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ORIGINAL ARTICLE

Therapeutic Implications of Ajwa Dates (*Phoenix dactylifera*) in the Inhibition of Liver Tissue Alterations through the Modulation of Vascular Endothelial Growth Factor and Phosphatase, and Tensin Homolog Gene

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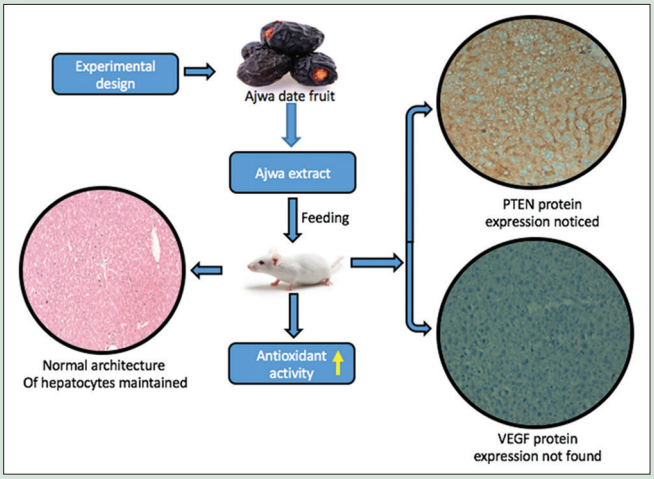
ABSTRACT

Background: Dates are a plant species in the palm family, *Arecaceae* and is used as staple food in the Middle East for thousands of years. **Aim:** The current study was undertaken to evaluate the protective role of ajwa dates extract (ADE) on carbon tetrachloride (CCl_4)-induced hepatotoxicity. **Materials and Methods:** The study was carried out on mice mode through different groups as control group without treatment of CCl_4, ADE and CCl_4-treated group, and CCl_4-treated group only. **Results:** This finding demonstrated that histological alterations including degeneration, congestion and infiltration of lymphocytes were seen in the liver tissue in CCl_4-treated groups. However, ADE-treated groups showed protection to attenuate CCl_4-induced liver toxicity through maintenance of architecture of hepatocytes as evident of congestion, necrosis, and degeneration was not noticed. Moreover, a few number of infiltrations of lymphocytes were noticed in ADE-treated groups. However, expression of phosphatase and tensin homolog (PTEN) and vascular endothelial growth factor (VEGF) protein was evaluated in all groups, and it was observed that PTEN was highly expressed in control group and ADE-treated group. Whereas, PTEN expression was also observed in CCl_4-treated group, but out of eight cases, one case showed less expression of PTEN protein. The difference in expression pattern of PTEN protein in CCl_4-treated group and ajwa-treated group was statically insignificant ($P > 0.05$). VEGF was not expressed in control and ajwa-treated group. While expression of VEGF protein was observed in CCl_4-treated group, and it was noticed that two out of eight cases showed expression of VEGF protein. **Conclusion:** The findings supported the idea that ADE might reduce liver tissue alterations or maintenance of architecture of hepatocytes. Ajwa dates-treated group showed decreased in the VEGF expression and such angiogenesis process involve in migration and differentiation of endothelial cells as well as prevent the loss of PTEN protein expression.

Key words: Ajwa dates, apoptosis, carbon tetrachloride, hepatoprotective activity, phosphatase and tensin homolog, vascular endothelial growth factor

SUMMARY

- Dates fruit is used as staple food in the Middle East for thousands of years and its play a significant role in the cure of various diseases
- The study was undertaken to evaluate the protective role of ADE on carbon tetrachloride (CCl_4)-induced hepatotoxicity
- ADE-treated mice showed protection to attenuate CCl_4-induced liver toxicity through maintenance of architecture of hepatocytes as evident of congestion, necrosis, and degeneration was not noticed
- Expression of PTEN and VEGF protein was evaluated in all groups, and it was observed that PTEN was highly expressed in control group as well ADE-treated group
- The findings supported the idea that ADE might reduce liver tissue alterations or maintenance of architecture of hepatocytes.



Abbreviations Used: CCl_4: Carbon Tetrachloride, VEGF: Vascular Endothelial Growth factor, IHC: Immunohistochemistry TUNEL assay: Terminal deoxynucleotidyl transferase dUTP nick end labeling, H and E: Hematoxylin-eosin staining, ADE: Ajwa dates extract.

