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The protective effects of fish oil and artichoke on hepatocellular carcinoma in rats

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Abstract. – *Background and Objectives:* **The present study aimed to evaluate the effect of fish oil and Artichoke (*Cynara scolymus l.*) against diethylnitrosamine (DEN) induced hepatocellular carcinoma in rats.**

Materials and Methods: **Animals were divided into 8 groups. Group 1, control rats. Group 2: rats injected with single dose of DEN (100 mg/kg body weight). Groups 3-8 supplemented with different concentrations of either fish oil or artichoke for 25 days before DEN injection.**

Results: **DEN treatment revealed a significant decrease in tissue xanthine oxidase (XO), glutathione, glutathione-s-transferase (GST), and a marked increase in malondialdehyde (MDA) and nitric oxide (NO) levels. Vascular endothelial growth factor (VEGF), alpha-fetoprotein (AFP), and ferritin levels showed a significant increase. A significant increase in serum aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin levels were found. A significant decrease in tissue total proteins and serum albumin was observed.**

The administration of DEN affected the liver cell through occurrence of hepatic cellular degeneration and necrosis. Treatment with fish oil (5%, 10%) or artichoke heads or leaves (0.5, 1 g) for 25 days led to significant amelioration of DEN-induced changes in the biochemical parameters. An almost normal histological architecture of the liver, in treated groups, was showed as compared to the controls.

Conclusions: **The results pointed that 10% fish oil and 1 g% leaves of artichoke succeeded to protect from hepatocellular carcinoma to a certain degree. In addition, they may be considered as protective foods against angiogenesis.**

Key Words:

Fish oil, Artichoke, Hepatocellular carcinoma, Diethylnitrosamine.

Introduction

Liver cancer is one of the major health problems, which have a high mortality rate to human, and its occurrence varies with respect to age, sex, rate of growth, invasiveness, metastatic potential, and response to prognosis of treatment[1]. It classified as primary, involving hepatic cells only and secondary that involves other parts of the body other than liver[2]. Primary liver cancer is subdivided into two types as cholangio carcinoma (CAC) and hepatocellular carcinoma (HCC).

HCC is one of the world's deadliest cancers, ranking the third among all cancer-related mortalities. Most cases occur in Asia and sub-Saharan Africa, where viral hepatitis is endemic; the incidence is rising in the West due to the prevalence of hepatitis C virus[3]. In Egypt, between 1993 and 2002, there was an almost two-fold increase in HCC amongst chronic liver patients[4].

Liver is the key organ of metabolic, secretory and excretory functions in the body, where its disorders are numerous and varied. For treatment of such disorders, conventional medicine does not provide enough remedies. However, remedies of both plant and animal origin have long been used for hepatobiliary diseases and the studies have proved them to be beneficial[5].

Fish oil is rich sources of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Compared to saturated fats, PUFAs are more readily used for energy when initially ingested. Increasing the degree of unsaturation at a given carbon chain length in-

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creases the relative mobility of stored fat, making PUFAs more bioavailable[6]. There are several circumstances where the requirements for DHA greatly exceed the rate of synthesis, making supplementation necessary. Epidemiological, experimental, and mechanistic data implicate n-6 PUFAs as stimulators and long chain n-3 PUFAs as inhibitors of development and progression of a range of human cancers[7]. Studies have found that the antitumor effect of EPA is mainly related to its suppression of cell proliferation. On the other hand, the effect of DHA appears to be related to its ability to induce apoptosis[8].

Studies also showed that fish oil inhibited growth of breast cancer cells and hepatocarcinoma in rats[9,10]. Moreover, fish oil as n-3 polyunsaturated fatty acid, eicosapentaenoic and docosahexaenoic acid inhibited human lung carcinoma cell growth and prostate cancer[11,12].

Artichoke, *Cynara scolymus L.*, is an ancient herbaceous plant, originating from the Mediterranean area. Today, it is widely cultivated all over the world. Its head is edible as a favorite vegetable and prepared for a variety of products such as salad, jam and canned beverages and its leaf is widely used for therapeutic purposes[13].

Artichoke leaves extracts used in the treatment of dyspeptic and hepatic disorders. *In vitro* assays, artichoke leaf extract has shown an increase in bile production, inhibition of hepatocellular cholesterol biosynthesis, inhibition of LDL-oxidation and have both antioxidant and apoptotic activities[14-17].

Therefore, the aim of the present study was to investigate the role of some functional foods either of animal origin (fish oil) or of plant origin (artichoke) during the initiation phase of hepatocellular carcinoma in rats using diethylnitrosamine. To asses this aim biochemical analyses for the related parameters were done and confirmed with the histopathological investigation.

Materials and Methods

Materials

N-Nitrosodiethylamine was purchased from Sigma (St. Louis, MO, USA). Other chemicals and reagents used were of highest purity. Fish oil purchased from Sigma, is derived from Menhaden fish, and is composed of different fatty acids[18]. The edible portions of Artichoke, heads and leaves, separated and dried in air-drying oven at 60°C. The dried materials grinded separately to a powder and stored until use.

Animals

Adult female rats (Sprague Dawley strain) (100-120 g) were purchased from the Laboratory of Animal House, National Research Centre, Giza, Egypt.

All animals received professional humane care and ethical guidelines were followed approved by the Ethical Committee of the National Research Centre.

Food intake was determined every day by subtract refusal diet from the given diet and the animals were weighed weekly to monitor the body weight changes and feed efficiency ratio (FER) was calculated as follows: FER = Gain in body weight/ Food intake.

Artichoke Extracts

1 and 0.5 g of dry powder of Artichoke heads or leaves was immersed in 100 ml boiled water, for 15 minutes then filtered[19]. This solution corresponds to the popular form of the use for beneficial health effects. They were prepared daily and *ad libitum* to rats in bottles to be the sole source of drinking fluid.

Preparation of Standard Diet

Animals were fed on standard diet according to American Institute of Nutrition[20]. It was prepared as follows: corn starch (465.69 g/kg diet), casein (140.0 g/kg diet), dextrinized corn starch (155.0 g/kg diet), sucrose (100.0 g/kg diet), corn oil (40.0 g/kg diet), fiber (50.00 g/kg diet), mineral mixture (35.00 g/kg diet), vitamin mixture (10.00 g/kg diet), L-cystine (1.80 g/kg diet), choline chloride (2.50 g/kg diet) and tert-butyl hydroquinone (0.008 g/kg diet).

Experimental Design

Sixty-four female adult albino rats were divided into eight groups:

Group 1: Served as control and fed on standard diet.

