STUDY OF THE ANTICANCER POTENTIAL OF CELERY SEED OIL AGAINST CHEMICALLY INDUCED HEPATOCELLULAR CARCINOMA IN RATS: A MECHANISTIC APPROACH.

BY

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Abstract

Hepatocellular carcinoma (HCC), the major primary malignant tumour of the liver, represents a complex and fatal malignancy driven primarily by oxidative stress and inflammation. Celery seed oil (CSO) is a rich source of phytochemicals endowed with potent antioxidant and anti-inflammatory properties. In this study, we examined the molecular mechanisms by which CSO inhibited diethylnitrosamine (DENA)-induced hepatocellular carcinoma in rats by analysing the expression patterns of proliferating cell nuclear antigen (PCNA), nuclear factor-kappaB (NF-kB), cyclooxygenase-2 (COX-2), and caspase-3 immunohistochemically. In addition, serum TNF-α level and liver nitric oxide content were investigated. Rats were administered CSO (300 mg/kg, p.o.) four weeks after DENA-induced HCC. There was a significant elevation in serum TNF-α level and liver nitric oxide content, upregulation of PCNA, NF-kB and COX-2 in DENA-exposed rats. Administration of CSO decreased the expression of PCNA and increased the expression of caspase-3, indicating antiproliferative and apoptotic effects, respectively. Moreover, CSO markedly suppressed all aforementioned elevated inflammatory parameters. Our study provides evidence that CSO exerts its anticancer effect through antiproliferative, proapoptotic and anti-inflammatory mechanisms.

Key words: Celery, hepatocellular carcinoma, PCNA, COX-2, NF-kB, apoptosis.

Introduction

Hepatocellular carcinoma (HCC) is a malignant neoplasm of hepatocytes that is considered as the most common primary cancer of liver, one of the world's deadliest cancers and now the second prominent cause of cancer-related mortalities, resulting in 782,000 cases estimated to have occurred in 2012 (El-Serag 2011; Wallace *et al.* 2015). HCC is a distinctive type of cancer that typically arises in the setting of chronic liver disease at a rate dependent upon the complex interaction between the host, disease and environmental factors (Wallace *et al.* 2015).

Cancer arises as a result of genetic changes leading to insensitivity to antigrowth signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Changes in the expression of molecules that regulate cell cycle and cell proliferation pathways are disturbed in malignant cells resulting in

loss of control of cell proliferation. PCNA functions as auxiliary protein for DNA polymerase δ, which is required for DNA synthesis and cell cycle progression. Furthermore, the rate of PCNA synthesis is linked with the proliferative rate of cells (Oyama *et al.* 2002; Stoimenov and Helleday 2009; Strzalka and Ziemienowicz 2011).

Dysregulation of apoptosis increases susceptibility to enhanced cell proliferation and cell survival (Plati *et al.* 2011). Moreover, apoptosis evasion is recognized as a prerequisite for cancer cell to acquire permissive environment for angiogenesis, invasion and metastasis. (Diaz-Cano 2008; Hanahan and Weinberg 2011; Plati *et al.* 2011).

Increasing evidences from preclinical and clinical studies support that dysregulated inflammatory response plays a pivotal role in a multitude of chronic ailments including cancer (Ben-Neriah and Karin 2011). The molecular mechanism(s) by which chronic inflammation drives cancer initiation and promotion include increased production of pro-inflammatory mediators, such as cytokines, chemokines, reactive oxygen intermediates, increased expression of oncogenes, COX-2 (cyclo-oxygenase-2), 5-LOX (5-lipoxygenase), and pro-inflammatory transcription factors, such as NF-kB (nuclear factor-kappaB), that mediate tumour cell proliferation, transformation, metastasis, survival, invasion and angiogenesis (Luqman and Pezzuto 2010; Tan *et al.* 2011). Moreover, transcription factor nuclear NF-kB regulates the expression of a wide variety of genes involved in cellular events such as inflammation, immune response, proliferation, apoptosis and cancer invasion (Chaturvedi *et al.* 2011).

Therefore, medicinal plants and dietary phytochemicals that are capable of targeting multiple molecules in disease signalling pathways are considered as promising candidate for chemoprevention and chemotherapeutic protocols.

Celery (*Apium graveolens)* seeds locally known as "Karfas" have been widely used in traditional medicine for the treatment of liver and spleen diseases, jaundice rheumatism, gout, and other inflammatory disorders (Al-Asmari *et al.* 2014). The phytochemical screening of celery showed the presence of various chemical constituents such as flavonoids, phenolic acids, tannins, volatile oils, alkaloids, sterols and/or triterpenes (Al-Howiriny *et al.* 2010). Numerous studies reported that celery seeds possesses anti-inflammatory, antioxidant and cytotoxic properties (Ren and Lien 1997; Sultana *et al.* 2005; Powanda *et al.* 2015).

