Hepatoprotective Effect of Artichoke Extract against Pre-cancerous Lesion of Experimentally Induced Hepatocellular Carcinoma in Rats

Ramadan, A.[1], Nehal A. Afifi[1], Nemat Z. Yassin[2], Rehab F. Abdel-Rahman[2], Azza H. M. Hassan[3] and Hany M. Fayed[2]

[1]Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Pharmacology Department, Medical Division, National Research Center, Egypt
[3]Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt
azzahassan99@gmail.com

Abstract: Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. It is now the third leading cause of cancer deaths worldwide. The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC. The present study was conducted to investigate the protective effect of *cynara scolymus* leaves extract against N-nitrosodiethylamine (NDEA) at a dose of 100 mg/kg b.wt and carbon tetrachloride (CCl_4) at a dose of 3ml/kg b.wt -induced hepatocarcinogenesis in male Wistar albino rats. Main methods: rats were pretreated with *Cynara scolymus* 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 CCl_4 once a week for 6 weeks and the pretreatment was continued for another six weeks. At the end of the experiment, blood samples were taken for biochemical analysis and liver tissues were histopathologically examined. Results of the current study showed significant increase in serum levels of AST, ALT, ALP, total and direct bilirubin and alpha fetoprotein (AFP) as well as significant decrease in albumin was observed in NDEA-intoxicated group, compared to normal control group. Pretreatment with *Cynara scolymus* extract, silymarin and their combination limited the increase in serum levels of AST, ALT, ALP, total and direct bilirubin and alpha fetoprotein (AFP) and produced significant increase in albumin, compared to NDEA- intoxicated group. Histopathological examination of liver of NDEA and Ccl4 treated group revealed obvious cellular damage and hepatocellular carcinoma with frequent mitotic activity and nuclear pleomorphism, meanwhile other groups pretreated with either silymarin, *Cynara scolymus* extract or a combination of silymarin and *Cynara scolymus* extract revealed improvement in the liver architecture . The current results indicated that *Cynara scolymus* extract possess hepatoprotective effect.
[Ramadan, A., Nehal A. Afifi, Nemat Z. Yassin, Rehab F. Abdel-Rahman, Azza H. M. Hassan and Hany M. Fayed. **Hepatoprotective Effect of Artichoke Extract against Pre-cancerous Lesion of Experimentally Induced Hepatocellular Carcinoma in Rats.** *Life Sci J* 2014;11(4):43-50]. (ISSN:1097-8135). http://www.lifesciencesite.com. 4

Key words: Hepatocarcinogenesis; NDEA; Artichoke; Silymarin; liver enzymes, oxidative stress, rats.

1. Introduction

Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histological subtype, accounting for 70% to 85% of the total liver cancer burden worldwide (Wang *et al.*, 2013). Globally, it is the fifth most common cancer and the second leading cause of cancer-related death (Zhang *et al.*, 2013). In Egypt, hepatocellular carcinoma contributes to 14.8% of all cancer mortality with a higher incidence in males (17.3%) than in females (11.5%). It is the second most frequent cancer type in Egyptian males after bladder cancer and the eighth most frequent in Egyptian females (Aleem *et al.*, 2012). Chronic inflammations of the liver and subsequent cirrhosis are highly correlated with hepatitis B and hepatitis C viral infections, alcohol abuse and metabolic liver diseases (diabetes and non alcoholic fatty liver disease). Additionally, obesity, environmental pollutants, consumption of food contaminated with the fungal toxins as aflatoxins that is produced by *Aspergillus flavus* in food grains and nitrosamine consumption are the strongest risk factors for HCC development (Farazi *et al.*, 2006).

Food plants, including fruits, vegetables, and spices are the primary sources of naturally occurring nutrients essential for human health. Due to their health benefits, vegetables and fruits have become popular among consumers and the number of medicinal plants being used in healthcare has increased worldwide. Phytochemicals with antioxidative, anti-inflammatory, antibacterial and antimutagenic properties are extremely attractive potential agents for preventing diseases in humans (Li *et al.*, 2012).

Cynara scolymus L. is an ancient herbaceous plant, originating from the Mediterranean area. Today, it is widely cultivated all over the world. Its leaf is widely used for 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 (*Cynara scolymus*) leaf extracts have traditionally been used to treat dyspeptic symptoms, increases bile flow and to exert hepatoprotective, lipid-lowering, antioxidant and antispasmodic effects (Holtmann *et al.*, 2003).