Group 2: DEN treated group, fed on standard diet for 25 days before i.p injection with a single dose of DEN (100 mg/kg body weight)[21], then fed with standard diet for another 25 days. The other groups (G3-G8) were fed on treated diet for 25 days before i.p injection with single dose of DEN. Rats were fed with treated diet for extra 25 days.

Group 3: Fed with standard diet supplemented with 5% fish oil.
Group 4: Fed with standard diet supplemented with 10% fish oil[22].
Group 5: Fed with standard diet and aqueous extract of artichoke heads (0.5 g %) is supplied in the drinking bottles.
Group 6: Fed with standard diet and aqueous extract of artichoke heads (1 g %) is supplied in the drinking bottles.
Group 7: Fed with standard diet and aqueous extract of artichoke leaves (0.5 g %) is supplied in the drinking bottles.
Group 8: Fed with standard diet and aqueous extract of artichoke leaves (1 g %) is supplied in the drinking bottles.

At the end of the experimental period (50 days), blood samples were withdrawn from sublingual vein after anesthetizing the animals with diethyl ether. Blood was collected in clean test tubes and allowed to clot, then centrifuged for ten minutes at 3000 r.p.m. Serum was separated and stored into Eppendorff tubes at – 20°C to be used for later biochemical analyses. Liver tissues were homogenized in bidistilled water using homogenizer instrument in the ratio of 1:10 w/v. The homogenate was centrifuged for ten minutes at 3000 r.p.m and the supernatant was collected used directly, or stored into Eppendorff tubes and kept at – 20°C for further use.

Biochemical Analysis

Xanthine oxidase was performed in the liver tissue according to the method described by Fried and Fried[23]. Nitric oxide was measured in the liver using the method described by Moshage et al[24]. Liver reduced glutathione was measured in the liver using the method described by Beutler et al[25]. Glutathione-S-transferase (GST) activity was measured in the liver using the method described by Habig et al[26]. Lipid peroxides were measured in the liver as malondialdehyde using the method described by Buege and Aust[27]. VEGF was measured in sera using the method described by Kim et al[28], alpha fetoprotein was performed according to the method described by Li et al[29] using AccuBind Elisa Microwells kits (Monobind Inc, Costa Mesa, CA, USA). Ferritin was determined according to the method described by Steiene-Martin et al[30] using AccuBind Elisa Microwells kits. AST and ALT activities were determined in sera using cromatest kits (Monobind Inc, Costa Mesa, CA, USA), according to the method described by Young[31] GGT was measured in sera of treated and non treated rats using Centronic GmbH-Germany Kits (Centronic GmbH, Germany) following the method described by Persijn and Vander Slik[32]. Alkaline phosphatase was determined in serum using the method described by Demetriou et al[33]. Serum bilirubin was carried out by the method described by Young[34], serum albumin was performed according to the method described by Webster[35] and total proteins were determined in liver of different groups using the method described by Cannon et al[36].

Histopathological Studies

After rats sacrificing, liver were plotted free of adhering blood, washed with cold saline, dried between filter papers and weighed to calculate the relative liver weight, then a part of liver tissue was rapidly removed and cut into small slices were directly used or frozen at –20°C for other biochemical analyses. Some of these tissues were fixed in 10% formal saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax. Sections of 5 mm thickness were prepared and stained with haematoxylin and eosin for histopathological investigation[37].

Statistical Analysis

Data were evaluated with SPSS (Statistical Package for the Social Sciences, version 9) software (SPSS Inc, Chicago, IL, USA). Hypothesis testing methods including one-way analysis of variance (ANOVA) followed by least significant differences (LSD) test at p value less than 0.05.

Results

A significant decrease in food intake was observed in DEN group as compared to control, but there were no changes in the groups treated with fish oil (5%, 10%) and artichoke heads or leaves (0.5 g, 1 g) as compared to DEN group. These groups still showed a marked decrease in food intake as compared to control group (Table I).

A highly significant decrease was observed in feeding efficiency ratio in DEN-treated group as compared with control group. Five % fish oil and artichoke heads (0.5 g) showed a non-significant change, while fish oil 10%, artichoke leaves (0.5

Table I. Food intake, feed efficiency, body weight gain, and relative liver weight in control and different experimental groups.

Groups: parameters	Food intake	Feed efficiency ratio	Body weight gain	Relative liver weight
Control	697.8 ± 11.82	0.152 ± 0.005	106.3 ± 4.87	3.27 ± 0.035
DEN	679 ± 8.9^{A}	0.126 ± 0.008^{A}	86 ± 6.7^{A}	3.78 ± 0.33^{A}
Fish oil 5%	679 ± 8.9^{A}	0.133 ± 0.002^{A}	89.4 ± 2.2^{A}	3.68 ± 0.28
Fish oil 10%	678.4 ± 11^{A}	0.146 ± 0.01^{B}	99.8 ± 11^{B}	3.91 ± 0.14^{A}
Artichoke (heads) 0.5 g	675.4 ± 7.9^{A}	0.130 ± 0.006^{A}	88.6 ± 5.3^{A}	3.61 ± 0.19
Artichoke (heads) 1 g	672.2 ± 10^{A}	0.138 ± 0.007^{ab}	93.6 ± 6.2^{A}	3.94 ± 0.32^{A}
Artichoke (leaves) 0.5 g	675 ± 7.9^{A}	0.144 ± 0.01^{B}	98 ± 8.6^{B}	3.28 ± 0.52^{B}
Artichoke (leaves) 1 g	679.4 ± 7.1^{A}	0.141 ± 0.01^{B}	96.7 ± 10^{ab}	3.14 ± 0.36^{B}

All data are presented as Mean ± S.D. $^{A}p \leq 0.01$; $^{a}p \leq 0.05$ where, results expressed as comparing treated groups and DEN group to healthy control. $^{B}p \leq 0.01$; $^{b}p \leq 0.05$ where, results expressed as comparing treated groups to DEN group.

g, 1 g) and heads (1 g) showed a significant increase. When these groups compared to control group, our results demonstrated that, treatment with fish oil (5%), artichoke heads (0.5 g) showed a marked decrease and treatment with artichoke heads (1 g) showed a slight decrease, while treatment with fish oil (10%) and artichoke leaves (0.5 g, 1 g) showed non significant change (Table I).

Data presented in table I showed a highly significant decrease in body weight gain of DEN administered group as compared control. The results demonstrated that treatment with fish oil (5%) and artichoke heads (0.5 g, 1 g) showed no significant change in body weight gain, while treatment with fish oil 10% and artichoke leaves (0.5 g) showed a highly significant increase in body weight gain and treatment with higher dose of artichoke leaves caused a significant increase in body weight gain.

