MOLECULAR MECHANISMS UNDERLYING HEPATOPROTective EFFECT OF ARTICHOKE EXTRACT: MODULATES TNF-INDUCED ACTIVATION OF NUCLEAR TRANSCRIPTION FACTOR (NF-KAPPA B) AND OXIDATIVE BURST INHIBITION

Nehal Afifi*,1, Ramadan, A.1, Nemat Z. Yassin2, Hany M. Fayed2 and Rehab F. Abdel-Rahman2

*Correspondence for Author
Dr. Nehal Afifi
Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt

1Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
2Pharmacology Department, Medical Division, National Research Center, Egypt

ABSTRACT

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. It is now the third leading cause of cancer deaths worldwide. The purpose of this study was to evaluate the effect of artichoke (Cynara scolymus leaves) extract against N-nitrosodiethylamine (NDEA) at a dose of 100 mg/kg b.wt and carbon tetrachloride (CCl4) at a dose of 3ml/kg b.wt -induced hepatocarcinogenesis in male Wister albino rats. Main methods: rats were pretreated with artichoke extract, silymarin or both for six weeks prior to the injection of NDEA. Then rats administered with a single intraperitoneal injection of NDEA followed by subcutaneous injections of CCl4 once a week for 6 weeks and the pretreatment was continued for another six weeks. Product of lipid peroxidation malondialdehyde (MDA), nitric oxide (NO), glutathione content (GSH), superoxide dismutase (SOD), hydroxyproline and DNA damage contents were evaluated in liver homogenate. Tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B (NF-kappa B) were determined in serum using ELSIA. Results: Pretreatment with Cynara scolymus extract, silymarin and their combination limited the increase in the levels of MDA, NO; TNF alpha, NF-kappa, hydroxyproline and DNA damage while diminished the loss of GSH and SOD. Conclusion: The obtained findings suggested that Cynara scolymus extract may have beneficial chemo preventive roles against hepatocarcinogenesis through their antioxidant, anti-inflammatory, anti-fibrotic, and anti-apoptotic activities.

KEYWORDS: (Hepatocellular carcinoma, N-nitrosodiethylamine (NDEA), Carbon tetrachloride (CCL₄), Cynara scolymus, silymarin, Rats).

1. INTRODUCTION
Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and it represents the fifth most common cancer in men worldwide, and the seventh most frequent in women, with over 600000-650000 new cases diagnosed annually (Cardin et al., 2014). It ranked 2nd most common cancer site among males and 7th among females in the National Cancer Institute (NCI), Cairo University, Egypt (Abd El Gawad et al., 2014). The chronic inflammatory state appears to be necessary for the initiation and development of liver cancer. Chronic infections with hepatitis viruses (hepatitis B virus, HBV and hepatitis C virus, HCV) are major risk factors for HCC development. Other risk factors include chronic alcohol abuse, biliary disease, metabolic disorders, drugs, toxins, and genetic conditions, such as hereditary hemochromatosis and 1-antitrypsin deficiency (Ramakrishna et al., 2013).

The chronic inflammation is characterized by the continued expression of cytokines and recruitment of immune cells to the liver. Activated inflammatory cells release free radicals, such as reactive oxygen species (ROS) and nitric oxide (NO) reactive species, which in turn can cause DNA damage and lead to gene mutations, thus fostering neoplastic transformation (Porta et al., 2011). The chronic inflammation also affects many cellular pathways, leading to fibrosis, cirrhosis and finally hepatocarcinogenesis. Liver injury induces tissue repair and liver regeneration, which involve deregulated growth and death of hepatocytes. High cell turnover induces several critical alterations for malignant transformation, including structural and/or functional modifications of proteins involved in cell-cycle control, apoptosis, oxidative stress, lipid peroxidation and DNA repair damage (Leonardi et al., 2012). Moreover, Tumor-Necrosis-Factor-alpha (TNF-α)-induced Nuclear Factor kappa B (NF-κB) activation which is a key transcriptional regulator of the inflammatory response and plays an essential role in the regulation of inflammatory signaling pathways in the liver (Capece et al., 2013).