The aim of the present study was to investigate the hepatoprotective effect of *Cynara scolymus* extract against N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCl_4) 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 (CCl_4) 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 CCl_4 (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 retro-orbital 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 liver function parameters.

Determination of liver function tests:

Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to the method of (Reitman *et al.*, 1957). Serum alkaline phosphatase (ALP) was calorimetrically determined according to the method described by (Babson *et al.*, 1966). Serum direct and total bilirubin determined colorimetrically as described by (Doumas *et al.*, 1973).Serum concentration of albumin was determined as described by (Bartholomew *et al.*, 1966).

Determination of serum alpha fetoprotein (AFP) (as a tumor marker):

Serum levels of alpha fetoprotein (AFP) were quantified as performed by (Gibbs *et al.*, 1987) using an enzyme-linked immunosorbent assay (ELISA) kit.

Histopathological examination:

For histopathological study, few-millimeters mid-sections of the left lobes of the liver excised from each group were processed for light microscopy. The processing involved fixing the tissue specimens in a 10% neutral buffered formalin solution, preparing the blocks in paraffin, cutting sections 5-6 µm in thickness, and staining the sections with haematoxylin-eosin stain. The sections were examined by an expert pathologist who was not aware of sample assignment to experimental groups (Carleton *et al.*, 1967).

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 GraphPad Prism (version 5.00). Difference was considered significant when $P<0.05$.

3. Results:

The results in Table (1) showed that administration of a single toxic dose of N-nitrosodiethylamine (NDEA; 100 mg/kg b.wt) followed by a weekly S.C. injections of CCl_4 (3ml/kg) for 6 weeks induced a significant increase in serum AST, ALT and ALP levels when compared to the 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 serum AST, ALT and ALP levels when compared to NDEA-intoxicated group.

Table 1: Effect of oral administration of *Cynara Scolymus* leaves extract on serum AST, ALT and ALP levels.

Groups	Dose (mg/kg b.wt)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	-	97.60±1.36□	38.53±1.08□	124.20±2.88□
NDEA–intoxicated rats	100	391.70±2.93□	171.30±4.31□	296.10±0.85□
NDEA–intoxicated rats + Artichoke extract	750	230.20±9.44□□	100.80±1.86□□	168.50±4.16□□
NDEA–intoxicated rats + Artichoke extract	1500	113.20±2.57□	50.73±1.07□	140.50±3.07□□
NDEA–intoxicated rats treated with Artichoke extract and Silymarin	750 50	111.60±3.42□	47.63±1.81□	134.30±3.24□
NDEA–intoxicated rats + Silymarin	50	113.20±2.48□	48.37±1.46□	135.70±1.72□

Data are presented as the mean ± S.E. for each group.
□ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
□ P<0.05: Statistically significant from NDEA –intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

The results in Table (2) showed that serum level of direct and total bilirubin significantly elevated in NDEA–intoxicated group, as compared to the normal control group.

Rats pretreated with *Cynara scolymus* extract (750 and 1500 mg/kg b.wt), silymarin and their combination prevented the elevation of serum direct and total bilirubin as compared with NDEA–intoxicated group.

Table 2: Effect of oral administration of *Cynara Scolymus* leaves extract on serum total and direct bilirubin levels.

Groups	Dose (mg/kg b.wt)	Direct Bilirubin (mg/dl)	Total Bilirubin (mg/dl)
Normal control	-	0.12±0.007□	0.32±0.01□
NDEA–intoxicated rats	100	2.52±0.09□	3.81±0.08□
NDEA–intoxicated rats +Artichoke extract	750	1.46±0.04□□	1.80±0.04□□
NDEA–intoxicated rats +Artichoke extract	1500	0.36±0.02□	0.42±0.02□
NDEA–intoxicated rats treated with Artichoke extract and Silymarin	750 50	0.23±0.01□	0.27±0.01□
NDEA–intoxicated rats +Silymarin	50	0.30±0.01□	0.39±0.01□

Data are presented as the mean ± S.E. for each group.
□ P <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
□ P<0.05: Statistically significant from NDEA –intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

The results in Table (3) showed that rats injected with NDEA and CCL_4 caused a significant reduction in serum albumin level as compared with the normal control rats.