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INTRODUCTION

Several factors such as exposure to toxic chemicals, environmental pollutants, and drugs cause cellular damage through reactive oxygen species metabolic activation.[1] Considerable evidence has concerned that inflammation and oxidative stress are the etiologies of liver injury[2] and carbon tetrachloride (CCl_4) notably enhances oxidative stress, hepatic inflammation, apoptosis, necrosis, and even liver cancer in mice.[3]

The present regime to liver diseases or liver cancer treatment, based on allopathic drugs are expensive, alters the various mechanisms of

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biological process and causes the toxic effect to the normal cells. Thus, recent evidence have established that natural products or derivatives of plant including *Cassia fistula*, olive fruits, and black seed shows pivotal role in the health management and killing of liver cancer cells.[4-6]

In this regards, dates fruits (*Phoenix dactylifera*) has proven therapeutic role in the management of several diseases.[7] *P. dactylifera* is one of the members of the palm family *Arecaceae*[8] and ajwa is types of dates that are only cultivated in Al-Madinah Al-Munawarah of Saudi Arabia. Its health-promoting effects have been mentioned in Islamic literature and are in practice to cure of diseases since very old time. Moreover, Prophet Muhammed (Peace Be Upon Him) said that the best assets are date palm, dates cure several disorders, Numerous pharmacological activities have been reported by earlier finding including tumor prevention, antioxidant, and anti-inflammatory activity. [9-12] Therefore, the present study was designed to examine the protective effects of ajwa dates fruits extract against CCl_4-induced liver toxicity and correlation of expression pattern of phosphatase and tensin homolog and vascular endothelial growth factor (VEGF) protein with the histopathological finding.

MATERIALS AND METHODS

Ajwa dates extract preparation

The ajwa dates fruits were purchased from Qassim region of Saudi Arabia, and extraction was made on the pulp of ajwa dates fruits. Ajwa pulp was separated from the fruits, dried, and extraction was performed as previous described methods with little modification.[13] Briefly, powder of dry pulp was made manually, and powered form of pulp was soaked in double-distilled water as 400 g powder pulp/liter of distilled water. Next morning, the solution was completely mixed and filtered and lyophilized to dryness through freezer dryer and stored at low temperature.

Animals grouping

This study was made on mice model and mice were housed in polypropylene cages. The mice model was kept under controlled environmental conditions of temperature (25°C) for 12 h light/dark cycles. Mice were acclimatized under these conditions for 1 week before the start of the experiments.

Mice were given normal chow and water *ad libitum* and the study was approved by the Animal Ethics Committee of College. After acclimatization, the mice animals were randomly grouped into three groups and carried out for 12 weeks as follows:

Group 1: 8 mice were selected as control group which received distilled water and chow.

Group 2: 8 mice served as the CCl_4-treated group twice in a weak.

Group 3: 8 mice: Ajwa dates extracts (ADE) (50 mg/kg/) body weight orally and CCl_4 administered orally three times per weeks.

Liver tissue samples of mice

A small part of the liver of the mice tissue was removed, thawed, and homogenized in buffer solution and centrifuged at high speed for 10 min. The supernatant was taken for the measurement of superoxide dismutase (SOD) and catalase enzyme activity.

Histopathological observation

A small part of livers were removed from each mouse and immediately fixed in 10% formalin. Tissues were processed and embedded in paraffin to make a paraffin-embedded block. Five micron serial sections from each block were made. Hematoxylin-eosin (H and E) staining was performed on sections to analyze the histological alterations including congestion, necrosis, fatty change, degeneration, and infiltration of lymphocytes under a light microscope.

Evaluation of phosphatase and tensin homolog and vascular endothelial growth factor protein expression

Expressional pattern of PTEN and VEGF protein was evaluated on each group through immunohistochemistry. Expressional evaluation of markers was performed on paraffin-embedded tissue sections as previously described method with minor medications.[14]

In brief, deparaffinization and rehydration were performed on each section. Endogenous peroxidase activity was blocked through the hydrogen peroxidase blocking agent (Abcam, USA). Moreover, protein blocking agent (Abcam, USA) was applied for 10 min to minimize the nonspecific binding. PTEN and VEGF monoclonal antibodies (Abcam, USA) were used as primary antibodies. Rest of the procedures including secondary antibody and tertiary antibody were performed according to manufactures guidelines. Finally, diaminobenzidine was applied on each section according to kits gridlines and section were counterstain with hematoxylin.

