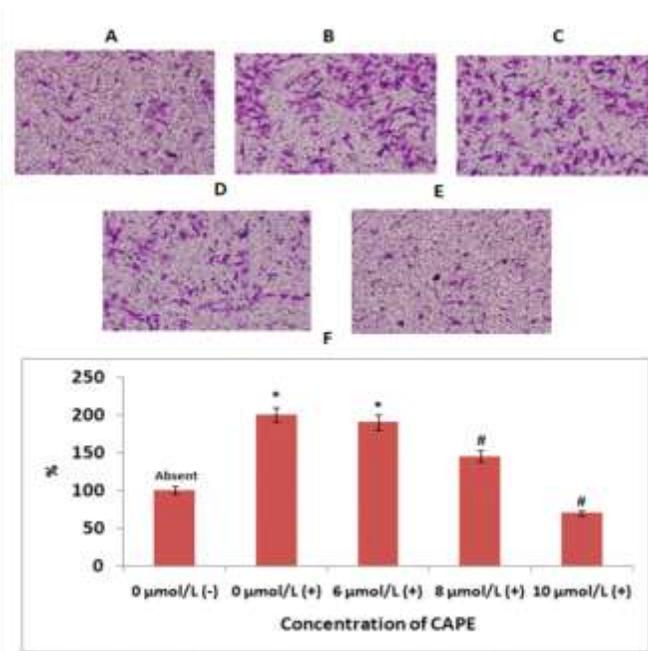
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Figure 3: Effects of CAPE on PMA mediated invasion of SW480 colon cancer cells.

CAPE reduces MMP-2 and MMP-9 activity in SW480 cancerous cell

PMA is established to increase activity of both MMP-2 and MMP-9 (Meyer et al. 2005; Han et al. 2000). The activities in SW480 cancerous cells exposed to PMA and incubated with CAPE. Results of study demonstrated decreased activity of both MMP-2 and MMP-9 with all concentrations of CAPE with significant results (Fig. 4) ($P<0.05$) were shown in dose dependent manner compared to untreated cells.
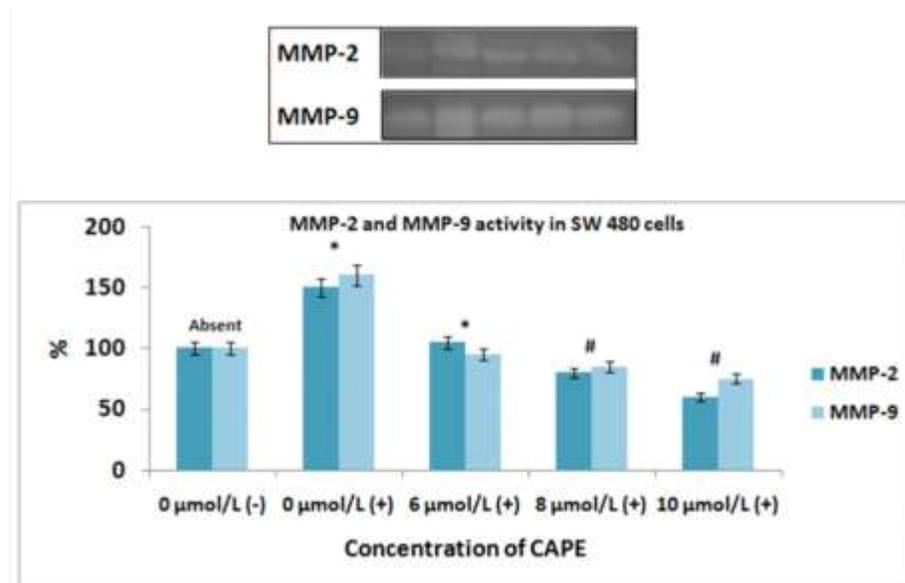

Figure 4: Effects of CAPE on PMA mediated MMPs activity

Inhibitory effect of CAPE on expression of protein MMPs (MMP-2 and MMP-9)