Groups pretreated with *Cynara Scolymus* extract (750 and 1500 mg/kg b.wt), silymarin and their combination maintained synthetic capacity of hepatocytes which exhibited by significant elevation of serum albumin as compared with NDEA–intoxicated group.

Table 3: Effect of oral administration of *Cynara Scolymus* extract on serum albumin level.

Groups	Dose (mg/kg b.wt)	Albumin (g/dl)
Normal control	-	5.62±0.18□
NDEA–intoxicated rats	100	2.73±0.07□
NDEA–intoxicated rats + Artichoke extract	750	4.50±0.14□□
NDEA–intoxicated rats + Artichoke extract	1500	5.60±0.16□
NDEA–intoxicated rats treated with Artichoke extract and Silymarin	750 50	5.77±0.26□
NDEA–intoxicated rats + Silymarin	50	5.58±0.22□

Data are presented as the mean ± S.E. of (n=5) for each group.
□ *P* <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
□ *P*<0.05: Statistically significant from NDEA –intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

The results in Table (4) showed a significant increase in serum α-fetoprotein (AFP) level in NDEA- intoxicated group, as compared to normal control group.

Pretreatment with *Cynara scolymus* extract (750 and 1500 mg/kg b.wt), silymarin and their combination limited the elevation of serum AFP level as compared with NDEA-intoxicated group.

Table 4: Effect of oral administration of *Cynara Scolymus* leaves extract on serum AFP level.

Groups	Dose (mg/kg b.wt)	α-fetoprotein (AFP) (ng/ml)
Normal control	-	2.98±0.06□
NDEA–intoxicated rats	100	8.06±0.10□
NDEA–intoxicated rats + Artichoke extract	750	4.02±0.04□□
NDEA–intoxicated rats + Artichoke extract	1500	3.62±0.08□□
NDEA–intoxicated rats treated with Artichoke extract and Silymarin	750 50	3.55±0.04□□
NDEA–intoxicated rats + Silymarin	50	3.60±0.08□□

Data are presented as the mean ± S.E. for each group.
□*P* <0.05: Statistically significant from control group (one way ANOVA followed by "Tukey's Multiple Comparison Test").
□ *P*<0.05: Statistically significant from NDEA –intoxicated group (one way ANOVA followed by "Tukey's Multiple Comparison Test").

Histopathological examination of liver sections of control rats showed normal hepatic architecture. On the other hand , liver of NDEA and Ccl4 treated group revealed marked disarrangement of hepatic lobules with characteristic hepatocellular carcinoma (HCC) formation in which the tumor cells arranged in a compact pattern (Fig.1). The tumor cells showed large hyperchromatic nuclei with prominent nucleoli, nuclear pleomorphism and frequent mitotic activity as well as presence of large amount of cytoplasmic fat and/or glycogen (Fig.3&3) that result in a clear cell appearance. Portal area revealed intense oval cell hyperplasia that arranged themselves in acinar like structure associated with lymphocytic cell infiltration (Fig.4). While Liver sections of rats pretreated with silymarin revealed less disarrangement of hepatic architecture with minimal mitosis and nuclear prominence, as well as presence of intracytoplasmic fat and/or glycogen globules especially at the periportal area (Fig.5). Liver sections of rats pretreated with *Cynara Scolymus* extract 750 and 1500 mg/kg b.wt showed nearly the same histopathological alterations represented by preserved hepatic architecture, minimal nuclear changes, vacuolation of hepatocellular cytoplasm and scanty mitosis (Fig.6&7). Liver sections of rats pretreated with a combination of *Cynara Scolymus* and silymarin restored many of normal hepatic architecture with less disarrangement and degeneration of hepatocytes, in addition to apoptosis and single cell necrosis (Fig.8) compared with NDEA treated group.