The anticancer effect of CSO against HCC in rats has been investigated in vivo (Sultana *et al.* 2005). However, the possible molecular and cellular mechanism of CSO against HCC is yet to be elucidated. In this study, we investigated the modulatory effects of CSO on the expression of various cellular markers, such as PCNA, caspase-3, COX-2 and the pro-inflammatory transcription factor NF-kB in DENA induced hepatocellular carcinoma in rats.

Materials and methods

Chemicals

Diethylnitrosamine (DENA) was purchased from Sigma Aldrich (St. Louis, MO, USA) and celery seed oil was obtained from a commercial source (Hashem Brothers for essential oils & aromatic products, Benisuef, Egypt). All other chemicals used were of the highest purity and analytical grade.

Animals and diet

Male Wistar albino rats weighing 90-120 g were used in the present study. They were obtained from the breeding colony maintained at the animal house of the Nile Pharmaceuticals Company (Cairo, Egypt). They were allowed an acclimatization period for at least one week prior to the experiment. Animals were kept under controlled environmental conditions; room temperature (24-27°C), constant humidity (60 ± 10%), with alternating 12 h light and dark cycles. Standard pellet diet and water were allowed ad libitum. All animals' procedures were performed in accordance with the ethical guidlines and policies approved by the Ethics Committee of Faculty of Pharmacy, Cairo University and complies with the *Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).*

Experimental design

After a period of adaptation, all animals except the normal group were intraperitoneally injected with DENA (20 mg/kg) five times weekly for 6 consecutive weeks to induce HCC (Ahmed *et al.* 2013). Animals were randomly assigned into a normal control group and diethylnitrosamine-induced HCC group (DENA). After tumour induction (6 weeks), the DENA group was distributed into 2 groups, a control HCC group and CSO treated group, each composed of 6-8 rats. CSO treatment was carried out by the daily oral administration of CSO (300 mg/kg) for four consecutive weeks from the 7^{th} week until the end of the 10^{th} week (Baananou *et al.* 2013) .

Blood sampling and serum preparation

At the end of the treatment period, blood samples were taken from retro-orbital sinus of rats under ether anaesthesia. Blood samples were allowed to clot at room temperature then serum was separated by centrifugation of blood at 3000 rpm for 15 minutes using a centrifuge (Hettich universal 32A, Germany). Serum samples were designated for the estimation of TNF-α and stored at -80ºC until analysis is performed.

Tissue sampling

Animals were then sacrificed by cervical dislocation, livers were carefully and rapidly excised.

Samples of the liver, from different lobes were homogenized in ice-cold saline, using a homogenizer (Heidolph Diax 900, Germany), to prepare 20% homogenate. The prepared homogenate was stored at -80 ºC until assayed later for estimation of the liver nitric oxide content. Samples from the remaining parts were fixed with 10% formaldehyde for the immunohistochemical examination. The dead bodies were frozen till incineration.

Estimation of Biochemical Parameters

Estimation of liver nitric oxide content

Liver homogenate was used for determination of nitric oxide content according to the method described by (Miranda *et al.* 2001).

Estimation of serum TNF-α level

Tumour necrosis factor alpha (TNF-α) was assessed using enzyme-linked immunosorbent assay (ELISA kits) according to the manufacturer specifications (R&D Systems, Inc., Minneapolis, USA).

Immunohistochemical analysis of liver tissue

Five µm thick sections were prepared from formalin fixed livers of different animal groups and immunohistochemistry was performed. Sections were placed on positively charged slides, deparaffinised, rehydrated and endogenous peroxidase activity was blocked with H_2O_2 in methanol. Sections were pre-treated in citrate buffer (pH 6.0) in a microwave. The sections were incubated with the mouse monoclonal antibody (PCNA) and various primary rabbit polyclonal antibodies NF-κB, COX-2 and caspase-3 (Thermo Scientific, USA). The sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with diaminobenzedine plus chromogen. The slides were visualized under light microscope and the extent of cell immunopositivity was assessed. The number of immunopositive cells was counted in 10 separate microscopic fields/animal, the results were expressed as percentage of total cell per field and the mean value for each slide was obtained, then the mean ± S.E.M. was calculated for each group (n=5) .

Statistical analysis

Data are expressed as means ± standard error (S.E.M). Comparisons between means were carried out using one way analysis of variance (ANOVA) test followed by Tukey-Kramer multiple comparison's test. For all statistical tests, the level of significance was fixed at $p< 0.05$. GraphPad Prism® software package, version 6 (GraphPad Software, Inc., USA) was used to carry out all statistical tests.