Comparing treated groups with healthy control group our results illustrated that treatment with fish oil (5%) and artichoke heads (0.5 g, 1 g) caused a marked decrease in body weight gain, while treatment with fish oil (10%) and artichoke leaves (0.5 g) showed no significant change and treatment with artichoke leaves (1 g) showed slightly decrease in body weight gain.

Regarding the relative liver weight, that shown in table I there was a highly significant increase in DEN group as compared to control group. When comparing treated groups with DEN group, there was a non significant change in case of treatment with fish oil (5%, 10%) and artichoke heads (0.5 g, 1 g), while treatment with artichoke leaves (0.5 g, 1 g) showed a highly significant decrease.

Biochemical Results

Data listed in table II showed that DEN caused high depletion in the level of xanthine oxidase activity. This decrease reached to 68.29% as compared to the control group. The results indicated that, treatment with higher dose of fish oil, artichoke heads (0.5 g, 1 g) and leaves (1 g) showed a highly significant elevation in XO activity, but lower dose of fish oil and artichoke leaves caused no significant difference when comparing with DEN group.

Comparing treated groups to control, the results showed that there was a marked reduction in the level of XO in case of treatment with lower dose of fish oil and artichoke leaves, while treatment with higher dose of fish oil and lower dose of artichoke heads enhanced the level of XO. Treatment with higher dose of artichoke heads or leaves improved the level of XO, which reached near the normal value.

As shown in Table II, the level of NO showed a highly significant increase in DEN group that reached to 48.64% as compared to the control group. Comparing treated groups with DEN group, fish oil (5%, 10%) and artichoke leaves (0.5 g, 1 g) showed highly significant decrease in nitric oxide level, while with the high dose of artichoke heads a significant decrease were observed and with low dose no significant difference was noted.

These groups compared to healthy control, demonstrated that, treatment with lower dose of fish oil and artichoke heads (0.5 g, 1 g) still caused a marked elevation in the level of NO, while higher doses caused slightly elevation in the level of NO.

DEN caused great depletion in the level of GSH (82.03%), while treatment with fish oil or

Table II. Effect of fish oil and artichoke (heads and leaves) on some antioxidants levels in control and different experimental groups.

Groups: parameters	XO (µmol/g tissue)	NO (µ mol/g)	GSH (mg/g)	GST (mmol/g protein)	LPO (nmol MDA/g tissue
Control	1.23 ± 0.33	7.38 ± 0.40	8.29 ± 1.05	4.62 ± 0.80	14.79 ± 1.39
DEN	0.39 ± 0.20^{A}	10.97 ± 0.87^{A}	1.49 ± 0.24^{A}	1.71 ± 0.40^{A}	26.39 ± 3.06^{A}
Fish oil 5%	0.27 ± 0.15^{A}	8.98 ± 0.68^{AB}	8.07 ± 0.83^{B}	2.18 ± 0.15^{A}	19.38 ± 1.85^{AB}
Fish oil 10%	0.81 ± 0.23^{aB}	8.72 ± 0.72^{aB}	7.47 ± 0.75^{B}	4.46 ± 0.44^{B}	19.56 ± 1.68^{AB}
Artichoke (heads) 0.5 g	0.85 ± 0.20^{aB}	10.21 ± 0.95^{A}	7.07 ± 0.93^{aB}	2.29 ± 0.29^{A}	14.14 ± 0.8^{1B}
Artichoke (heads) 1 g	1.04 ± 0.23^{B}	9.92 ± 0.57^{Ab}	7.87 ± 0.75^{B}	2.18 ± 0.51^{A}	14.18 ± 0.93^{B}
Artichoke (leaves) 0.5 g	0.50 ± 0.15^{A}	7.56 ± 0.52^{B}	7.48 ± 0.74^{B}	2.99 ± 0.33^{AB}	14.92 ± 2.03^{B}
Artichoke (leaves) 1 g	1.00 ± 0.20^{B}	8.61 ± 0.99^{aB}	8.20 ± 0.23^{B}	4.47 ± 0.58^{B}	15.17 ± 0.30^{B}

All data are presented as Mean ± S.D. $^{A}p \leq 0.01$; $^{a}p \leq 0.05$ where, results expressed as comparing treated groups and DEN group to healthy control. $^{B}p \leq 0.01$; $^{b}p \leq 0.05$ where, results expressed as comparing treated groups to DEN group.

artichoke head and leaves showed highly significant elevation in GSH levels, while treatment with fish oil (5%, 10%), artichoke heads (1 g) and artichoke leaves (0.5 g, 1 g) improved the level of GSH which becomes semi normal values (Table II).

DEN led to a marked reduction in GST activity (62.99%) as compared to control group, but treated groups with higher doses of fish oil and artichoke leaves (0.5 g, 1 g) showed a highly significant elevation in GST activity, while treatment with lower dose of fish oil and artichoke heads (0.5 g, 1 g) showed no significant difference. These groups, compared to the control, showed a marked reduction in the level of GST in treated groups with lower dose of fish oil, artichoke leaves and artichoke heads (0.5 g, 1 g). While treatment with higher dose of fish oil or artichoke leaves enhanced the activity of GST and reached to near normal value (Table II).

Data in Table II showed that DEN caused a significant increase (78.43%) in the level of MDA in the liver compared to the control group, while in all treated groups with fish oil and artichoke, a highly significant reduction in MDA levels were noticed as compared to DEN group. As compared to the control group, these groups showed a marked increase in the level of MDA in the case of fish oil (5%, 10%), while treatment with two doses of artichoke heads or leave improved the level of MDA.

As shown in Table III, the results indicated that the level of VEGF showed a marked in-

Table III. Serum vascular endothelial growth factor (VEGF), alpha fetoprotein (AFP) and ferritin levels of control and treated groups.

Groups: parameters	VEGF (pg/ml)	AFP (ng/ml)	Ferritin (ng/ml)
Control	7.72 ± 1.82	8.20 ± 0.86	60.83 ± 2.04
DEN	12.01 ± 2.68^{A}	13.42 ± 0.65^{A}	79.64 ± 1.65^{A}
Fish oil 5%	8.33 ± 1.39^{B}	13.03 ± 0.75^{A}	74.31 ± 2.49^{AB}
Fish oil 10%	8.22 ± 1.57^{B}	10.67 ± 0.92^{AB}	65.86 ± 3.39^{AB}
Artichoke (heads) 0.5 g	10.93 ± 1.36^{a}	13.06 ± 1.30^{A}	75.20 ± 2.99^{AB}
Artichoke (heads) 1 g	10.81 ± 2.51^{a}	12.66 ± 1.28^{A}	68.58 ± 1.79^{AB}
Artichoke (leaves) 0.5 g	10.11 ± 1.56^{a}	12.97 ± 1.32^{A}	74.58 ± 2.77^{AB}
Artichoke (leaves) 1 g	7.12 ± 2.31^{B}	12.75 ± 1.60^{A}	67.15 ± 2.18^{AB}

All data are presented as Mean ± S.D. $^{A}P \leq 0.01$; $^{a}p \leq 0.05$ where, results expressed as comparing treated groups and DEN group to healthy control. $^{B}p \leq 0.01$; $^{b}p \leq 0.05$ where, results expressed as comparing treated groups to DEN group.

crease in DEN group (55.57%) as compared to the control one. In comparing the treated groups with DEN group, the results indicated that treatment with two doses of fish oil and higher dose of artichoke leaves caused a highly significant decrease in the level of VEGF, while treatment with artichoke heads (0.5 g, 1 g) and lower dose of artichoke leaves showed no significant difference.