Artichokes, especially Cynara scolymus L.(Asteraceae), is an ancient herbaceous plant, originating from the Mediterranean area and it is widely cultivated all over the world for its therapeutic purposes (Metwally et al., 2011). Cynareae tribe present in Egypt are known for their efficacy in relieving some liver disorders (El-Sohafy et al., 2013). Artichoke leaf extracts (LE) have long been used in traditional folk medicine, mainly because of their
choleretic, diuretic and hypocholesterolemic activities (Speroni et al., 2003). Studies of the secondary metabolites of Cynara spp. have shown that polyphenolic compounds, mainly caffeic acid derivatives, as well as triterpenoid saponins and flavonoids, play an important biological role in the action of these extracts (Jacociunas et al., 2014).

The aim of the present study was to investigate anti-oxidant, anti-inflammatory, anti-fibrotic, and anti-apoptotic activities of Cynara scolymus extract against N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCl₄) induced hepatocarcinogenesis in rats, comparing with silymarin as a standard drug.

2. MATERIAL AND METHODS

Rats and Diet: Sixty; adult male Wistar albino rats, 200-250 g were obtained from the animal house colony, National Research Centre, Giza, Egypt. All animals were housed in metal cages in a well-ventilated environment at (22 ± 3°C, 55 ± 5% humidity and 12h dark & light cycles); received standard rat food pellets and water was provided ad libitum throughout the experimental period. All experiments were carried out according to the ethical guidelines for care and use of experimental animals approved by the Ethical Committee of the National Research Centre.

Chemicals: N-Nitrosodiethylamine (NDEA) was purchased from Sigma Chemical Company, USA. Carbon tetrachloride (CCl₄) was obtained from El-Gomhorya Company, Cairo, Egypt. Biochemical kits for serum analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Plants: Cynara scolymus dry extract and silymarin were purchased from MEPACO, Egypt as a fine powder and stored in an airtight container in a refrigerator below 10 ºC.

Experimental Design: Adult male Wistar albino rats weighing 200–250 g (10–12 weeks old) were divided into six groups. Group I (Normal control), rats were IP injected with Dimethyl sulfoxide (DMSO) at a dose of 1 ml/kg and injected SC with liquid paraffin (3 ml/kg,). The other five groups were given a single IP injection of N-nitrosodiethylamine (NDEA; 100 mg/kg b.wt.) followed by weekly SC injections of CCl₄ (3ml/kg b.wt.) for six weeks as reported by (Sundaresan et al., 2003) and then divided as follow: Group II Kept as hepatotoxic group. Group III (standard group) was given silymarin orally at a dose of 50 mg/Kg b.wt. per day for six weeks before induction of hepatocarcinogenesis and continued
for another six weeks (Shaarawy et al., 2009). Groups IV and V were orally administered Cynara scolymus leaves extract at doses of (750 and 1500 mg/kg b.wt) per day for six weeks before induction of hepatocarcinogenesis and continued for another six weeks (Mehmetcik et al., 2008). Groups VI, rats were received Cynara scolymus extract at a dose of 750 mg /kg b.wt in combination with silymarin (50mg/kg b.wt.) per day orally for six weeks before induction of hepatocarcinogenesis and continued for another six weeks.

At the end of the experimental period (12 weeks), blood samples were collected from the retroorbital venous plexus of rats under light ether anesthesia and collected in clean test tubes, allowed to clot, then centrifuged for 10 minutes at 3000 r.p.m. Serum was separated and stored into Eppendorff tubes at – 20 °C to be used for determination of nuclear factor-kappa beta (NF-κ B) and tumor necrosis factor-alpha (TNF-α). Liver samples were kept at (-80 °C) for determination of hepatic nitric oxide (NO) and malondialdehyde (MDA) levels (as a free radicals increase in liver disease), determination of hepatic reduced glutathione (GSH) and superoxide dismutase (SOD) levels (constitutes an important antioxidant defense), determination of the level of liver DNA damage (Molecular Pharmacology, as a marker of cell death in chronic liver diseases), determination of liver hydroxyproline content (as a fibrosis marker).