The effect of CAPE on expression of proteins matrix metalloproteinase was assayed by western blot technique in cancerous SW480 cells. Both MMPs were over expressed in PMA exposed cells whereas significant decrease ($P<0.05$) in expression of both proteins was in a dose mediated pattern linearly (Figure 5).
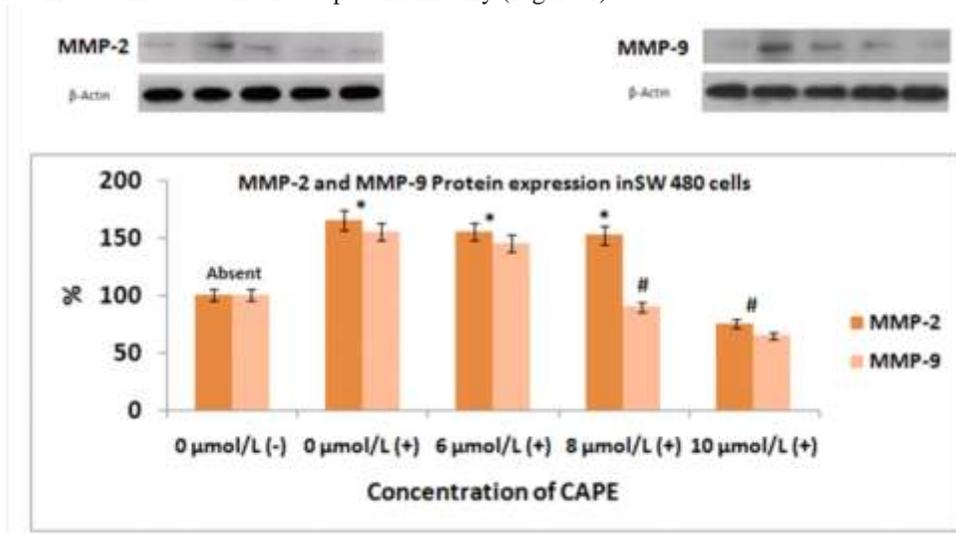

Figure 5: Effects of CAPE on PMA mediated MMPs expression in SW480 cells.

CAPE decreases metastasis in colon of BALB/c mice

For the study BALB/c mice were selected randomly and were induced to metastasis by CT-26 cells and were subjected to CAPE treatment. The cancerous cells and CAPE treatment had no effects on weight of animals (table 1 and Figure 6). CAPE treatment significantly reduced colonies induced by cancerous cells in lungs ($P<0.05$). CAPE treatment significantly reduced the plasma activity of both MMP-2 and MMP-9 ($P<0.05$) in mice (Figure 7).
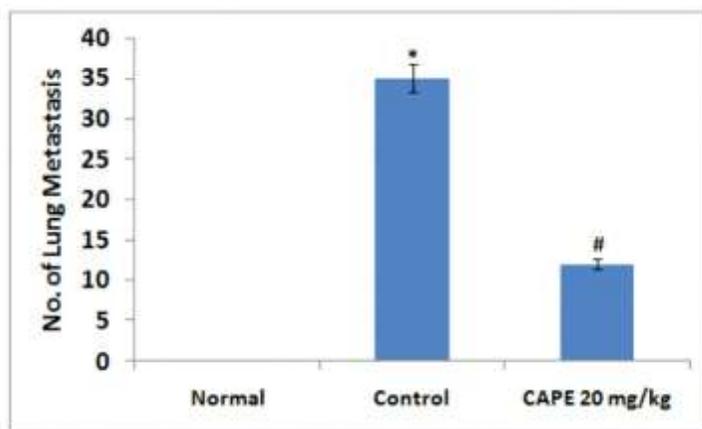