Comparing these groups with the control one demonstrated that treatment with fish oil (5%, 10%) and higher dose of artichoke leaves enhanced the level of VEGF, but still slightly elevation in the level of VEGF was noticed in the case of treatment with artichoke heads (0.5 g, 1 g) and lower dose of artichoke leaves.

Data listed in Table III showed a highly significant increase in serum AFP level in DEN group reached 63.66% as compared to healthy control group. Comparing treated groups with DEN group, all treated groups showed no significant difference except treatment with higher dose of fish oil showed a highly significant reduction in AFP level. While these groups compared to standard healthy control demonstrated that, all treated groups still showed marked increases in the level of AFP.

As shown in table III, the present results revealed that the level of ferritin as a tumor marker was increased by the treatment of DEN (30.92%) as compared to healthy control. While treatment with fish oil (5%, 10%); artichoke heads and leaves (0.5 g, 1 g) showed a highly significant decrease in serum ferritin level. This decrease in ferritin level recorded 6.69, 17.3, 5.58, 13.89, 6.35 and 15.68%, respectively as compared to DEN group. As comparing treated groups with healthy control, it was clear that, there was still a marked elevation in the level of ferritin in all treated groups.

The results in Table IV showed a marked increase in the level of AST in animals treated with DEN (186.76%) as compared to healthy control group. While treatment of these groups with fish oil and artichoke (head & leaves) showed a highly significant decrease in AST activity amounting, 45, 63.76, 46.03, 51.18, 62.86 and 58.81%, respectively. Comparing with standard healthy control, our results demonstrated that treatment with higher dose of fish oil and lower dose of artichoke leaves improved the level of AST activity, which reached nearly to the normal value, while treatment with lower dose of fish oil and two doses of artichoke heads still represent a marked increase in AST.

As shown in Table IV DEN group showed the marked elevation in the level of ALT activity reached 89.30% as compared to control group. Comparing treated groups with DEN group, our results indicated that, all treated groups showed a highly significant reduction in ALT activity. When these groups compared to control, the results revealed that treatment with two doses of fish oil or artichoke heads still showed a marked elevation in the level of ALT activity, while treatment with artichoke leaves (0.5 g, 1 g) enhanced the level of ALT activity.

The results in Table IV revealed clear elevation in the level of GGT in the pretreated group with DEN (147.32) as compared to healthy control group, while treatment of these groups with fish oil (5%, 10%); artichoke heads and leaves

Table IV. Effect of fish oil and artichoke on liver function enzymes in rat serum of control and different experimental groups.

Groups: parameters	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)
Control	30.51 ± 1.71	29.16 ± 2.15	13.17 ± 1.59	37.66 ± 3.06
DEN	87.49 ± 5.69^{A}	55.20 ± 4.43^{A}	147.32 ± 12.5^{A}	113.32 ± 5.05^{A}
Fish oil 5%	48.12 ± 6.25^{AB}	36.25 ± 2.85^{AB}	64.32 ± 9.31^{AB}	45.26 ± 2.43^{aB}
Fish oil 10%	31.71 ± 1.13^{B}	37.21 ± 2.86^{AB}	47.04 ± 4.94^{AB}	40.42 ± 0.96^{B}
Artichoke (heads) 0.5 g	47.22 ± 4.78^{AB}	48.40 ± 5.03^{AB}	43.05 ± 2.85^{AB}	62.01 ± 6.58^{AB}
Artichoke (heads) 1 g	42.71 ± 2.81^{AB}	47.44 ± 5.69^{AB}	39.37 ± 4.66^{AB}	57.69 ± 3.63^{AB}
Artichoke (leaves) 0.5 g	32.49 ± 2.15^{B}	31.04 ± 2.08^{B}	66.94 ± 8.93^{AB}	50.44 ± 5.11^{AB}
Artichoke (leaves) 1 g	36.04 ± 2.99^{aB}	29.16 ± 3.67^{B}	27.93 ± 2.03^{AB}	46.30 ± 4.30^{AB}

All data are presented as Mean ± S.D. $^{A}p \leq 0.01$; $^{a}p \leq 0.05$ where, results expressed as comparing treated groups and DEN group to healthy control. $^{B}p \leq 0.01$; $^{b}p \leq 0.05$ where, results expressed as comparing treated groups to DEN group.

(0.5 g, 1 g) inhibited GGT activity. Comparing with standard healthy control the efficacy of the selected functional foods are not significant.

The findings in table IV revealed that, DEN group showed a marked elevation in ALP activity reached 200.1% as compared to healthy control group, treatment of DEN-initiated rats with fish oil (5%, 10%); artichoke heads and leaves (0.5g, 1g) caused a significant decrease in the activity of ALP as compared to DEN group. Comparing with standard healthy control, treatment with higher dose of fish oil enhanced the level of ALP activity, which reached close to the normal value while the lower doses caused slight improvement. Furthermore, a marked increase in the level of ALP activity was noticed in the case of treatment with artichoke heads, leaves (0.5 g, 1 g).

Data in Table V showed that, the DEN group showed a high increase in total bilirubin level and the increment reached 237.34% as compared to healthy control group. Comparing treated groups with DEN group, treatment with fish oil (5%, 10%), artichoke heads and leaves (0.5 g, 1 g) caused a highly significant depletion in total bilirubin level, while these groups compared to standard healthy control, demonstrated that treatment with higher dose of fish oil and lower dose of artichoke leaves improved the level of total bilirubin which reached near the normal value, while still a marked elevation in the level of total bilirubin was noticed in the case of treatment with lower dose of fish oil, artichoke heads (0.5 g, 1 g) and higher dose of artichoke leaves.