**Determination of hepatic nitric oxide (NO) level:**
Nitric oxide was determined in rat liver homogenate (20%) using a colorimetric method based on the Griess reaction according to the method of (Miranda et al., 2001) with minor modification.

**Determination of hepatic malondialdehyde (MDA) (lipid peroxidation):** The lipid peroxides content in liver homogenate (20%) was determined by monitoring the thiobarbituric acid reactive substance formation as described by (Ruiz-Larrea et al., 1994).

**Determination of reduced glutathione content in liver homogenate:** Determination of reduced glutathione content (GSH) in liver homogenate (20%) was determined according to the colorimetric method of (Ellman, 1959) as modified by (Bulaj et al., 1998).

**Determination of hepatic superoxide dismutase (SOD) activity:** The superoxide dismutase (SOD) activity in rat liver cytosol was measured as the degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH by the method described by (Marklund et al., 1974).
Determination of serum tumor necrosis factor-alpha (TNF-α): Serum levels of TNF-α were quantified as performed by (Pennica et al., 1984) using an enzyme-linked immunosorbent assay (ELISA) kit.

Determination of serum nuclear factor-kappa beta (NF-κ β): Serum levels of NF-κ β were quantified as performed by (Adams, 2009) using an enzyme-linked immunosorbent assay (ELISA) kit.

Determination of liver DNA damage: DNA fragmentation assay using diphenylamine method (DPA):DNA was quantified by using diphenylamine reaction as previously described by (Burton, 1956). Results were expressed as percentage of fragmented DNA.

Determination of liver hydroxyproline content: Total liver collagen was determined as hydroxyproline according to the method of (Woessner, 1961). The method resides on the acid digestion of collagen, and produced hydroxyproline was left to react with Ehrlich's reagent to form a reddish-brown color that was measured spectrophotometrically.

Statistical analysis: In the present study, all results were expressed as mean ± standard error of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using software program Graph Pad Prism (version 5.00). Difference was considered significant when P<0.05.

3. RESULTS
Administration of a single toxic dose of N-nitrosodiethylamine (NDEA; 100 mg/kg b.wt) followed by a weekly S.C. injections of CCl4 (3ml/kg) for 6 weeks induced a significant increase in nitric oxide (NO) and malondialdehyde (MDA) levels of liver homogenate, as compared to normal control group. Pretreatment of rats with Cynara scolymus extract at doses of 750 and 1500 mg/kg b.wt, silymarin and their combination limited the elevation of nitric oxide (NO) and malondialdehyde (MDA) levels when compared to NDEA intoxicated group (Table 1).
Table 1: Effect of oral administration of Cynara Scolymus leaves extract on hepatic nitric oxide (NO) and malondialdehyde (MDA) levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>Nitric oxide (NO) (µM/g liver tissue)</th>
<th>Malondialdehyde (MDA) (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>16.11±0.78ᵇ</td>
<td>44.49±0.28ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>152.60±3.99ᵃ</td>
<td>98.78±2.94ᵃ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>83.46±4.89ᵃᵇ</td>
<td>67.63±1.79ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>34.81±3.18ᵃᵇ</td>
<td>60.26±1.14ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>20.65±1.89ᵃᵇ 31.08±3.00ᵃᵇ</td>
<td>58.85±1.09ᵃᵇ 60.06±1.79ᵃᵇ</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

ᵃ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
ᵇ P<0.05: Statistically significant from NDEA–intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

NDEA administration led to significant depletion in hepatic GSH and SOD content, as compared with normal control rats. Pretreatment of rats with Cynara scolymus extract at doses of 750 and 1500 mg/kg b.wt, silymarin and their combination for six weeks before induction of hepatocarcinogenesis and continued for another six weeks maintained hepatic GSH and SOD content when compared to NDEA–intoxicated group (Table 2).