Interpretation of marker expression

Four different fields from section were chosen, and positive cells for PTEN and VEGF were counted and the mean percentage positivity was made. The cases were considered as positive for marker when more than of the cells showed expression either nuclear or cytoplasmic or both.

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is a method for detecting DNA fragments through labeling the 3′-hydroxyl termini. Apoptotically fragmented cellular DNA was identified by TUNEL assay (Apoptosis Detection Kit, Abcam, USA). Briefly, an enzyme called terminal deoxynucleotidyl transferase catalyzes the addition of dUTP nucleotides to the free 3' ends of fragmented DNA. Moreover, dUTPs that are labeled produce color product, and finally, apoptotic cells were identified by light microscope.

Statistical analysis

All values are expressed as mean ± SD. Comparison between any two groups was performed using one-way analysis of variance. A level of $P \leq 0.05$ was taken as statically significant. Chi-square $(\lambda)^2$ test was used to make the correlation of marker with histopathological findings.

RESULTS

Measurement of enzymes activities

Antioxidant enzyme activities were measured in all groups, and it was observed that level of antioxidant enzymes such as SOD and catalase were low in CCl_4-treated groups while ADE-treated group showed increased level of enzymes and that difference in enzymes level was statically significant ($P \leq 0.05$).

Analysis of liver tissue through hematoxylin and eosin staining

Microscopic analysis was made on all treated and untreated liver tissue to interpret the histological alterations. In control cases, the histological alteration was not seen, and normal architecture of hepatic lobules in the form of hepatocytes was maintained [Figure 1]. Whereas, in CCl_4-treated groups, liver tissue showed various types of histological alterations including congestion, loss of architecture of hepatocytes, degeneration, blood vessel dilation, infiltration of lymphocytes, [Figure 2a-c]. In addition, ADE with CCl_4-treated group mice did not show any severe

alteration ($P \leq 0.05$). High number of infiltration of leukocytes was observed in CCl_4-treated group, whereas it was not seen or few number of infiltration of leukocytes were observed in ADE with CCl_4-treated group ($P \leq 0.05$) [Figure 3].

Expressional evaluation of phosphatase and tensin homolog protein

Expression pattern of PTEN protein was examined in all groups including treated and untreated group. Control group showed high expression of PTEN protein and all control cases were positive for PTEN protein. In the CCl_4-treated group, all cases showed PTEN protein expression, but one case showed less expression as compared to control groups [Figure 4]. In the ADE-treated group, all cases were also positive or showed expression of PTEN protein [Figure 5]. The expression pattern of PTEN protein in CCl_4-treated group and ADE with CCl_4 group was statically insignificant ($P > 0.05$).

Immunohistochemical detection of vascular endothelial growth factor protein

The expression pattern of VEGF protein was examined in all studied groups. The cytoplasmic expression of VEGF was detected in 2 (25%) out of 8 cases of CCl_4-treated group [Figure 6], whereas untreated group (control cases) did not show any expression and all control cases were negative for VEGF protein. Moreover, expression of VEGF protein was not noticed in ADE-treated groups [Figure 7]. These findings supported the idea that ADE might reduce decreased angiogenesis process.

Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

Cells were evaluated through TUNEL assay for apoptosis (Apoptosis Detection Kit, Abcam, USA). Apoptosis was not noticed in treated and untreated cases [Figures 8 and 9].

DISCUSSION

The liver shows an important role in the biological system that is accountable for the metabolism and clearance of xenobiotics and drugs.[15] Several liver toxicant including CCl_4, nitrosamines, and polycyclic aromatic hydrocarbons needed metabolic activation, mainly by liver cytochrome P450 enzymes, to form reactive, toxic metabolites, finally that cause liver injury.[16]

Recent studies have proven that natural product or plants derivatives shows role in the hepatoprotection or reduction of CCl_4-induced hepatotoxicity. In this regard, an earlier finding based on Dates seeds, which reported that *P. dactylifera* seeds significantly improved the CCl_4-induced alterations in liver function parameters.[17] Other study finding supported the role of *P. dactylifera* as hepatoprotective activity and it revealed that treatment with *P. dactylifera* extract caused noticeable ameliorations of transaminase enzymes activity.[18]