Results

Effect of celery seed oil on PCNA expression in DENA-induced HCC in rats.

As shown in [Figure (1)A-D], the immunohistochemical staining depicts the differential expression of levels of the proliferative marker, PCNA, in liver sections obtained from the various experimental rats groups. Near to complete absence of PCNA-positive cells was observed in normal control group [Figure (1)A]. An increase in hepatic PCNA expression was contrastingly observed in rats treated with DENA alone [Figure (1)B]. CSO (300 mg/kg) after HCC induction resulted in a significant decrease in PCNA expression relative to the untreated HCC rats [Figure (1)C].

[Figure (1)D] represent the quantitative analysis in the frequency of PCNA expressing hepatocytes in the different experimental group. In comparison to the DENA rats, CSO treatment is shown to significantly reduce the number of PCNA-positive cells by 41.64 %.

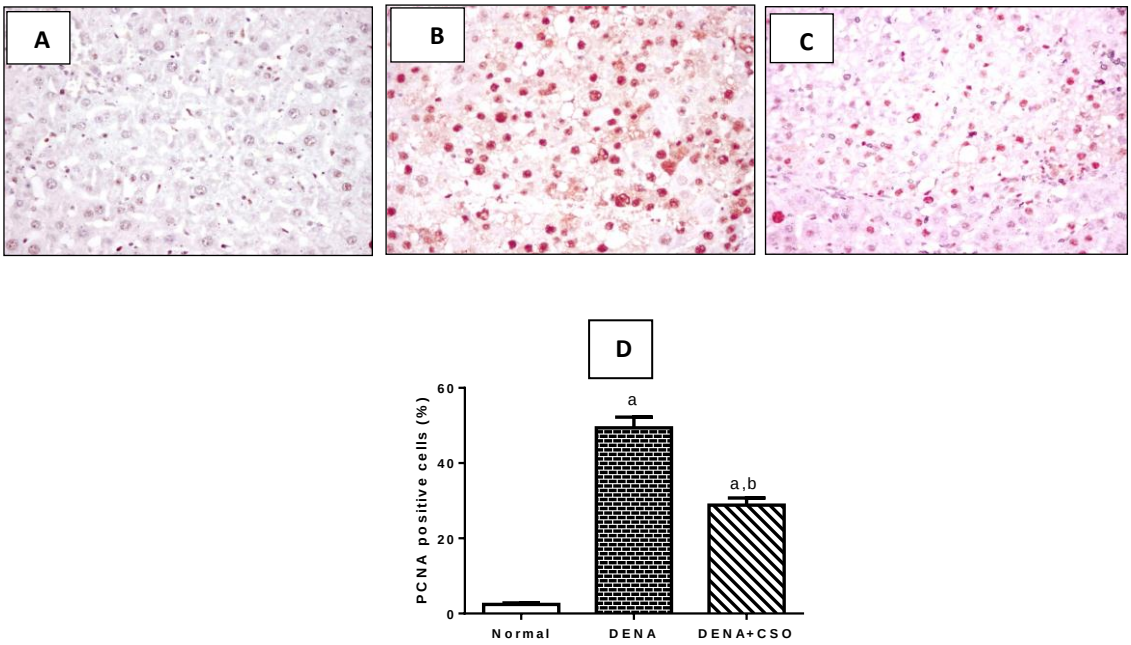

Figure (1): Effect of celery seed oil (300mg/kg) on PCNA expression in DENA-induced HCC in rats.

Representative photomicrograph of immunohistochemical staining of PCNA in rat liver from: (A, 400×) normal control group showing no expression of PCNA; (B, 400×) DENA-induced hepatocellular carcinoma showing a significant increase in PCNA immunoreactivity in the nucleus of hepatocytes; (C, 400×) DENA + CSO group showing a significant reduction in PCNA immunostaining. Brown colour indicates PCNA positivity; (D) percentage expression of PCNA.

DENA = diethylnitrosamine, CSO = celery seed oil and HCC= hepatocellular carcinoma .

Values are expressed as means ± S.E.M. (n=5).

The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

[a] Significantly different from control, [b] Significantly different from DENA at $p< 0.05$.

Effect of celery seed oil on caspase-3 expression in DENA-induced HCC in rats.

The changes in caspase-3 expression are illustrated in [Figure (2)A-D], caspase-3 positive cells was not detected in normal control rats [Figure (2)A]. However, DENA exposed rats showed a rise in caspase-3 expression relative to the normal control [Figure (2)B. CSO (300 mg/kg) administration displayed profound apoptotic activity and resulted in a marked upregulation of caspase-3 expression as compared to DENA-induced HCC rats group [Figure (2)C]. [Figure (2)D] represent the quantitative analysis of caspase-3 expressing hepatocytes in the different experimental groups. CSO treatment is shown to significantly augment (1.2 folds) the number of caspase-positive cells relative to the untreated DENA-induced HCC rats.