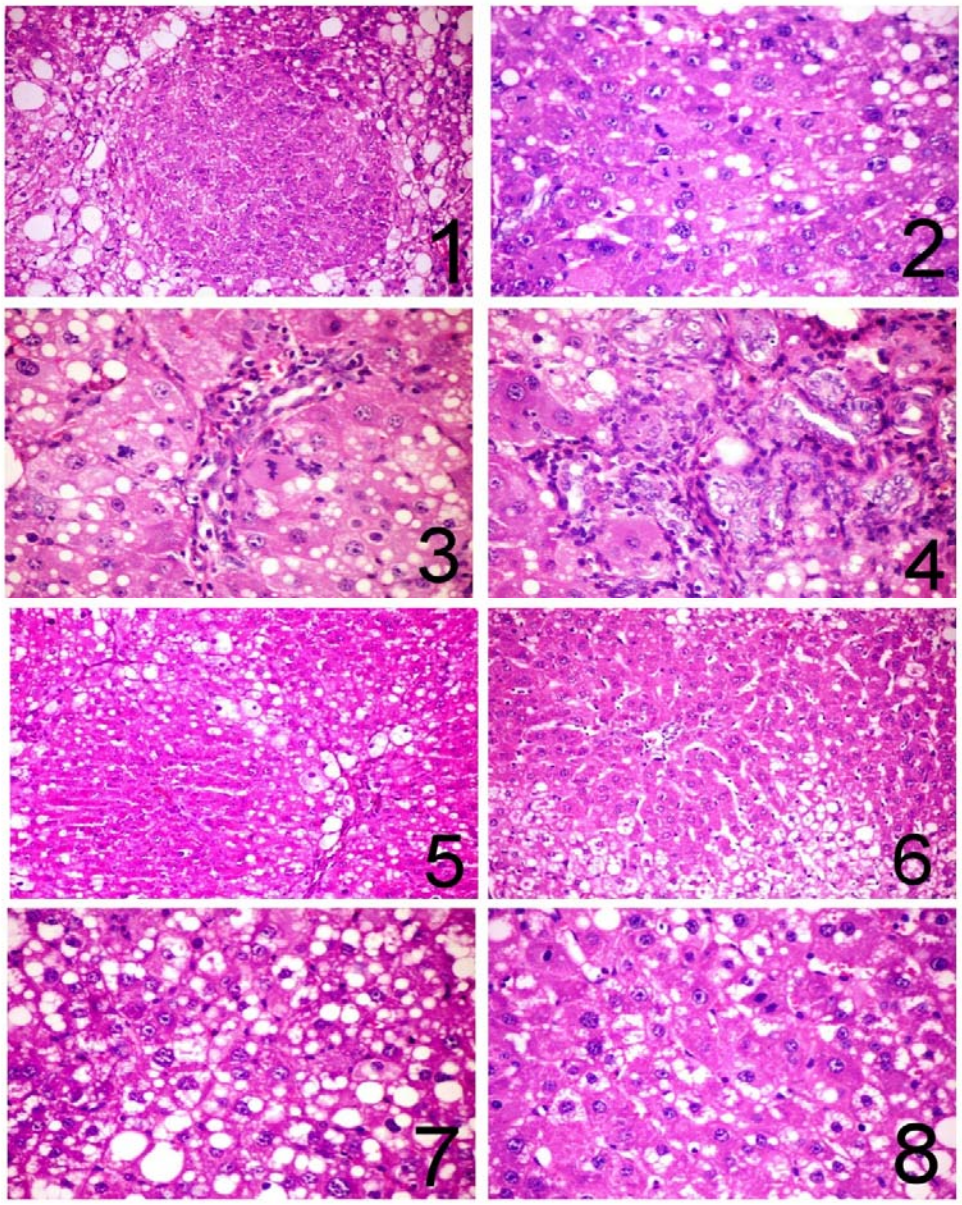

Fig. 1: liver of NDEA and Ccl4 treated rat showing compact hepatocellular carcinoma (HCC) (H&E x200).
Fig. 2: Liver of NDEA treated rat showing large hyperchromatic nuclei with prominent nucleoli, nuclear pleomorphism and frequent mitotic activity (arrow) (H&E x400).
Fig. 3: Liver of NDEA treated rat showing cytoplasmic fat and/or glycogen as well as mitotic activity (H&E x400)
Fig.4: Liver of NDEA treated rat showing intense oval cell hyperplasia arranged in acinar like structure (arrow) associated with lymphocytic cell infiltration (H&E x400).
Fig.5: Liver of rat pretreated with silymarin showing intracytoplasmic fat and/or glycogen globules (H&E x200).
Fig. 6: Liver of rat pretreated with *Cynara scolymus* at a dose of 750 mg/kg b.wt showing preserved hepatic architecture, minimal nuclear changes and vacuolation of hepatocellular cytoplasm (H&E x200).
Fig. 7: Liver of rat pretreated with *Cynara scolymus* at a dose of 1500 mg/kg b.wt vacuolation of hepatocytes (H&E x400).
Fig. 8: Liver of rat pretreated with *Cynara scolymus* and silymarin showing vacuolar degeneration of hepatocytes , apoptosis (arrow) and single cell necrosis (H&E x400).