Dates fruits are a good source of antioxidants due to the carotenoids and phenolics.[19] In the current study, the activities of antioxidant enzymes including SOD and catalase were measured, and it was observed that such antioxidant enzymes notable decreased in CCl_4-treated. On the other side, activities of antioxidant enzymes levels were considerably increased in the ADE-treated groups. From the finding, it is suggested that ADE contained numerous constituents and that has potential role in the restore or enhancement of antioxidant enzymes activities. A recent study reported that pretreatment with date palm fruit extract restored the liver damage and amelioration of SOD, GPx, and CAT activities.[20]

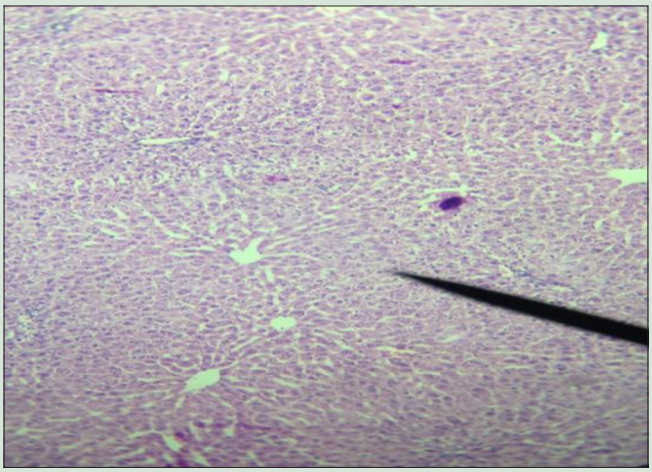

Figure 1: Control group: Normal architecture of hepatic lobules in the form of hepatocytes is maintained and central vein is normal (×40)

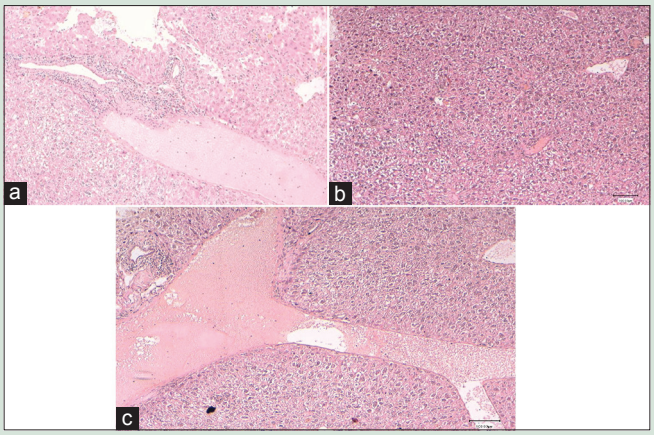

Figure 2: (a) Carbon tetrachloride-treated group showing infiltration of lymphocytes and congestion (×40). (b) Carbon tetrachloride-treated group showing fatty degeneration (×40). (c) Carbon tetrachloride-treated group showing congestion (×40)

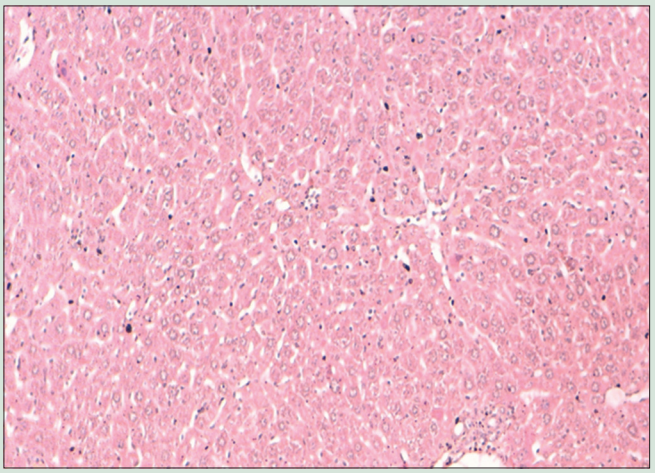

Figure 3: Ajwa dates extract plus CCL4 treated group: Normal architecture of hepatocytes was maintained and few inflammatory cells was seen (x40)