Figure 6: CAPE inhibits lung colonization by CT- 26 cells in BALB/c mice

Table 1: Effect on body weight of BALB/c mice

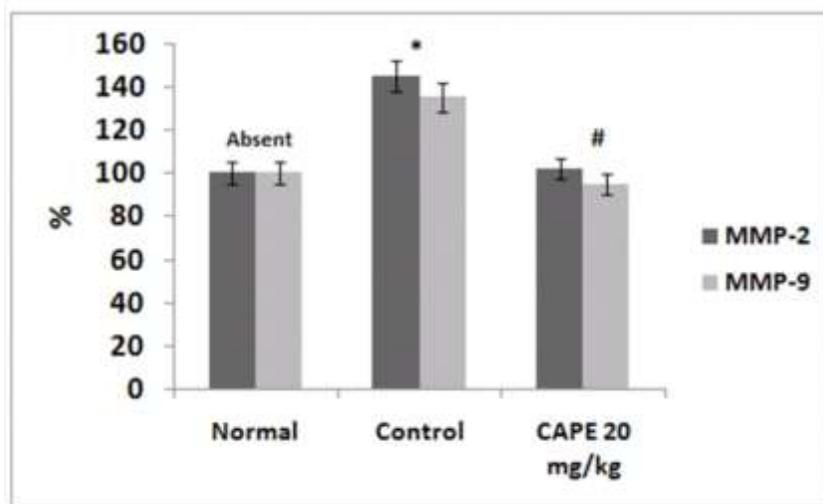| Groups	Body weight (g) (Initial)	Body weight (g) (Final)
Normal	22.10 ± 0.5	21.05 ± 0.60
Control	22.12 ± 0.2	22.10 ± 0.22
CAPE (20 mg/kg)	22.20 ± 0.1	22.15 ± 0.20

Figure 7: Effects of CAPE on MMPs activity of CT-26 cancerous cells in tumor-inducted BALB/c mice.

Discussion

Metastasis of colon tumors has contributed largely causing deaths of patients suffering from colon cancer. Associated toxicity of synthetic anticancer agents in treatment regimen is the biggest drawback. Therefore, research on natural compounds possessing anti-metastatic activity and lesser side effects is demanding. Current research is first to establish anti-metastatic role of CAPE both in-vitro and in-vivo.

In the current research CAPE significantly reduced proliferation of SW480 cells after 48 h of incubation with highest anti-proliferative effect achieved post 96 h of incubation. CAPE has been reported to show cytotoxic activity for cancer cell lines for breast cancer whereas it has shown no toxicity against normal cells (Wu et al., 2011; Omene et al., 2012). CAPE has shown to inhibit growth both in-vivo and in-vitro of MDA-MB 231, MCF-7 and BC cells (Iishi et al., 1997) (Harlozinska, 2005). In this study, the aim was to evidence anti-metastatic role of CAPE which was independent to suppression of tumor cell growth. Results of study showed that CAPE minified PMA mediated mobility and invasion; treatment also suppressed levels of MMPs and expression of proteins in cancerous cells suggesting the suppressive action of CAPE on metastasis was autonomous of growth of tumor cells.

One of the main step in metastasis is tumor invasion which also include adhesion of tumor cells, migration followed by degradation of ECM and basement membrane mediated by enzyme (Harlozinska, 2005). Cell migration and invasion includes MMPs and serine proteinase mediated extracellular proteolysis (Pepper 2001). PMA has been found to be a potent promoter of tumor via migration/invasion of tumors cells causing increased MMPs activity (Torricelli et al., 2006). PMA is also reported in regularize number of cellular activities by protein kinase pathways (Lee et al., 2002) which can play a possible role in invasion and metastatic characters of cancerous cells (Heider et al., 2004). In the study, it seems that CAPE curbs motility and also the invasion via subduing activation and expression of genes of MMPs in cancerous SW480 cells by protein kinase pathway. Oral intake of CAPE reduced the count of nodules in mice's and plasma activity of both matrix metaloprotenase (MMP-2, MMP-9). It was found that activity was dose dependent. Studies conclude role of MMPs to play important role in metastasis of cancer (Shibata et al. 2001).

In conclusion of experiments, the study has proved that CAPE inhibits metastatic character of cancerous SW480 cells in vitro also the oral treatment in BALB/c mice inhibits metastasis of cancerous CT-26 cells. Treatment with CAPE may cause inhibition of metastasis by diminishing both activity and expression levels of MMPs (MMP-2, MMP-9).

Declaration: Authors declare that this research presents no conflict of interests.