The results showed a marked depletion in albumin level (54.37%) in DEN treated group as compared to healthy control group. Comparing treated groups with DEN group, all treated groups showed a highly significant elevation in albumin level except treatment with lower dose of fish oil, which showed no significant difference (Table V). As comparing with control one. Higher doses of fish oil and artichoke leaves and lower dose of artichoke heads led to increase the level of albumin. While the treatment with lower dose of fish oil still caused a marked depletion in the level of albumin, while a slight decrease in the level was noticed in the case of treatment with lower dose of artichoke leaves and higher dose of artichoke heads. The data in Table V revealed that the induction of DEN caused a highly depletion in the level of total protein by 20.76% as compared to healthy control group. While treated rats with fish oil (5%, 10%); artichoke heads (0.5 g, 1 g); and leaves (0.5 g) caused a highly significant increases in total proteins level as compared to DEN group. Treatment with higher dose of artichoke leaves showed no significant difference in the level of total proteins in rat liver. Comparing treated groups with standard healthy control, our findings pointed out improvement in the level of total proteins.

Histopathological Results

The hepatic lobules are the structural units of the liver; each is formed of cords of hepatocytes and blood sinusoids in-between. The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and abundant cytoplasm. The cytoplasm of such cells is granular and strongly eosinophilic. The nuclei of the hepatocytes are large with peripherally dispersed chromatin and prominent nucleoli. Hepatocytes are oriented in cords composed of a single row of cells separat-

Table V. Serum total bilirubin, albumin and liver total protein levels in control and different experimental groups.

Groups: parameters	Total bilirubin (gm/dl)	Albumin (gm/dl)	Total proteins (mg/g tissue)
Control	3.91 ± 0.86	5.26 ± 0.54	549 ± 0.59
DEN	13.19 ± 1.71^{A}	2.40 ± 0.65^{A}	435 ± 0.14^{A}
Fish oil 5%	5.63 ± 0.65^{AB}	2.66 ± 0.43^{A}	507 ± 0.29^{B}
Fish oil 10%	4.26 ± 0.44^{B}	4.87 ± 0.76^{B}	511 ± 0.26^{B}
Artichoke (heads) 0.5 g	7.24 ± 0.99^{AB}	4.87 ± 0.47^{B}	553 ± 0.45^{B}
Artichoke (heads) 1 g	6.44 ± 0.99^{AB}	4.23 ± 0.14^{AB}	515 ± 0.25^{B}
Artichoke (leaves) 0.5 g	4.05 ± 0.47^{B}	4.38 ± 0.13^{aB}	538 ± 0.29^{B}
Artichoke (leaves) 1 g	6.26 ± 0.45^{AB}	5.58 ± 0.48^{B}	473 ± 0.29^{A}

All data are presented as Mean ± S.D. $^{A}p \leq 0.01$; $^{a}p \leq 0.05$ where, results expressed as comparing treated groups and DEN group to healthy control. $^{B}p \leq 0.01$; $^{b}p \leq 0.05$ where, results expressed as comparing treated groups to DEN group.

ed from vascular sinusoids by endothelial cells. The sinusoids run radially, converging at the centre of the hepatic lobule to form the central vein (Figure 1A). The administration of a single dose of DEN (100 mg/kg b.w., i.p.) on day 26 and left for 50 days caused disorganization of architecture of the liver and cellular damage. The hepatocytes seen paler, vacuolated, lost their normal shape, lost their arrangement and the cell membranes were not obvious. The hepatocytes exhibited a large pale cytoplasm and large nuclei in the group of cells in the center. The portal triads were less obvious (Figure 1B).

Examination of sections of liver of rat administered of a single dose of DEN then treated with 5% fish oil showed the loose of hepatocytes to their normal appearance and many of it appeared vacuolated (Figure 2A). However, sections of liver of rat treated with 10% fish oil after DEN administration showed normal hepatocytes (Figure 2B). Examination of liver sections of rats administered of DEN and treated with 0.5 g of the extract of the artichoke head showed an improvement in the hepatocytes as compared with DEN treated group. Some of the hepatocytes still paler, vacuolated and lost their normal shape, arrangement and the cell membranes are not obvious (Figure 3A). Examination of liver sections of rats received a single dose of DEN and treated with 1g artichoke head showed the hepatocytes appearance with more or less like control except some cells that appeared vacuolated and lost their normal shape (Figure 3B). The histopathological investigation of the sections of rats liver administered DEN then treated with 0.5 g artichoke extract leaves showed the hepatocytes appeared more or less like normal except few of it appeared vacuolated (Figure 4A). Sections of liver of rat treated with 1g of the extract of the artichoke leaves showed that the hepatocytes appearance were more or less like normal except few of it that appeared vacuolated (Figure 4B).

Discussion

The present results showed the tendency towards the decrease in food intake, feed efficiency ratio, body weight gain and significantly increase in relative liver weight in DEN group than in normal control group. These data were confirmed by Granado-Serrano et al[38] who reported that food intake, feed efficiency ratio and body weight gain were suppressed in DEN group as compared with the untreated control. Reduction of food intake and consequently, the reduction of body weight gain observed in DEN treated animals may be due to losses of skeletal muscle and adipose tissue as mentioned by Sreepriya and Bali[39], and it could be considered as an indirect indication of the declining hepatic function following exposure to DEN. Sivaramakrishnan et al[40] reported that body weight decreased significantly in DEN-induced animals compared to control and administration of DEN to animals also caused a significantly increase in liver weight due to appearance of liver nodules.

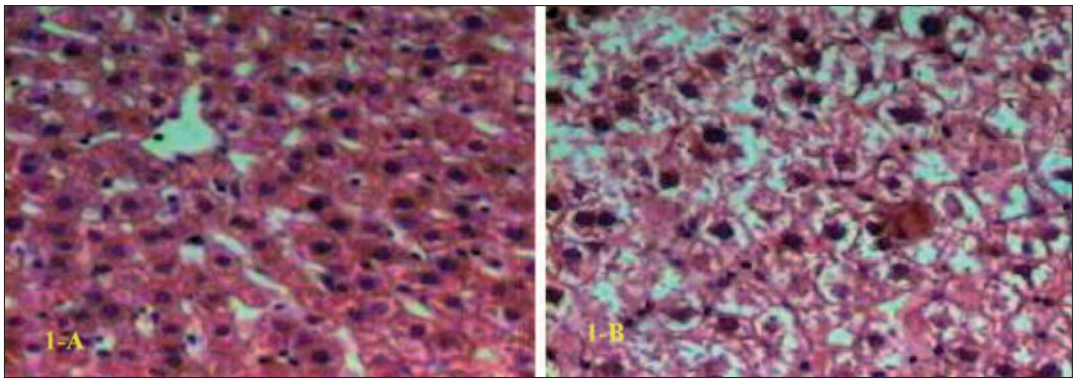

Figure 1. A, Section of control liver shows the architecture of a hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm, and distinct nuclei. Between the strands of hepatocytes, the hepatic sinusoids are shown. ***B,*** Section of liver of rat administered a single dose of DEN shows disorganization of liver architecture with cellular damage and necrosis in some areas. The hepatocytes are paler, vacuolated, lost their normal shape and arrangement and the cell membranes are not obvious. The hepatocytes exhibit a large pale cytoplasm and large nuclei in the centre of image (H & E stain- × 300).