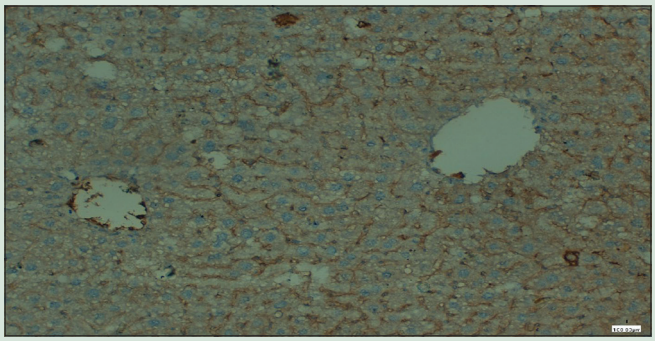

Figure 4: Phosphatase and tensin homolog protein showing expression in carbon tetrachloride-treated groups (×40)

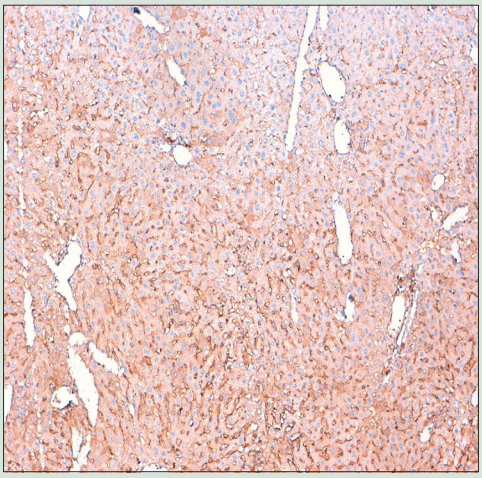

Figure 6: Vascular endothelial growth factor protein showing cytoplasmic expression in carbon tetrachloride-treated groups (×40)

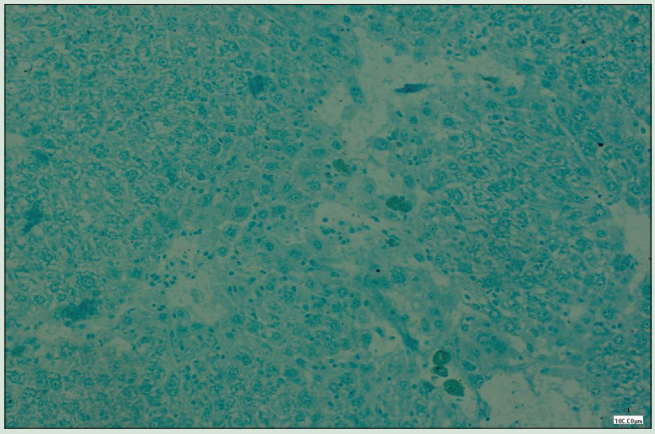

Figure 8: Apoptosis was not detected in carbon tetrachloride-treated group (×40)

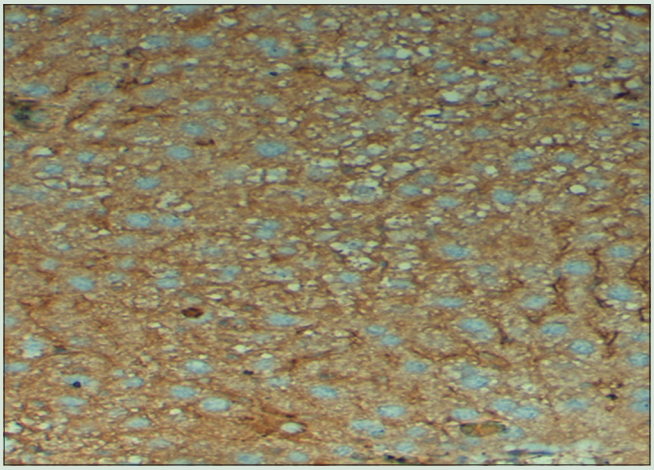

Figure 5: PTEN protein showing expression in Ajwa dates extract plus CCL4 treated group (x40)