4. Discussion:

In the present study, NDEA and CCl4 administration to rats led to a marked increase in the levels of serum AST, ALT and ALP compared to the normal group, which indicating that NDEA could induce a liver damage in the rats. These results are in

agreement with Bansal *et al*. (2005) who attributed the elevation of serum transaminases and alkaline phosphatase to the injured structural integrity of the liver as these enzymes released from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. These results are also in agreement with Mittal *et al*. (2006) who found that activities of AST, ALT and ALP were increased significantly following nitroso compounds treatment in rats due to substantial liver damage.

In the present investigation, there was a marked increase in serum levels of direct and total bilirubin as compared to the normal group. These findings are in agreement with the data presented by Rezaie *et al*. (2013) who revealed a highly significant increase in serum level of both total and direct bilirubin in NDEA treated rats. These results may be due to inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes Rajkapoor *et al*. (2006). Concerning the level of serum albumin, the current study showed a highly significant decrease in their level after administration of NDEA compound. These results supported with Cross *et al*. (1987) who attributed the decreased in albumin level to the increased rate of catabolism rather than impairment of synthesis due to the highly toxic effect of carcinogen, which leads to increase in formation of reactive oxygen species (ROS) that are capable of damaging biological molecules such as proteins. Vandenberghe (1996) explained that hypoalbuminemia may be due to kidney function disorder caused by the toxic effect of NDEA which leads to increase in albumin loss.

In the current study, daily administration of *cynara scolymus* extract (0.75g/kg, 1.5g/kg), silymarin and their combination significantly limited the elevation of serum AST, ALT and ALP levels compared to NDEA intoxicated group. These findings are in agreement with Speroni *et al*. (2003) who revealed that these effects due to the active ingredients of *Cynara scolymus* which are known as caffeoylquinic acid derivatives (cynarin and chlorogenic acid) via their strong hepatoprotective effect and antioxidant capacity.

In this study, daily administration of *cynara scolymus* extract (0.75g/kg, 1.5g/kg), silymarin and their combination significantly limited the elevation of serum total and direct bilirubin levels. These results are in agreement with Ghanem *et al*. (2009) who attributed this reduction in serum bilirubin to cynarin which stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the liver damage.

Administration of *cynara scolymus* extract (0.75g/kg, 1.5g/kg), silymarin and their combination significantly increased the level of serum albumin compared with NDEA intoxicated rats. These results are in agreement with El Saeed *et al*. (2012) who attributed this elevation of serum albumin to the anabolic effect of flavonoids. Also Jimenez-Escrig *et al*. (2003) explained that the hepatoprotective activity of artichoke may play a role by increasing biosynthesis of proteins (including albumin) by the liver.

Serum AFP level was significantly increased in NDEA-intoxicated group as compared to the normal group. The obtained results are in agreement with those obtained by Song *et al*. (2013); they reported that NDEA administration led to increased level of alpha-fetoprotein. Motalleb *et al*. (2008) attributed the reinitiation of AFP synthesis by neoplastic hepatocytes to increased transcription of AFP gene. The present study showed that daily administration of *Cynara scolymus* extract at doses of (0.75 and 1.5g/kg b.wt), silymarin and their combination significantly limited the elevation of serum AFP level as compared to NDEA-intoxicated group, suggesting that *Cynara scolymus* extract might delay the NDEA-induced HCC in rats.

Histopathology of the liver tissue of NDEA and Ccl4 treated group revealed hepatocellular carcinoma with cellular and nuclear pleomorphism and frequent mitosis. These alterations could be attributed to the toxic effect of NDEA through generation of reactive oxygen species (ROS) with production of malondialdehyde (MDA) and 4-hydroxynonenal (Metwally etal,2011) which attack cellular targets including DNA, thereby inducing mutagenicity and carcinogenicity (Jeyabal etal,2005). Liver sections of rats pretreated with silymarin revealed improvement in the hepatocytes that exhibited less dissarangement and few mitosis. This improvement could be attributed to the antioxidant activity of silymarin which reduce intracellular ROS level of hepatocytes ,preventing oxidative stress-induced cellular damage (El Mesallamy etal,2011). Liver sections of rats pretreated with *Cynara scolymus* revealed marked improvement in the hepatocytes compared with NDEA treated group. This improvement may be attributed to the antioxidant capacity of *Cynara scolymus* and suppression of tumor promotion (Yasukawa et al ,2012).