Table 2: Effect of oral administration of Cynara scolymus leaves extract on hepatic GSH and SOD levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>Reduced glutathione (GSH) (µmol/g)</th>
<th>Superoxide dismutase (SOD) (U/g liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>9.53±0.28ᵇ</td>
<td>7.43±0.29ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>3.45±0.13ᵃ</td>
<td>3.14±0.29ᵃ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>7.80±0.20ᵃᵇ</td>
<td>5.14±0.35ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>8.43±0.10ᵃᵇ</td>
<td>7.14±0.45ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>9.16±0.35ᵇ 8.49±0.27ᵃᵇ</td>
<td>8.00±0.35ᵇ 7.71±0.35ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

ᵃ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
ᵇ P<0.05: Statistically significant from NDEA–intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
A significant elevation of serum NF-κ B level in NDEA–intoxicated group as compared to normal control group. In comparing pretreated groups with NDEA-intoxicated group, the results indicated that pretreatment with Cynara scolymus extract (750 and 1500 mg/kg b.wt), silymarin and their combination for six weeks inhibited the elevation of serum NF-κ B level, while these groups compared to normal control group showed a marked increases in the level of NF-κ B (Table 3).

Table 3: Effect of oral administration of Cynara scolymus leaves extract on serum NF-κ B level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>Nuclear factor-κB (NF-κ B) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>0.98±0.04ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>5.76±0.16ᵃ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>3.70±0.02ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>3.35±0.05ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>3.00±0.13ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Silymarin</td>
<td>50</td>
<td>3.12±0.11ᵃᵇ</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

ᵃ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
ᵇ P<0.05: Statistically significant from NDEA –intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

NDEA–intoxicated group showed a significant increase in serum level of TNF-α, as compared to the normal control group. The elevation of serum TNF-α level was prevented in all groups pretreated with Cynara scolymus extract (750 and 1500 mg/kg b.wt), silymarin and their combination for six weeks prior the hepatotoxicity induction and continued for another six weeks when compared to NDEA–intoxicated group (Table 4).
Table 4: Effect of oral administration of Cynara scolymus leaves extract on serum TNF-α level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>Tumor necrosis factor α (TNF-α) (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>110.50±4.51ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>174.30±1.04ᵃ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>132.10±2.41ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>114.10±6.16ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>96.67±17.32ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Silymarin</td>
<td>50</td>
<td>116.80±1.14ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

ᵃ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
ᵇ P<0.05: Statistically significant from NDEA–intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

The level of hydroxyproline content in liver homogenate of NDEA–intoxicated rats was increased as compared to normal control group. Rat groups administered Cynara scolymus extract, silymarin and their combination for six weeks revealed significant decrease in hydroxyproline content in liver homogenate as compared to NDEA–intoxicated group (Table 5).

Table 5: Effect of oral administration of Cynara scolymus leaves extract on liver hydroxyproline.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>Hydroxyproline (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>12.70±0.80ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>30.45±1.13ᵃ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>20.75±0.86ᵃᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>15.55±0.76ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>12.13±0.59ᵇ</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Silymarin</td>
<td>50</td>
<td>13.55±0.42ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

ᵃ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
ᵇ P<0.05: Statistically significant from NDEA–intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
DNA fragmentation % was significantly increased in NDEA–intoxicated group as compared with normal control group. It was noticed that DNA fragmentation was inhibited in groups pretreated with Cynara scolymus extract (750 and 1500 mg/kg b.wt), silymarin and their combination for six weeks before induction of hepatocarcinogenesis and continued for another six weeks as compared to NDEA–intoxicated group (Table 6).

Table 6: Effect of oral administration of Cynara scolymus leaves extract on liver DNA damage.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt)</th>
<th>DNA fragmentation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>30.92±1.21</td>
</tr>
<tr>
<td>NDEA–intoxicated rats</td>
<td>100</td>
<td>80.08±2.71</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>750</td>
<td>43.16±1.60</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Artichoke extract</td>
<td>1500</td>
<td>30.02±1.20</td>
</tr>
<tr>
<td>NDEA–intoxicated rats treated with Artichoke extract and Silymarin</td>
<td>750 50</td>
<td>29.89±0.57</td>
</tr>
<tr>
<td>NDEA–intoxicated rats + Silymarin</td>
<td>50</td>
<td>30.66±1.37</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E. of (n=5) for each group.