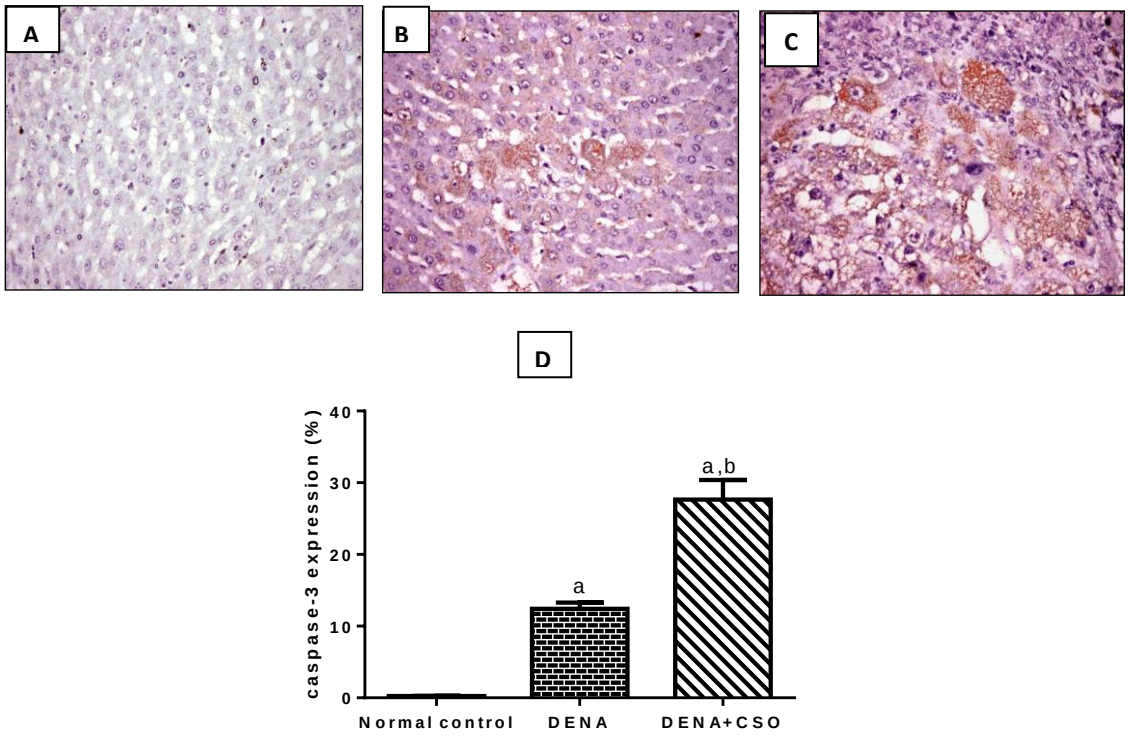

Figure (2): Effect of celery seed oil (300mg/kg) on caspase-3 expression in DENA-induced HCC in rats.

Representative photomicrograph of immunohistochemical staining of caspase-3 in rat liver from: (A, 400×) normal control group showing no expression of caspase-3; (B, 400×) DENA-induced hepatocellular carcinoman showing a significant increase in caspase-3 immunoreactivity in the cytoplasm of hepatocytes; (C, 400×) DENA + CSO group showing an additional significant increase in caspase-3 immunostaining. Brown colour indicates caspase-3 positivity; (D) percentage expression of caspase-3.

DENA = diethylnitrosamine, CSO = celery seed oil, HCC= hepatocellular carcinoma and ND = non detectable

Values are expressed as means ± S.E.M. (n=5).

The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

[a] Significantly different from control, [b] Significantly different from DENA at $p< 0.05$.

Effect of celery seed oil on serum TNF-α and liver nitric oxide in DENA-induced HCC in rats.

As shown in Table (1), induction of HCC by DENA significantly increased the serum TNF-α to (51.83 pg/ml) relative to the normal control (23.93 pg/ml). Additionally, liver nitric oxide content was markedly augmented to (201.26 μmol/g

liver**)** as compared to the normal control (79.97 µmol/g liver). CSO (300 mg/kg) administration after HCC induction resulted in a remarkable decrease in the upsurge of TNF-α serum level (25.97 %) and liver nitric oxide content (47.19 %) relative to the untreated DENA –induced HCC rats.

Table (1): Effect of celery seed oil (300 mg/kg) on serum TNF-α level and liver nitric oxide content in DENA-induced HCC in rats.