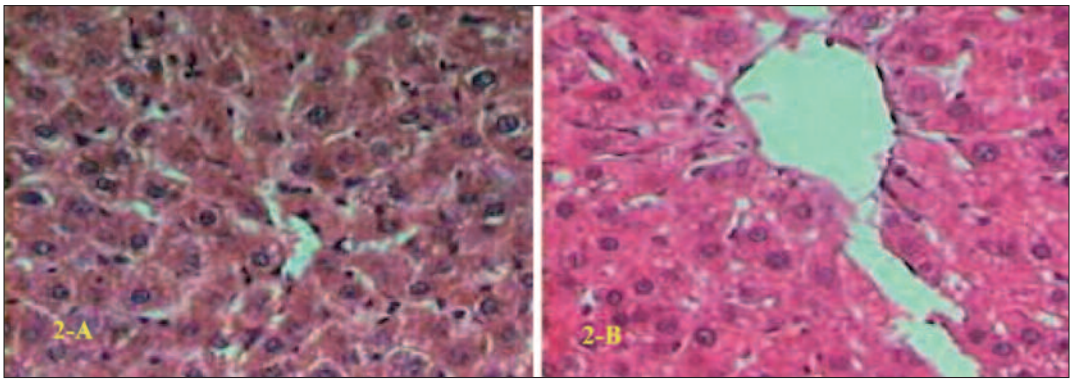

Figure 2. ***A,*** Section of liver of rat administered of a single dose of DEN and treated with 5% fish oil shows more or less normal hepatocytes. Notice the dilation of blood sinusoids. ***B,*** Section of liver of rat administered a single dose of DEN and treated with 10% fish oil shows more or less normal hepatocytes (H & E stain- × 300).

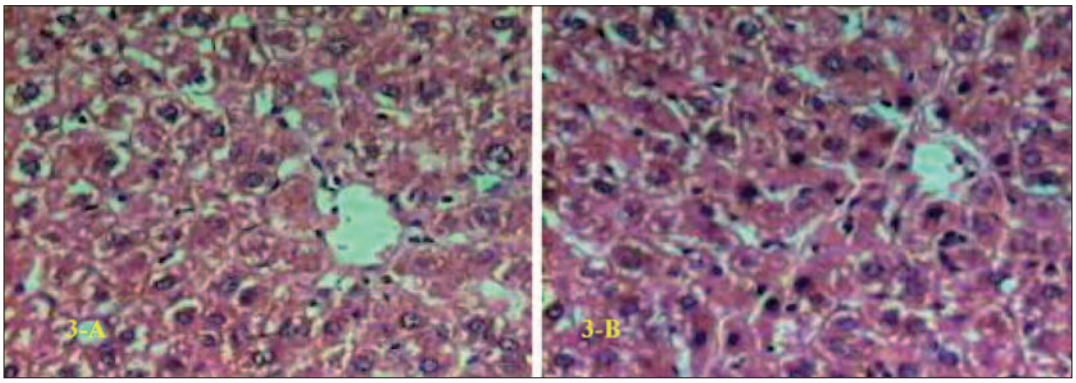

Figure 3. ***A,*** Section of liver of rat given a single dose of DEN and treated with 0.5 g of the extract of the artichoke head shows some improvement in liver architecture. However, some of the hepatocytes at the upper left appear paler, vacuolated, lost their normal shape and arrangement and the cell membranes are not obvious. ***B,*** Section of liver of rat given a single dose of DEN and treated with 1g of the extract of the artichoke head shows normal hepatocytes except some cells that appear vacuolated and lost their normal shape at the upper left (H & E stain- × 300).

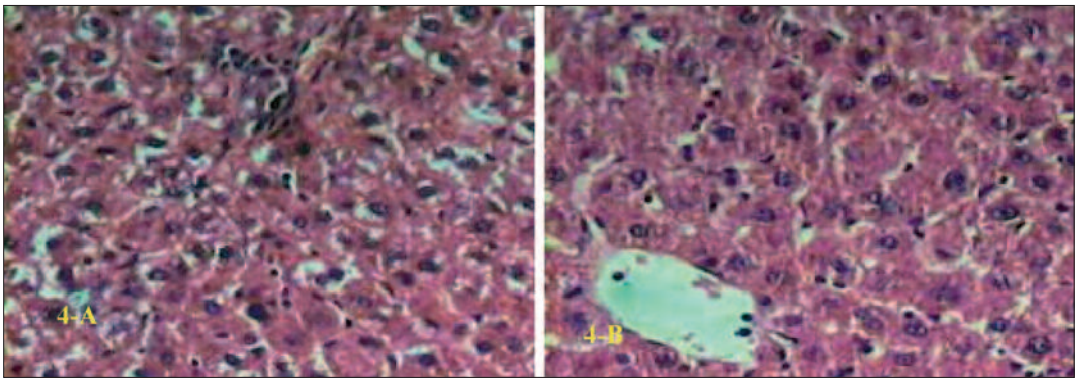

Figure 4. ***A,*** Section of liver of rat given a single dose of DEN and treated with 0.5 g of the extract of the artichoke leaves showing hepatocytes with more or less normal shape except few of them appear vacuolated. ***B,*** Section of liver of rat given a single dose of DEN and treated with 1g of the extract of the artichoke leaves showing hepatocytes appear with more or less normal shape except few of them appear vacuolated (H & E stain- × 300).

The present findings represented a highly significant depletion in XO level after DEN administration to rats as compared to the healthy group. Our results agree with Stripe et al[41] and Sadik et al[42] who reported a significant decrease in XO activity in rat liver administrated DEN as compared to the control group. The low activity of XO in hepatocellular carcinoma might be attributed to the lack of expression of the enzyme as a sign of low differentiation level in the transformed cells and presence of necrosis in the tumor tissue[43]. Furthermore, reactive oxygen species (ROS) produced during the oxidative stress process were believed to be responsible for aggravation of the damage, amplification of the lesion area and causing damage even at other sites, this damage caused release of the enzymes from liver or any other tissues into the circulation[44].