\( ^a P <0.05: \) Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

\( ^b P<0.05: \) Statistically significant from NDEA–intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

4. DISCUSSION
In the present study, the level of malondialdehyde (MDA) was significantly elevated by NDEA indicating increased lipid peroxidation and oxidative stress in liver. This may be due to lipid peroxidation induced by NDEA that plays an important role in carcinogenesis which lead to the formation of several toxic products, such as malondialdehyde (MDA) that can attack cellular targets including DNA, thereby inducing mutagenicity and carcinogenicity (Banakar et al., 2004). Also (Pradeep et al., 2007) attributed the increase in lipid peroxidation level to the oxidative stress leading to peroxidative membrane damage, loss of membrane integrity and subsequent release of the cytosolic contents. Pretreatment with Cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination prevented lipid peroxidation as compared with NDEA-intoxicated group. This may be due to anti-oxidative capabilities of artichoke (Heidarian et al., 2013).
Increased NO production and plasma nitrite/nitrate levels are found during chronic hepatic inflammation, suggesting a role for NO in the hepatic response to inflammatory stimuli (Geller et al., 1993). The present study revealed a significant elevation in the level of liver nitric oxide in NDEA-intoxicated rats as compared to the normal group which may be due to host cells, mainly monocytes/macrophages, that produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage (Raso et al., 2001). Regarding our results, oral administration of Cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination showed a significant decrease in hepatic NO level as compared to NDEA-intoxicated group. This may be attributed to four well-known artichoke compounds (cynarin, cyaniding, luteolin and cynaroside) which led to a downregulation of inducible nitric oxide synthase (iNOS) mRNA and protein expression, with cynarin being the most potent one (Xia et al., 2014).

Reduced glutathione (GSH) and superoxide dismutase (SOD) are major antioxidants, which involved in defense mechanism against lipid peroxidation in biological system and convert active oxygen molecules into non-toxic compounds (Jia et al., 2012). A reduction in GSH and SOD is associated with the accumulation of high-living free radical, leading to injury of cell function (Okamoto et al., 1998). Our results revealed a significant depletion in reduced glutathione (GSH) level after NDEA administration which may be attributed to the depletion in reduced glutathione (GSH) level in rats administered with NDEA due to the decreased expression of these antioxidants during hepatocellular damage and its utilization in inactivating the free radicals generated during NDEA metabolism (Granado-Serrano et al., 2009). In addition to that NO production by cytotoxicity caused an inhibition of glutamyl cysteine synthetase, a cytosolic enzyme help in GSH synthesis, leading to GSH depletion (Andre et al., 2003).

NDEA produced a significant decrease in superoxide dismutase (SOD) activities. These results are attributed to the decrease of SOD activity in liver of NDEA-treated rats due to the enhanced lipid peroxidation or inactivation of the antioxidant enzymes (Shaban et al., 2013). Moreover, the decreased activities of SOD in NDEA-treated rats could have been due to over-utilization of these enzymatic antioxidants to scavenge the products of lipid peroxidation and the tumor cells have been reported to sequester essential antioxidants from the circulation in order to meet the demands of the growing tumor cells (Sundaresan et al., 2003). In our study, daily administration of Cynara scolymus extract at doses of (0.75 and
1.5g/kg b.wt), silymarin and their combination diminished loss of hepatic GSH content and maintained SOD activity as compared with NDEA-intoxicated group. The antioxidant activity of artichoke could be attributed to its constituents of many bioactive polyphenolic compounds, mainly cynarin, luteolin and chlorogenic acids which are abundant in both heads and leaves (Magielse et al., 2014).