Parameters	Serum TNF-α (pg/ml)	Liver nitric oxide (µmol/g liver)
Normal control	**23.93 ± 1.49**	**79.97 ± 8.24**
(DENA) (20 mg/kg, i.p.)	**51.83 [a] ± 2.35**	**201.26 [a] ± 19.53**
DENA + CSO (300 mg/kg, p.o.)	**38.37 [a,b] ± 4.43**	**106.28 [b] ± 10.68**

DENA = diethylnitrosamine, CSO = celery seed oil, TNF-α = tumour necrosis factor alpha.

Values are expressed as means ± S.E.M. (n = 6-8).

The significance of the difference between means was tested by ANOVA followed by Tukey- Kramer multiple comparisons test.

* Significantly different from control, @ Significantly different from DENA at $p< 0.05$.

Effect of celery seed oil on NF-κB expression in DENA-induced HCC in rats.

[Figure (3)A-D] depicts the immunohistochemical staining of the differential expression of NF-κB, in liver sections obtained from the different rat groups. Absence of NF-κB was observed in normal control group [Figure (3)A]. However, a marked increase in hepatic NF-κB expression was observed in rats administered DENA alone relative to the normal control group [Figure (3)B]. Treatment with CSO (300 mg/kg) after HCC induction caused a significant decrease in NF-κB expression as compared to the HCC untreated rats [Figure (3)C]. [Figure (1)D] shows the quantitative analysis in the hepatic NF-κB expression in the different experimental group. Relative the DENA induced HCC rats, CSO treatment markedly reduced the number of NF-κB -positive cells by 48.51 %.

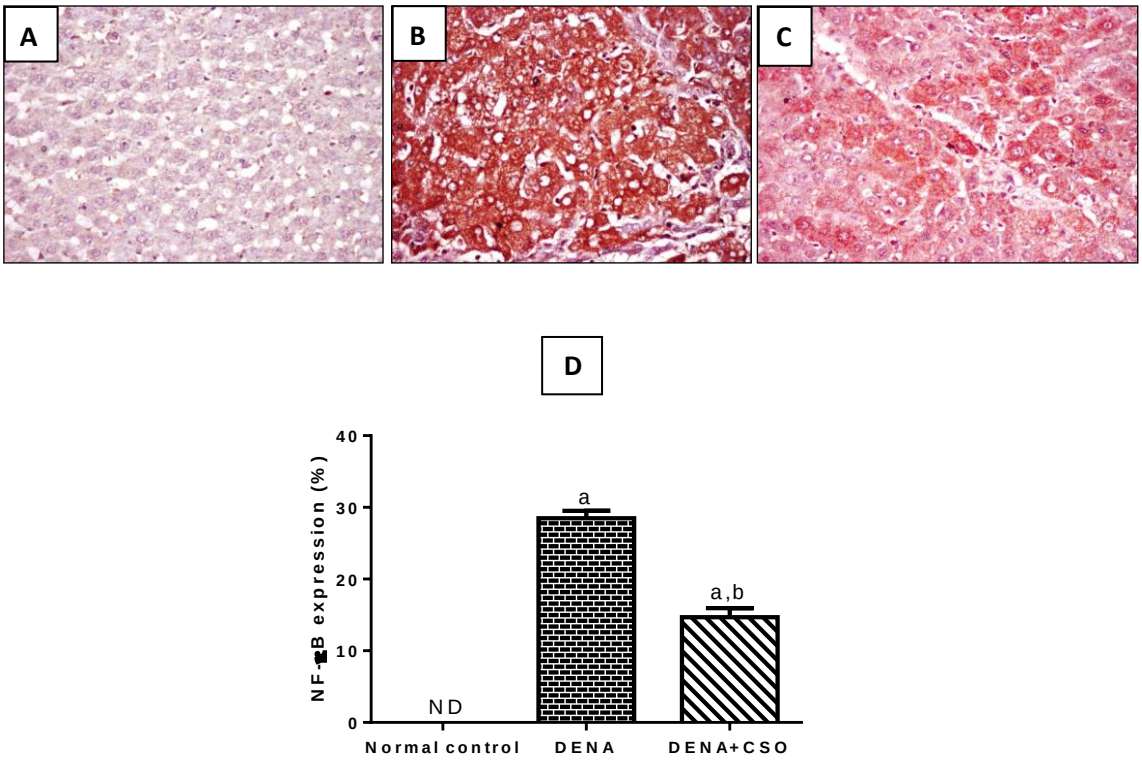

Figure (3): Effect of celery seed oil (300mg/kg) on NF-κB expression in DENA-induced HCC in rats.