References

1. Borrelli F, Izzo A.A., Di Carlo, G., Maffia, P., Russo, A. (2002) Effect of a propolis extract and caffeic acid phenethyl ester on formation of aberrant crypt foci and tumors in the rat colon. Fitoterapia., 73 Suppl 1: S38-43.
2. Burdock, G.A. (1998) Review of the biological properties and toxicity of bee propolis (propolis). Food Chemcal Toxicology., 36: 347-363.
3. Chih-Pin, C., Hui-Ping, L., Mark, F.C., John, M.K., Ronald, J., Hause, J., Richard, A.H., Shutsung, L., Richard, B.J. (2012) Caffeic Acid Phenethyl Ester Suppresses the Proliferation of Human Prostate Cancer Cells through Inhibition of p70S6K and Akt Signaling Networks. Cancer Prevention Research. 5(5):788-797.
4. Forsyth, P.A.,Wong, H., Laing, T.D., Rewcastle, N.B., Morris, D.G., Muzik, H. (1999) Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas. British Journal of Cancer., 79:1828–35.
5. Frenkel, K., Wei, H., Bhimani, R., Ye, J., Zadunaisky, J.A. (1993) Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. Cancer Research., 53: 1255-1261.
6. Han, S.Y., Lee, M.S., Kim, H.R., Baek, S.H., Ahn, D.H., Chae, H.S., Erickson, R.H., Sleisenger, M.H., Kim, Y.S. (2000) Phorbol 12-myristate 13-acetate induces alteration in mucin gene expression and biological properties of colon cancer cells. International Journal of Oncology., 17:487-494.
7. Harlozinska, A. (2005) Progress in molecular mechanisms of tumor metastasis and angiogenesis. Anticancer Research., 25:3327-3333.
8. Heider, I., Schulze, B., Oswald, E., Henklein, P., Scheele, J., Kaufmann, R. (2004) PAR1-type thrombin receptor stimulates migration and matrix adhesion of human colon carcinoma cells by a PKCepsilon-dependent mechanism. Oncology Research., 14:475-482.
9. Huang, M.T., Ma, W., Yen, P., Xie, J.G., Han, J. (1996) Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA and protein in HeLa cells. Carcinogenesis., 17: 761-765.
10. Huang, Q., Shen, H.M., Ong, C.N. (2004) Inhibitory effect of emodin on tumor invasion through suppression of activator protein-1 and nuclear factor-kappaB. Biochemical Pharmacology., 68:361-371.
11. Iishi, H., Tatsuta, M., Baba, M., Uehara, H., Nakaizumi, A., Shinkai, K., Akedo, H., Funai, H., Ishiguro, S., Kitagawa, I. (1997) Inhibition by ginsenoside Rg3 of bombesin-enhanced peritoneal metastasis of intestinal adenocarcinomas induced by azoxymethane in Wistar rats. Clincal Experimental Metastasis.,15:603-611.
12. Kim, E.J., Kang, I.J., Cho, H.J., Kim, W.K., Ha, Y.L., Park, J.H. (2003) Conjugated linoleic acid downregulates insulin-like growth factor-I receptor levels in HT-29 human colon cancer cells. Journal of Nutrition.133:2675-2681.
13. Koltuksuz, U., Mutu, H.M., Kutlu, R., Ozyurt, H., Cetin, S. (2001) Effects of caffeic acid phenethyl ester and epidermal growth factor on the development of caustic esophageal stricture in rats. Journal of Pediatric Surgery., 36: 1504-1509.
14. Lee, H.S., Seo, E.Y., Kang, N.E., Kim, W.K. (2008) [6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. Journal of Nutritional Biochemistry.,19:313-319.
15. Lee, H.W., Ahn, D.H., Crawley, S.C., Li, J.D., Gum, J.R., Basbaum, C.B., Fan, N.Q., Szymkowski, D.E., Han, S.Y., Lee, B.H. (2002) Phorbol 12-myristate 13-acetate up-regulates the transcription of MUC2 intestinal mucin via Ras, ERK, and NF-kappa B. Journal of Biological Chemistry.,277:32624-32631.
16. LEE, K.W., Nam, J.K., Jong, H.K., Kyoung, M.L., Dong, E.L., Haeng, J.H., Hyong, J.L. (2008) Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B. Genes and Nutration., 2(4): 319–322.
17. Meyer, E., Vollmer, J.Y., Bovey, R., Stamenkovic, I. (2005) Matrix metalloproteinases 9 and 10 inhibit protein kinase C-potentiated, p53-mediated apoptosis. Cancer Research., 65:4261-4272.
18. Michaluart, P., Masferrer, J.L., Carothers, A.M., Subbaramaiah, K., Zweifel, B.S. (1999) Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer Research., 59: 2347-2352.
19. Okegawa, T., Pong, R.C., Li, Y., Hsieh, J.T. (2004) The role of cell adhesion molecule in cancer progression and its application in cancer therapy. Acta Biochimica Polonica.,51:445-457.
20. Omene, C.O., Wu, J., Frenkel, K. (2012) Caffeic Acid Phenethyl Ester (CAPE) derived from propolis, a honeybee product, inhibits growth of breast cancer stem cells. Invest New Drugs., 30:1279-1288.
21. Park, J.H., Lee, J.K., Kim, H.S., Chung, S.T., Eom, J.H., Kim, K.A., Chung, S.J., Paik, S.Y., Oh, H.Y. (2004) Immunomodulatory effect of caffeic acid phenethyl ester in BALB/c mice. International Immunopharmacology. 4(3):429-36.