Nuclear factor kappaB (NF-kappaB) is a transcription factor that promote tumorigenesis after being activated by inflammatory agents, carcinogens and tumor promoters (Aggarwal et al., 2004). The present results showed a significant increase in serum nuclear factor kappaB (NF-κB) level in NDEA-intoxicated group in comparison with the healthy control group. Administration of cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination inhibited the elevation of serum NF-κB level as compared to NDEA-intoxicated group. This may be due to components mainly luteolin and apigenin in Cynara scolymus have been found to block IkBα phosphorylation and degradation which in turn could reduce NF-κB level (Mohamed et al., 2013). Also cynaropicrin, which is a sesquiterpene lactone component, inhibited the NF-κB-mediated transactivation (Tanaka et al., 2013). NF-κB is constitutively active in most tumor cells, and its suppression in these cells leads to inhibition of proliferation, arrest of cell cycle and apoptosis (Bharti et al., 2002). Thus, inhibition of NF-κB signaling pathway might be a therapeutic strategy in conjunction with the usage of chemopreventive agents (Surh, 2003).

In the present experiment, there was a marked increase in serum tumor necrosis factor alpha (TNF-α) level in NDEA-intoxicated group as compared to healthy control group. Daily administration of cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination significantly prevented elevation of serum TNF-α level as compared to NDEA-intoxicated group. This effect is attributed to the presence of luteolin and apigenin in Cynara scolymus extract which could inhibit the inflammatory cytokine production (Kotanidou et al., 2002).

Hydroxyproline is an amino acid unique to all of the collagens and represents 12% of amino acids in the major fibrillar collagen types I and III. Therefore, the measurement of hydroxyproline content serves as an excellent standard of fibrosis (Friedman, 1993). In this study, the liver hydroxyproline content showed a significant increase following administration of NDEA as compared to normal control group. This attributed to disruption of liver architecture and enhanced expression of α-smooth muscle actin (αSMA), a sensitive
marker of fibroblast formation and an established molecular marker of identifying activated hepatic stellate cells (HSCs). A continuous increase in the number of activated HSCs adds more of collagenous connective tissue to liver, resulting in the formation of distinct fibrotic areas (Ahmad et al., 2014).

Administration of Cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination reduced the development of hepatic fibrosis. These results were confirmed by a significant decrease of hepatic hydroxyproline content, a marker of liver collagen deposition as compared to NDEA-intoxicated group which may be attributed to luteolin (an active constituent of Cynara scolymus) (Domitrovic et al., 2009).

Apoptosis is a term used to describe the terminal morphological and biochemical events seen in programmed cell death (Wyllie, 1992). Apoptosis has been the subject of great interest because it has been clearly demonstrated to mediate cell death, not only during development but also in neoplasia in response to cancer chemotheraphy and radiation (Collins et al., 1997). One such feature, which is a hallmark of apoptosis, is DNA fragmentation. In dying cells, DNA is cleaved by an endonucleases that fragments the chromatin into nucleosomal units, which are multiples of about 180-bp oligomers and appear as a DNA ladder using agarose gel electrophoresis (Matassov et al., 2004).

The findings of DNA fragmentation using the diphenylamine (DPA) assay revealed that; a significant increase was observed in DNA fragmentation in the hepatocytes after NDEA administration followed by CCl₄ injection as compared to normal control group which may be due to that the enzymes of CYP₂E₁ subfamily play a role in the biotransformation of a range of compounds, including NDEA, producing the promutagenic DNA lesions which play an important role in DNA damage and induction of hepatocarcinogenesis (Verna et al., 1996). Pretreatment with Cynara scolymus extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination inhibited DNA fragmentation when compared to NDEA-intoxicated group. Cynara scolymus extract triggered apoptosis via a mitochondrial and a death-receptor pathway (Mileo et al., 2012).

CONCLUSION
From this study it can be concluded that Cynara scolymus extract possess significant antioxidant, anti-inflammatory, anti-fibrotic and anti-apoptotic properties. The effect of Cynara scolymus extract at a dose of (1.5g/kg b.wt) was more prominent than that of
(0.75g/kg b.wt). However, the combination of Cynara scolymus extract at a dose of (0.75g/kg b.wt) and silymarin was the strongest among other groups. Therefore it is recommended to use Cynara scolymus as a hepatoprotective in patients with chronic liver diseases which can prevent the possible development of HCC.

CORRESPONDING AUTHOR

Dr. Nehal A.
Afifi, Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt.

REFERENCES