Representative photomicrograph of immunohistochemical staining of NF-κB in rat liver from: (A, 400×) normal control group showing no expression of NF-κB; (B, 400×) DENA-induced hepatocellular carcinoma showing a significant increase in NF-κB immunoreactivity in the cytoplasm of hepatocytes; (C, 400×) DENA + CSO group showing a significant reduction in NF-κB immunostaining. Brown colour indicates NF-κB positivity; (D) percentage expression of NF-κB.

DENA = diethylnitrosamine, CSO = celery seed oil, HCC= hepatocellular carcinoma and ND = non detectable

Values are expressed as means ± S.E.M. (n=5).

The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

[a] Significantly different from control, [b] Significantly different from DENA at $p < 0.05$.

Effect of celery seed oil on COX-2 expression in DENA-induced HCC in rats.

As shown in [Figure (4)A-D], the immunohistochemical staining shows the differential expression levels of COX-2 in liver sections obtained from the various experimental rat groups. An almost complete absence of COX-2-positive cells was observed in normal control group [Figure (4)A]. An increase in hepatic COX-2 expression was contrastingly observed in rats treated with DENA alone [Figure (4)B]. Administration of CSO (300 mg/kg) after HCC induction resulted in a significant decrease in COX-2 expression [Figure (4)C].

[Figure (4)D] represent the quantitative analysis in the frequency of COX-2 expressing hepatocytes in the different experimental group. As compared to the DENA-induced HCC rats, CSO treatment is shown to significantly reduce the number of COX-2-positive cells to 49.79 %.

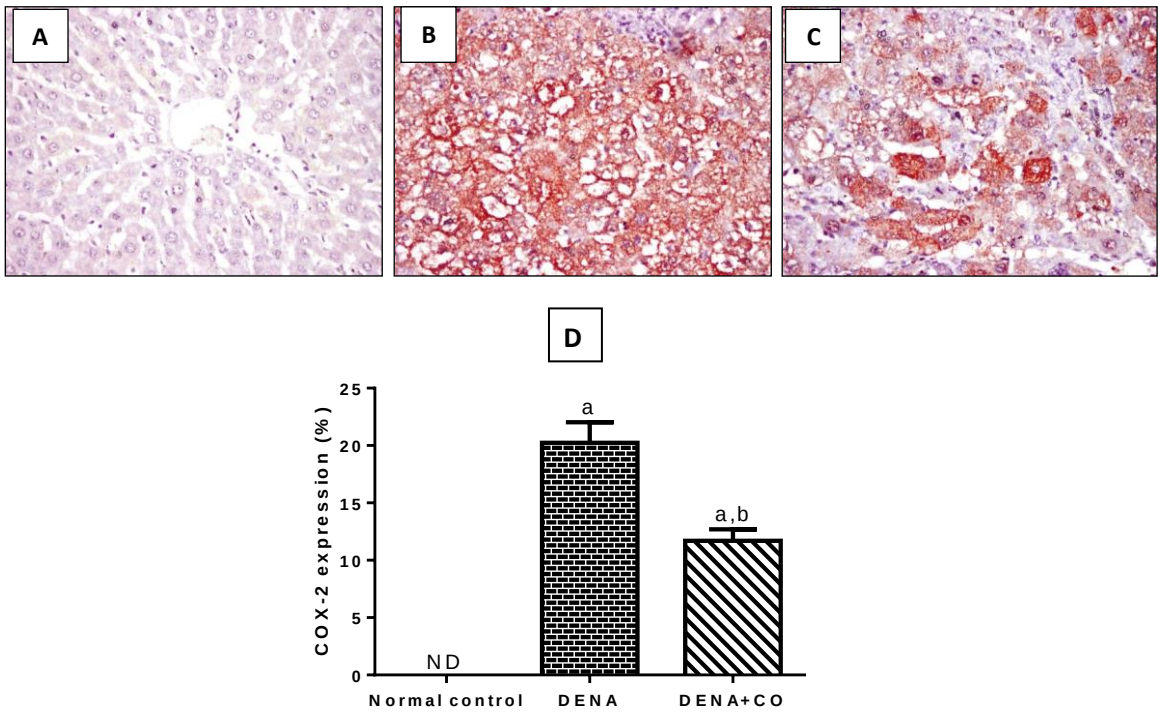

Figure (4): Effect of celery seed oil (300mg/kg) on COX-2 expression in DENA-induced HCC in rats.

Representative photomicrograph of immunohistochemical staining of COX-2 in rat liver from: (A, 400×) normal control group showing no expression of COX-2; (B, 400×) DENA-induced hepatocellular carcinoma showing a significant increase in COX-2 immunoreactivity in the cytoplasm of hepatocytes; (C, 400×) DENA + CSO group showing a significant reduction in COX-2 immunostaining. Brown colour indicates COX-2 positivity; (D) percentage expression of COX-2.