Conclusion:

From this study it can be concluded that *Cynara scolymus* extract possess hepatoprotective activity and the protective 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 food supplements in patients with chronic liver diseases.

Corresponding Author
Dr. Azza H. M. Hassan
Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.
azzahassan99@gmail.com

References

1. Aleem, E. Elshayeb, A. Elhabachi, N. Mansour, A.R. Gowily, A. Hela, 2012. Serum IGFBP-3 is a more effective predictor than IGF-1 and IGF-2 for the development of hepatocellular carcinoma in patients with chronic HCV infection. Oncol Lett., 3: 704-712.
2. Babson, A.L. Greeley, S.J. Coleman, C.M. Phillips, 1966. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. Clin Chem., 12: 482-490.
3. Bansal, A.K., Bansal, M. Soni, G. Bhatnagar, 2005. Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem Biol Interact, 156: 101-111.
4. Bartholomew, R. Delaney, 1966. Sulphonphthaleins as specific reagents for albumin: determination of albumin in serum. Proc Aust Assoc Clin Biochem., 1: 214-218.
5. Carleton, H.M. Drury, R.A.B. Wallington, 1967. Carleton's Histological technique. New York, Oxford University Press.
6. Cross, C.E. Halliwell, B. Borish, E.T. Pryor, W.A. Ames, B.N. Saul, R.L. McCord, J.M. Harman, 1987. Oxygen radicals and human disease. Ann Intern Med., 107: 526-545.
7. Doumas, B.T. Perry, B.W. Sasse, E.A. Straumfjord, 1973. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clin Chem., 19: 984-993.
8. El Mesallamy, H.O., Nadia S Metwally, Mahmoud S Soliman, Kawkab A Ahmed and Mai M Abdel Moaty, 2011. The chemopreventive effect of Ginkgo biloba and Silybum marianum extracts on hepatocarcinogenesis in rats. Cancer Cell International, 11:38
9. El Saeed, T.A. Taha, M.E. Hassan, M.S. Amany, 2012. Modulatory Effects of Artichock Leave Extract on Nandrolone Decanoate-Induced Biochemical Alterations in Rats. Glob J Biotechnol Biochem., 7: 68-78.
10. El-Sohafy, S.M. Alqasoumi, S.I. Metwally, A.M. Omar, A.A. Amer, M.M. Abou Shoer, M.I. El Toumy, S.A. Abdel-Kader, 2013. Evaluation of the hepatoprotective activity of some plants belonging to the tribe Cynareae growing in Egypt. J Med Plants Res., 7: 324-328.
11. Farazi, P.A. DePinho, 2006. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer, 6: 674-687.
12. Ghanem, K.Z. Ramadan, M.M. Farrag, A.R.H. Ghanem, H.Z. Farouk, 2009. Egyptian artichoke volatile compounds protect against lead-induced hepatic and renal toxicity in male rats. Pol J Food Nutr Sci., 59: 175-181.
13. Gibbs, P.E. Zielinski, R. Boyd, C. Dugaiczyk, 1987. Structure, polymorphism, and novel repeated DNA elements revealed by a complete sequence of the human alpha-fetoprotein gene. Biochem., 26: 1332-1343.
14. Holtmann, G. Adam, B. Haag, S. Collet, W. Grunewald, E. Windeck, 2003. Efficacy of artichoke leaf extract in the treatment of patients with functional dyspepsia: a six-week placebo-controlled, double-blind, multicentre trial. Aliment Pharmacol Ther., 18: 1099-1105.
15. Jeyabal PVS, Syed,MB, Venkataraman ,M., Sambandham JK, and Sakthisekaran D. 2005 Apigenin inhibits oxidative stress-induced macromolecular damage in Nnitrosodiethylamine induced hepatocellular carcinogenesis in Wistar albino rats. Mol Carcinogen; 44: 11-20.
16. Jimenez-Escrig, A. Dragsted, L.O. Daneshvar, B Pulido, R. Saura-Calixto, 2003. In vitro antioxidant activities of edible artichoke (Cynara scolymus L.) and effect on biomarkers of antioxidants in rats. J Agric Food Chem, 51: 5540-5545.
17. Li, C.J. Huang, S.Y. Wu, M.Y. Chen, Y.C. Tsang, S.F. Chyuan, J.H. Hsu, 2012. Induction of apoptosis by ethanolic extract of Corchorus olitorius leaf in human hepatocellular carcinoma (HepG2) cells via a mitochondria-dependent pathway. Mol., 17: 9348-9360.
18. Mehmetcik, G. Ozdemirler, G. Kocak-Toker, N. Cevikbas, U. Uysal, 2008. Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress. Exp Toxicol Pathol., 60: 475-480.
19. Metwally, N.S. Kholeif, T.E. Ghanem, K.Z. Farrag, A.R. Ammar, N.M. Abdel-Hamid, 2011. The protective effects of fish oil and artichoke on hepatocellular carcinoma in rats. Eur Rev Med Pharmacol Sci, 15:1429-1444.
20. Mittal, G. Brar, A.P. Soni, 2006. Impact of hypercholesterolemia on toxicity of N-nitrosodiethylamine: biochemical and histopathological effects. Pharmacol Rep., 58: 413-419.
21. Motalleb, G. Hanachi, P. Fauziah, O. Asmah, 2008. Effect of Berberis vulgaris fruit extract on alpha-fetoprotein gene expression and chemical carcinogen metabolizing enzymes activities in