As concerning to the level of liver nitric oxide, the present results revealed a highly significant elevation in DEN-treated rats as compared to the normal healthy group. This data is in accordance with Rakov et al[45] who mentioned that nitric oxide is involved in all stages of carcinogenesis including initiation, promotion, progression, invasion, and metastasis as well as in cytotoxicity. The Authors attributed the involvement of NO in the induction and development of tumors to its capacity to damage the structure of DNA and that peroxynitrite modifies DNA in a special way that may be one of the causes of cellular immortalization or provokes desamidization and/or nitrofication leading to changes in the structure of DNA. The latter one leads not only to cellular death, but may be inherited as mutation and causes induction of neoplasia[46]. One of the well-characterized functions of NO is its role as a mediator of vascular dilatation and vascular remodeling[47]. In addition, the increased levels of liver NO enhanced vascular endothelial growth factor synthesis in vascular smooth muscle cells[48]. Hence, angiogenesis which is affected by vascular endothelial factor (VEGF) is mediated by the sustained formation of NO. In addition, VEGF exerts a stimulatory effect on NO system expression in endothelial cells resulting an enhancement of the generation of bioactive NO[49].

Our findings revealed a highly significant depletion in reduced glutathione (GSH) and glutathione S-transferase (GST) levels in rat liver after intoxication with DEN compound. In agreement with our data, Czuczejko et al[50] referred that reduced red cell GSH content has been reported in patients with liver diseases of alcoholic, non-alcoholic and viral infection. Andre and Felley-Bosco[51] found that GSH conjugates with nitric oxide (NO) to form s-nitroso glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO. An increase in NO production by cytotoxicity caused an inhibition of glutamyl cysteine synthetase, a cytosolic enzyme help in GSH synthesis, leading to GSH depletion. Pradeep et al[52] and Granado-Serrano et al[38] added that the hepatocellular carcinoma induced in animals with DEN showed a subsequent decrease in glutathione level and GST activity due to the decreased expression of these antioxidants during hepatocellular damage. GSH also decreased due to its utilization in inactivating the free radicals generated during DEN metabolism. Also Sivaramakrishnan et al[40] observed reduction in glutathione S-transferase (GST) enzyme activity in rat liver post intoxication with DEN compound which may be due to the excessive utilization of this enzyme in scavenging free radicals in the body.

Our findings revealed a highly significant increase in lipid peroxides level after DEN administration to rats. This is in harmony with Ramakrishnan et al[53] and Pradeep et al[52] who noticed that administration of DEN increases the level of lipid peroxides in rat liver. In addition, DEN administration also causes generation of lipid peroxidation products like MDA and 4-hydroxy nonenal, which interact with various molecules to enhance carcinogenesis[54]. This dynamic action may further lead to uncompromised production of free radicals overwhelming the cellular antioxidant defense system[55]. Therefore, measurement of lipid peroxidation is considered to be a convenient method to monitor oxidative membrane damage as membrane lipids are susceptible to the deleterious actions of ROS. This oxidative stress may be the reason for the elevated lipid peroxidation level in the liver of DEN treated animals. So, in hepatocellular carcinoma there is disequilibrium between oxidant and antioxidant balance which is tilted towards oxidant side[56]. Our findings also agree with Pradeep et al[52] who attributed the increase in LPO level to the oxidative stress leading to peroxidative membrane damage, loss of membrane integrity and subsequent release of the cytosolic contents.

The present study revealed a highly significant elevation in serum VEGF level after injection with DEN compound. VEGF was previously re-

ported to be expressed in serum of patients with HCC, since HCC is characterized by hypervascularity, it is likely to produce angiogenic factors such as VEGF, causing proliferation of the hepatic sinusoidal endothelial cells[57]. VEGF overexpresses in HCC tissues compared to the non cancerous liver tissues as it is secreted by hepatoma cells and hepatic stellate cells and up regulated during tumor dedifferentiation and vascular development[58]. A few studies have reported that increased expression of VEGF might be associated with venous invasion and metastasis in HCC. VEGF was over expressed and localized around periportal area of liver sections intoxicated with diethylnitrosamine[59,60].

The present investigation showed a highly significant elevation in serum level of AFP in DEN group as compared to the control one. This agrees with of Sadik et al[42] and Sivaramakrishnan et al[40] who reported that rats injected with DEN resulted in increased level of alpha-fetoprotein. The possible explanations for the reinitiation of AFP synthesis by neoplastic hepatocytes are either increased transcription of AFP gene or posttranslational modification affecting AFP production. In rats which have been exposed to chemical carcinogens or HCC, AFP production is roughly proportional to the amount of transplantable mRNA present[61].

Concerning the ferritin level, the present work demonstrated that serum ferritin level was highly significantly elevated after DEN injection as compared to the normal healthy control rats. Previously, Lee[62] reported that serum ferritin level is detected in many malignant diseases and correlated with tumour mass and stage of the disease. The same Author added that, the high serum ferritin concentration has been attributed to reticuloendothelial disturbances, release of ferritin from damaged cells or increased synthesis of ferritin by neoplastic cells. Therefore, serum ferritin concentrations may be a helpful tool in management of malignant diseases and provide additional information about progression and remission of the disease.

Serum ferritin is a frequently used marker of iron. Liver dysfunction and inflammatory factors may interfere with the synthesis and clearance of ferritin, thereby increasing serum ferritin level[63]. Wu et al[64] reported that ferritin-H was over-expressed in the DEN-induced HCC in Wistar rats due to hepatocellular damage, which leads to hepatic iron overload in hereditary haemochromatosis.

In this study, a highly significant increase in AST, ALT and ALP were observed in DEN initiated HCC in rats serum. This is in accordance with Sadik et al[42] and Sivaramakrishnan et al[40] who found significant elevation of serum ALT, AST and ALP in DEN-induced animals as compared to normal healthy ones. Ha et al[65] mentioned that hepatospecific enzymes are increased when hepatocellular damage persist especially in hepatoma. Barbisan et al[21] mentioned that DEN induced acute elevation in ALT activity in a dose-dependent manner. The same Authors added that the degree of liver necrosis induced by DEN occurred also in a dose-dependent manner and attributed this changes in liver histology to the changes in serum ALT activity and not to the compensatory hepatocytes proliferation data. Wills et al[60] reported that rats intoxicated with DEN had elevated activities of serum AST and ALT. In addition, Ramakrishnan et al[66] attributed the increases in serum aminotransferase enzyme activities to their intracellular location in the cytosol, so toxicity affecting the liver with subsequent breakdown in membrane architecture of the cells leads to their spillage into serum where their concentration rises. The same Authors added that this significant increase in serum enzyme levels might be due to over production of these enzymes in tumor cells, which might have caused increase in the permeability of cell membrane resulting in liberation of this enzyme into serum.