DENA = diethylnitrosamine, CSO = celery seed oil, HCC= hepatocellular carcinoma and ND = non detectable

Values are expressed as means ± S.E.M. (n=5).

The significance of the difference between means was tested by ANOVA followed by Tukey Kramer multiple comparisons test.

[a] Significantly different from control, [b] Significantly different from DENA at $p < 0.05$.

Discussion

DENA usually causes genomic damage in exposed cells. As a consequence, the damaged cells may be triggered to proliferate with genomic damage, leading to the formation of cancerous cells that showed apoptosis evasion, increased cell proliferation, angiogenesis, invasion and metastasis (Hanahan and Weinberg 2011). In the present study, we have demonstrated that CSO was capable of modulating important proteins involved in cell proliferation, inflammation and apoptosis resulting in suppression of hepatocarcinogenesis induced by administration of DENA.

Abnormal cell proliferation is the main feature of carcinogenesis, making inhibition of the excessive proliferation of tumour cells is an effective therapeutic approach. PCNA, a marker of cell proliferation, is associated with DNA synthesis phase of the cell cycle (Oyama *et al.* 2002). The positive expression of PCNA is considered a common index for hepatocyte proliferation at late G1- and early S-phase. Moreover, PCNA represents an important cellular marker for assessing the proliferation in hepatocellular carcinoma (Qin and Tang 2002). In our study, the expression of PCNA was examined immunohistochemically in livers from the different animal groups. DENA-treated animals resulted in a drastic increase in PCNA expression, indicating accelerated cell proliferation in rat liver tumour. In agreement with our findings, it has been reported that DENA enhances hepatocytes proliferation and augments PCNA expression in rats (Song *et al.* 2013). Significant decrease in PCNA-positive hepatocytes due to CSO treatment clearly indicates a decrease in hepatocyte proliferation, possibly through inhibition of DNA synthesis in experimentally induced HCC in rats. Our data showing diminished hepatic PCNA expression and enhanced cell proliferation are concordant with a previous study showing antiproliferative effect of celery seed extract in vivo cancer model (Sultana *et al.* 2005).

Apoptosis or programmed cell death is involved in tissue homeostasis through targeted elimination of cells without disrupting the normal physiological function of the tissue. Dysregulation of apoptosis disturbing the balance between cell proliferation and cell death are involved in the development and progression of hepatic cancer (Fabregat 2009; Schattenberg *et al.* 2011). Apoptosis detection in tumour has emerged as valuable diagnostic tool and induction of tumour cell death by apoptosis has been recognized as one of the essential objectives of liver cancer therapy (Karamitopoulou *et al.* 2007). Apoptosis enhancement in tumour-target tissues has been identified as an innovative mechanism of potential chemopreventive and chemotherapeutic drug.

In the present study, caspase-3 was immuhistochemically detected to identify cells undergoing apoptosis. Our results show a low order of hepatic caspase-3 expression in DENA-exposed rat livers indicating apoptosis evasion (Bhatia *et al.* 2013). CSO treatment showed a manifest increase in the caspase-3 expression, which provides considerable evidence of induction of cell death by apoptosis.

TNF-α, a potent pleiotropic proinflammatory cytokines, affects the growth, differentiation and survival of all cells (Anderson *et al.* 2004). Existing evidence

indicates that high levels of TNF-α can favour cell survival and tumour progression (Mocellin *et al.* 2005).

In the present study, there was a marked increase in serum TNF-α in DENA-induced tumour group animals. This finding is consistent with a previous study that DENA-induced HCC in rats led to an increase in serum TNF-α level (Song *et al.* 2013). The diminished level of TNF-α in rats with DENA-induced HCC by the administration of CSO may be related to its anti-inflammatory and anti-tumour activities (Atta and Alkofahi 1998; Sultana *et al.* 2005).

Increased nitric oxide production in liver tissue was reported to be involved in the pathogenesis of HCC (Bishayee *et al.* 2010; Lee and Lim 2012)**.** This can be explained by the ability of TNF-α to up-regulate the iNOS enzyme (Morris, Jr. and Billiar 1994; Du *et al.* 2009). Moreover, several studies on human cancer and animal tumour model have documented that increased tumour expression of COX-2, was associated with more aggressive lesions that sustain inflammation, tumour growth and metastasis (Noriyuki *et al.* 2007; Liao *et al.* 2010).