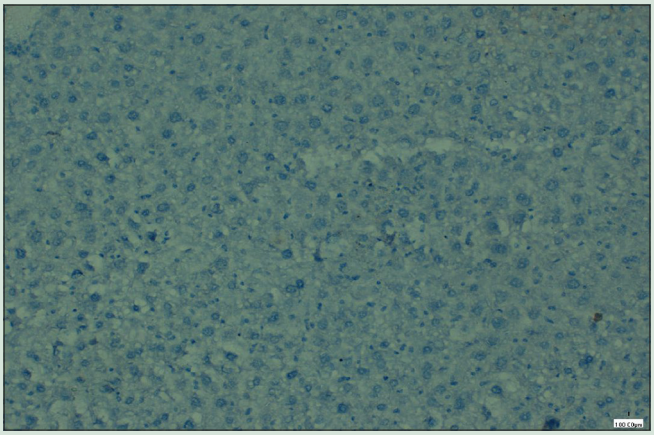

Figure 7: Vascular endothelial growth factor protein did not show any expression in ajwa dates extract-treated group (×40)

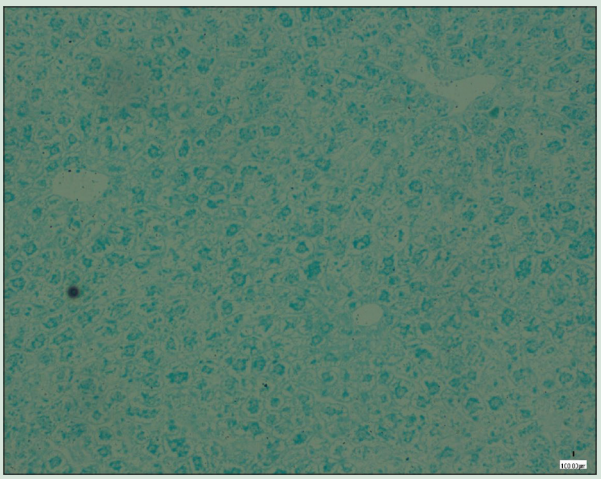

Figure 9: Apoptosis was not detected in ajwa dates extract and carbon tetrachloride-treated group (×40)

Histological alterations were also observed in the liver tissue of CCl_4-treated group, and it was noticed as lymphocytic infiltration, and congestion. ADE-treated group showed an improvement of hepatic tissues, normalization in liver architectures, and only few inflammatory cells were observed. In this regard, earlier finding reported that ADE-treated groups showed a decrease in the histological alterations caused by the treatment of CCl_4 and area occupied by collagen fibers was considerably reduced in ADE-treated groups.[21] In addition, another

study based on ajwa extract reported that extract-treated group showed a pivotal role in the reduction of congestion and cellular degeneration.[22]

Expressional evaluation of PTEN and VEGF protein was evaluated in all groups of animals and its interpretation was made accordingly. PTEN is a tumor suppressor gene and downregulation of PTEN gene has been noticed in many cancers.[14] In the current study, high expression of PTEN was noticed in all studies groups including control, CCl_4-treated and ADE-treated group. Moreover, 1 case (out of 8) of CCl_4-treated groups showed less expression of PTEN protein or loss of expression of PTEN protein in CCl_4-treated group was observed. Previous finding reported that loss of PTEN protein was observed in cancer cases, whereas normal cases or inflammatory lesion showed high expression of PTEN protein.[14] In our study, there were no cancer cases and CCl_4-treated cases just showed congestion, degeneration, necrosis, and infiltration of lymphocytes. High expression of PTEN protein was noticed in all studied group of the current study because of there was no tumor cases in the study.

Angiogenesis is an independent prognostic tumor marker in tumors and VEGF is vital factor in this phenomenon.[23] High expression of VEGF has been noticed in cancer cases, whereas low or undetectable expression was noted in benign or normal tissue.[24] In the current study, all control cases did not show any expression either in cytoplasm or nucleus. In addition, 2 out of 8 cases of CCl_4-treated group showed expression of VEGF protein, whereas ADE did not show any expression. These findings supported the idea that ADE decreased angiogenesis process and such process involve in migration and differentiation of endothelial.

TUNEL assay was performed to identify the apoptotic bodies, and it was noticed that apoptosis was not observed in treated and untreated groups. Earlier finding reported that apoptotic bodies were observed in cancer cases, whereas in control cases were not observed.[24]

CONCLUSION

The findings suggested that ADE has potential role in the hepatoprotection against CCl_4-induced liver toxicity which may act by preventing enzymes activities and enhance the antioxidant defense system/enzymes. These findings supported the idea that ADE might reduce liver tissue alterations or maintenance of architecture of hepatocytes and decreased the angiogenesis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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