Studies have shown that hepatic metabolism of DEN generates ROS resulting in oxidative stress and cellular injury[67] which give an additional support of the changed liver function enzyme activities after hepatic injury. Alkaline phosphatase, as one of the liver function enzymes, is closely connected with lipid membrane in the canalicular zone. Therefore, any interference with the bile flow, whether extra-hepatic or intra-hepatic leads to increased serum level of ALP activity[68].

Concerning gamma-glutamyl transferase (GGT), the present data revealed a highly significant increase in DEN- administered rat sera as compared to the healthy negative control group. This is in agreement with Wills et al[60] who reported an increased level of γ-glutamyl transferase in DEN treated rats. The significant elevation of GGT in rat sera may be attributed to the liberation of this enzyme from the plasma membrane into the circulation indicating damage of cell membrane as a result of carcinogenesis[69]. In addition, GGT is a more sensitive indicator of hepatobiliary disease and measurement of serum

GGT is considered to be frequently used parameter in liver diseases, where its activity is higher in the embryonic state and decreases rapidly to the lowest levels after birth[70]. Moreover, Yao et al[71] added that GGT is a membrane-bound enzyme, which exhibits a tissue specific expression and modified under various physiologic and pathologic conditions, such as development and carcinogenesis. The ubiquity of elevated GGT levels in many rodent and human hepatic or extrahepatic carcinomas have led to the hypothesis that GGT provides a growth advantage of focal cells during carcinogenesis[72].

We observed a highly significant increase in bilirubin level in DEN treated rats as compared to the control group. These data are in accordance with Shanab et al[73] who revealed a highly significant increase in serum level of both total and direct bilirubin among liver diseases, hepatocarcinoma as well as viral hepatitis. This increase may be due to a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes[74]. In addition, Marx[75] reported that this increase might be due to the toxic effect of carcinogen on hepatocytes and sinusoidal cells, which causes the reticulin network surrounding the central vein to collapse producing hemorrhage and increasing bilirubin formation. Also, Fabris et al[76] reported that there is a direct relationship between hyperbilirubinemia and an increased level of plasma lipoperoxide in several types of liver diseases. Moreover, Stohs and Bagchi[77] found that, the oxidative stress generated by the action of toxic compounds leads to the induction of liver oxygenase (a stress protein) as well as hyperbilirubinemia.

Concerning the level of serum albumin and liver total protein, the present results showed a highly significant decrease in their levels after administration of DEN compound. These data are in harmony with Cross et al[78] who attributed the hypoalbuminemia state 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 ROS. These free radicals are capable of damaging biological molecules such as proteins that have an impact on cell activities as well as membrane functions and structure. In addition, Vandenberghe[79] noticed that hypoalbuminemia may occur as a result of disorder of the kidney function by the toxic effect of N-nitrosamine which leads to increase in albumin loss. The same Author also revealed that, hypoalbuminemia may result from liver disorders, which are accompanied by reduction in albumin synthesis.

All of these drastic changes occurring in the biochemical parameters after intoxication with DEN compound were confirmed by our histopathological observation in rat liver of DEN treated group. The histopathological picture of the liver revealed that the hepatocytes appear paler, vacuolated, lost their normal shape and arrangement, exhibit large nucleoli and the cell membranes were not obvious. This agree with Bansal et al[80] who reported that the histology of liver in DEN-treated rats showed vacuolization of cells as compared to normal control rats, while necrosis was expressed with the increased period of treatment. The present observations indicated marked changes in the hepatic architecture, which could be explained on the basis that DEN treatment manifested its toxic effects through the generation of ROS. The resulting effect was the production of elevated amounts of malondialdehyde and conjugated dienes, which caused deleterious effects on the membranous components of hepatocytes. Wills et al[60] showed the histopathological picture of normal rat liver which is characterized by a uniform arrangement of liver plates with oval hepatocytes of uniform size. The same Authors added that the histopathological observation of rat liver after diethylnitrosamine administration showed irregularly formed cell plates, scattered masses of necrotic tissues with enlarged nuclei. Ramakrishnan et al[53] showed that the hepatocytes of DEN treated rats showed a tendency to spread by intrahepatic veins. The same Authors added that, both the hepatic and portal veins showed a significant tumor thrombi within portal vessels, where the histological appearance of hepatocellular carcinoma is also extremely variegated. Sadik et al[42] Sivaramakrishnan et al[40] and Singh et al[81] added that liver sections from control animals revealed normal architecture, where the cells exhibit granulated cytoplasm and small uniform nuclei. DEN group showed loss of architecture, where the tumor cells appear with granular cytoplasm, large hyperchromatic nuclei and scattered mitosis. Gupta et al[82] showed also DEN group revealed vacuolization, loss of normal hepatocellular architecture and presence of pycnotic nuclei.

Because of poor prognosis and high recurrence of HCC, there is an urgent need to develop novel chemopreventive or therapeutic strategies that selectively target key molecules aberrantly expressed during hepatocarcinogenesis to minimize the hazardous effect of the disease and improve the patient life. Dietary habits have been

regarded as one of important etiologic factors that lead to the wide variations in the risk and incidence of cancers. It has also been shown that energy rich diets composed of meat, dairy products, processed food with refined carbohydrates and fewer fibers along with lower consumption of fruits and vegetables are directly correlated with higher incidence and death of cancer[83].

Dietary factors could be an important component in regulating tumor dormancy as they have an important impact on cellular physiology and homeostasis, hence it could influence the equilibrium between anti- and pro-angiogenic factors. Dietary restrictions in various studies on animal models with limitation of fat or carbohydrate consumption suppress vascular endothelial growth factor expression, tumor angiogenesis and induce apoptosis[84]. Moreover, omega- 3-fatty acid-rich diets suppress tumor growth and angiogenesis while omega-6-fatty acid-rich diets promote tumor growth. Hence, the identification of pro- and anti-angiogenic dietary components could be a potential strategy for cancer prevention and control[85].

Because ROS play an important role in tumor angiogenesis, treatment with dietary antioxidants such as food phytochemicals, which have antioxidant capacity, seems to be a promising anti-angiogenic strategy. Dietary polyphenols are involved in protection against not only cardiovascular risk factors such as atherosclerosis but also cancer angiogenesis by inhibiting oxidative stress, inhibiting migration and proliferation of vascular cells[86].

Conclusions

The administration of fish oil and artichoke with balanced diet have an improvement effect to liver, also it caused an improvement in the histopathological changes in liver sections. Fish oil 10% and artichoke leaves then heads (1 g%) were found to have a more improvement effect on DEN rats and may thus be helpful in treatment and safe recovery of liver diseases.

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