In agreement with previous studies, DENA caused a significant increase in the liver nitric oxide content (Bishayee *et al.* 2010; Lee and Lim 2012). CSO administration after HCC induction markedly diminished the elevated liver NO content, an effect that might be related to its anti-inflammatory activities (Momin and Nair 2002; Sultana *et al.* 2005)

Numerous studies reported that increased expression of NF-kB with subsequent inflammatory reactions were responsible for hepatic injury in HCC (Ueno *et al.* 2005; Yokoo *et al.* 2011)**.** It has been reported that activation of NF-kB can convert inflammatory stimuli into tumour growth signals that are mediated by cytokines, chemokines, prostaglandins, nitric oxide and leukotrienes, which contribute to tumour promotion by altering normal cellular signalling cascades (Morrison 2012). Additionally, elevated TNF-α is known to be an important step for activation of the NF-kB signalling pathway (Du *et al.* 2009; Shukla *et al.* 2011)**.** In the current investigation, HCC induction resulted in a remarkable increased expression of NF-kB. In agreement with our findings, DENA administration caused upregulation of NF-kB that binds to DNA and results in transcription of genes that contribute to tumourigenesis, such as cell proliferation, inflammatory, anti-apoptotic and positive regulators of cell proliferation (Sivaramakrishnan and Niranjali 2009; Khan *et al.* 2011). Treatment with CSO significantly suppressed the overexpression of NF-kB, an effect that may be related to its anti-inflammatory properties(Atta and Alkofahi 1998; Momin and Nair 2002)

Moreover, result from a different study have shown that NF-kB induces the activation of COX-2, a primary mediator of the inflammatory cascade, which ensues directly after hepatic cancer mediated liver injury (Sivaramakrishnan and Niranjali 2009; Bishayee *et al.* 2013). Studies have also shown that selective COX-2 inhibition exerted a chemopreventive effect in human colorectal carcinoma(Bottone, Jr. *et al.* 2004). In agreement with earlier studies, DENA-induced HCC resulted in a marked overexpression of COX-2 immunohistochemically (Bishayee *et al.* 2013; Bayomi *et al.* 2015). CSO treated HCC rat liver manifested a significant downregulation of COX-2 expression related to the untreated DENA-induced HCC animals, this ameliorative effect may be attributed to the cyclooxygenase inhibitory effect of celery seeds (Momin and Nair 2002).

Conclusion

Our study demonstrates that the inhibition of cell proliferation, upregulation of apoptosis and down regulation of inflammatory markers may be, at least in part, the underlying mechanisms related to the liver tumour inhibition by CSO. The present study allows us to conclude that CSO being a dietary supplement, may be a promising candidate against hepatocellularcarcinoma.

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دراسة الإمكانية المضادة للسرطان لزيت بذور الكرفس ضد سرطان الكبد المحدث كيميائيا في الجرذان: بحث الآلية

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سرطان الخلايا الكبدية، يمثل ورما خبيثا معقدا وقاتلا تكون في المقام الأول بسبب الاكسدة وإحداث التهابات في خلايا الكبد. و يحتوي زيت بذور الكرفس على العديد من المواد الكيميائية النباتية المضادة للأكسدة و للالتهابات. في هذه الدراسة، درسنا الآليات الجزيئية التي منع بها زيت بذور الكرفس سرطان الكبد المحدث كيميائيا بعقار الداي ايثايل نيتروزامين في الجرذان عن طريق تحليل مستوى تعبيرالبروتينات الخاصة بتكاثر الخلايا، و الالتهابات، والكاسباز في انسجة الكبد. وبالإضافة إلى ذلك، تم قياس مستوى TNF-α في الدم ومحتوى أكسيد النيتريك في الكبد. في هذه التجربة كانت تعالج الفئران بزيت بذور الكرفس (٣٠٠ ملغ / كغ) يوميا لمدة أربعة أسابيع بعد احداث سرطان الكبد في الجرذان. و قد لوحظ في ان مادة الداي ايثايل نيتروزامين تسببت في ارتفاع كبير في مستوى ال TNF-α في الدم وزيادة ملحوظة في محتوى أكسيد النيتريك في الكبد، بالاضافة الى زيادة تعبيرالبروتينات الخاصة بتكاثر الخلايا و الالتهاب في انسجة الكبد. معالجة الجرذان بزيت بذور الكرفس ادى الى انخفاض في التعبير عن البروتينات الخاصة بتكاثر الخلايا وزيادة التعبير عن الكاسباس-٣، مشيرا الى قدرة زيت بذرة الكرفس على منع تكاثر الخلايا و قدرته على تحفيز الأبوبتوزس، على التوالي. وعلاوة على ذلك ، فإن زيت بذور الكرفس قمع بشكل ملحوظ جميع دلائل التهابات مرتفعة التي تم قياسها. توفر دراستنا دليلا على أن زيت بذور الكرفس قد يكون له تأثير مضاد للسرطان من خلال الآليات المضادة لتكاثر الخلايا و المحفزة للأبوبتوزس والمضادة للالتهابات ايضا .