hepatocarcinogenesis rats. Iran J Cancer Prev., 1: 33-44.
22. Rajkapoor, B. Jayakar, B. Murugesh, N. Sakthisekaran, 2006. Chemoprevention and cytotoxic effect of Bauhinia variegata against N-nitrosodiethylamine induced liver tumorsand human cancer cell lines. J Ethnopharmacol, 104: 407-409.
23. Reitman, S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol., 28: 56-63.
24. Rezaie, A. Fazlara, A. Haghi Karamolah, M. Shahriari, A. Najaf, H.Z. Pashmforosh, 2013. Effects of Echinacea purpurea on Hepatic and Renal Toxicity Induced by Diethylnitrosamine in Rats. Jundishapur J Nat Pharm Prod., 8: 60-64.
25. Shaarawy, S.M. Tohamy, A.A. Elgendy, S.M. Elmageed, Z.Y. Bahnasy, A. Mohamed, M.S. Kandil, E. Matrougui, 2009. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci., 5: 549-557.
26. Song, Y. Jin, S.J. Cui, L.H. Ji, X.J. Yang, 2013. Immunomodulatory Effect of Stichopus japonicus Acid Mucopolysaccharide on Experimental Hepatocellular Carcinoma in Rats. Mol., 18: 7179-7193.
27. Speroni, E. Cervellati, R. Govoni, P. Guizzardi, S. Renzulli, C. Guerra, 2003. Efficacy of different Cynara scolymus preparations on liver complaints. J Ethnopharmacol., 86: 203-211.
28. Sundaresan, S. Subramanian, 2003. S-allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N-nitrosodiethylamine-induced carcinogenesis. Pol J Pharmacol., 55: 37-42.
29. Vandenberghe, 1996. Hepatotoxicology: Structure, function and toxicological pathology. In Toxicology: Principles and Applications Edited by Niesink, JM, Vries J, Hollinger MA Vries J, Hollinger MA, 669-721.
30. Wang, Y. Shen, 2013. Unresectable hepatocellular carcinoma treated with transarterial chemoembolization: clinical data from a single teaching hospital. Int J Clin Exp Med., 6: 367-371.
31. Yasukawa, Ken (2012). Medicinal and Edible Plants as Cancer Preventive Agents, Drug Discovery Research in Pharmacognosy. ISBN 978-953-51-0213-7.p.181-208
32. Zhang, X.L. Yu, H. Xiong, Y.Y. Ma, S.T. Zhao, L. She, 2013. Resveratrol down-regulates Myosin light chain kinase, induces apoptosis and inhibits diethylnitrosamine-induced liver tumorigenesis in rats. Int J Mol Sci., 14: 1940-1951.

2/19/2014