22. Pepper, M.S. (2001) Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arteriosclerosis Thrombosis and Vascular Biology., 21:1104-1117.
23. Pepper, M.S., Montesano, R., Mandriota, S.J., Orci, L., Vassalli JD. (1996) Angiogenesis: a paradigm for balanced extracellular proteolysis during cell migration and morphogenesis. Enzyme Protein., 49:138-162.
24. Rujirek, C., Siriwan, O., Phorani, B., Prachya, K., Ampai. P., Vichai, R. (2013) Active compound of Zingiber Cassumunar roxb. down-regulates the expression of genes involved in joint erosion in a human synovial fibroblast cell line. African Journal of Traditional Complementary Alternative Medicine. 10(1):40-48.
25. Shao-Rong, H., Xiao-Yan, Lv., Yan-Ming, W., Hai, G., Cong, Z., An-Na, T., Ning, Y. (2013) A Study on the effect of aqueous extract of lobelia chinensis on colon precancerous lesions in rats. African Journal of Traditional complementary alternative medicine., 10(6):422-425
26. Shibata, S. (2001) Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. Journal of Korean Medical Sciences.,16 Suppl: S28-S37.
27. Soel, S.M., Choi, O.S., Bang, M.H., Yoon, J.H., Kim, W.K. (2007) Influence of conjugated linoleic acid isomers on the metastasis of colon cancer cells *in vitro* and *in vivo*. Journal of Nutritional Biochemistry., 18:650-657.
28. Son, S., Lewis, B.A. (2002) Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure-activity relationship. Journal of Agricultural Food Chemistry. 50: 468-472.
29. Stetler-Stevenson, W.G., Yu, A.E. (2001) Proteases in invasion: matrix metalloproteinases. Cancer Biology., 11:143–52.
30. Torricelli, C., Fortino, V., Capurro, E., Sacchi, G., Ponzo, P., Pacini, A., Muscettola, M., Maioli, E. (2006) Role of PTHrp and PTHrp-engaged pathways in MCF-7 cells migration/invasion. Matrix Biology., 25:104-111.
31. Vince, G.H.,Wagner, S., Pietsch, T., Klein, R., Goldbrunner, R.H., Rosen, K. (1999) Heterogeneous regional expression patterns of matrix metalloproteinases in human malignant gliomas. International Journal of Devlopmental Neuroscience., 17: 437–45.
32. Wang, C.Z., Li, X.L., Wang, Q.F., Mehendale, S.R., Fishbein, A.B., Han, A.H., Sun, S., Yuan, C.S. (2009) The mitochondrial pathway is involved in American ginseng-induced apoptosis of SW- 480 colon cancer cells. Oncolgy Reports.,21:577-584.
33. Wang, M., Wang, T., Liu, S., Yoshida, D., Teramoto, A. (2003) The expression of matrix metalloproteinase-2 and -9 in human gliomas of different pathological grades. Brain Tumor Pathology., 20:65–72.
34. Wu, J., Omene, C., Karkoszka, J., Bosland, M., Eckard, J. (2011) Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. Cancer Letters., 308: 43-53.
35. Yang, C., Wu, J., Zhang, R., Zhang, P., Eckard, J. (2005) Caffeic acid phenethyl ester (CAPE) prevents transformation of human cells by arsenite (As) and suppresses growth of As-transformed cells. Toxicology., 213: 81-96.