

ASSESSMENT OF THE ACTIVITY OF PROVITAMIN A AGAINST HEPATOCARCINOGENESIS IN RATS

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SUMMARY

The present work was performed to study the influence of chemoprotective and preventive activity of dietary carrot which was consumed daily on hepatocarcinogenesis induced by Dibutylnitrosamine (DBN) in male albino rats.

Sixty six adult male albino rats (Sprague Dawley Strain) were randomly allocated in groups of eleven rats fed continuously for 60 days on six diets. The first three groups were served as control and put on the following diets throughout the experimental period, (G1) commercial diet; (G2) commercial diet with 50g of fresh carrot daily; (G3) commercial diet and treated with Dibutylnitrosamine (DBN) in drinking water as hepatocarcinogenic agent. The other experimental groups were fed on commercial diet + 50g of fresh carrot, 30 days before (G4) or after treatment with DBN (G5) respectively, while G6 received commercial

diet + 50 g of fresh carrot daily and treated at the same time with DBN throughout the experimental period, 60 days.

Fasting blood samples were taken on the day 60 for the determination of antioxidative state by measuring reduced glutathione (GSH); complete blood picture (CBC) and blood indices; serum and liver malondialdehyde (lipid peroxidations marker); liver GSH; serum AST, ALT, ALP, LDH, GGT, total and direct bilirubin; serum total protein and albumin; and pathologic evaluations were made.

Fresh carrot administration revealed a protective and preventive effects on the rats hepatocyte treated with DBN which was reflected by the significant reduction in the liver function tests (AST, ALT, ALP, GGT, LDH and total bilirubin), while no significant improvement in either total protein or albumin could be detected. This reduction is

ordered among the different treated groups as following G5 , G6 and G4. The oxidative state was determined by measuring liver and whole blood GSH, exhibiting significant increase in blood GSH in contrast to significant reduction in liver GSH. While liver and serum MDA concentration as lipid peroxidation index, showed significant reduction in serum and liver MDA.

Histopathologically, liver of rats fed carrot before, during or after hepatocarcinogenesis, showed highly improvement on preneoplastic lesions, less fibrosis and oval cells development than positive control, but in different degree of lesions.

It was concluded that, carrot consumption was very effective in preventing hepatocarcinogenesis, when it is administrated daily after short exposure to hepatocarcinogen, while before or during the carcinogenesis carrot intake may have mild improvement effect on hepatocarcinogenesis which was revealed by decrease in the severity of illness

INTRODUCTION

Interest in a potential role of diet in the etiology of cancer emerged in the late 1960s when extensive international variations in the number of deaths from cancer were reported. It was estimated that 80-90% of human cancer are caused by environmental factors , Southon , (2001)

The discovery of β -carotene (BC) as a possible anti-cancer agent opened a new avenue in the field of cancer chemo-prevention. A large number of epidemiological studies evaluating the relationship between the consumption of carotene-rich fruits and vegetables and cancer incidence at several sites have demonstrated strong inverse associations, Gerster, (1995).

β -carotene even when administered at high dose for long periods of time does not cause toxicity. On the contrary, high doses of vitamin A and retinoid, if used as possible prophylactic agents for cancer prevention could lead to acute hepatotoxicity and produce other adverse physiological effects, O'Neill and Thurnham, (1998).

Nitrosamines are a major candidate class of carcinogens likely to be causally related to human cancer. Over three hundred nitrosamines have been tested for carcinogenicity and 90% of these compounds show activity. Careful dose response studies have shown that many of these compounds have a high degree of potency, and nitrosamine has been found to be carcinogenic in more than 20 species of animals, Hecht , (1997).

Based on their chemical structure, N-nitroso compounds (NOC) are divided into two major groups: N-nitrosamines and N-nitrosamides. Both groups of NOC are characterized by a nitroso group (-N=O) attached to a nitrogen atom (-N-N=O). Both are formed by the reaction of a nitrite compound

with amines or amides., Dietrich et al., (2005).

Vegetables rich in β -carotene may cause beneficial effects on human health. One of the main sources of the high β -carotene content in diets is the regular intake of carrots, which contain relatively large amounts of this carotenoid, Hart and Scott, (1995).

Falcarinol is a polyacetylene which can also be found in carrots which are the main source of this compound, Brandt and Christensen, (2000). Falcarinol, like many other polyacetylenes, is unstable, being sensitive to heat and light. The concentration of falcarinol in carrots varies depending on the cultivar, and is affected by storage and processing. Hansen et al., (2003) found that, the falcarinol content of diced carrots stored long-term decreased by approximately 35%, while boiling reduced the content by 70%; in raw carrots, the content ranged from 22.3-24.8 mg of falcarinol per kilogram of carrots, depending on the cultivar.

Polyacetylene falcarinol [(9z)-heptadeca-1,9-dien-4,6-diyne-3-ol], has been pointed out as the most bioactive polyacetylene present in carrot, showing a pronounced cytotoxic activity against human tumor cells, which provides a new perspective on the known epidemiological association between high intake of carrots and reduced incidence of cancer, Zidorn et al., (2005).

Aim of the Work: The primary focus of this study was conducted to gain more information on chemoprotective and preventive efficiency of dietary carrot which are consumed daily on hepatocarcinogenesis, induced by dibutyl nitrosamine, and to identify the particular stage (s) at which fresh carrot might act in male albino rats.

MATERIALS AND METHODS

1-Preparation of carcinogenic material:

Dibutyl nitrosamine was prepared by mixing dibutylamine and sodium nitrite at ratio 2:1 ppm (using a magnetic stirrer for 15 minutes until the solution became turbid), and added to tap water.

2-Experimental Design:

Six groups of eleven adult male albino rats, Sprague Dawley Strain, mean weight was 118 g \pm 4 were used. They were obtained from (National Research Center, Giza, Egypt). The animals were divided into six homogenous groups and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25 - 30 °C with about 50% relative humidity. The room was lighted on a daily photoperiod of 12 hr. light and dark. Then they were allocated to the various experimental treatments for 60 day as follows:-

1-Control group (-ve) of rats only given commercial diet.

- 2-Control group (-ve) of rats given commercial diet + 50 g of fresh carrot daily till the end of the experimental period. Fresh carrots were washed with tap water, then blotted on filter paper, and weighed, where each 100 g carrot was containing (5.63 mg β -carotene and 2.095 mg β -carotene that represents 1.12 mg retinol), according to Berry, (1993).
- 3- Control group (+ve) of rats given commercial diet and treated with dibutylamine and sodium nitrite 2:1 ppm in the drinking water, continued till the end of the experimental period.
- 4-Group of rats given 50 g. of fresh carrot daily starting 30 days before treatment with drinking water containing hepatocarcinogenic agent.
- 5-Group of rats treated with drinking water containing 2:1 ppm of DBA and sodium nitrite daily starting 30 days before treatment with 50 g fresh carrot.
- 6-Group of rats treated with drinking water containing 2:1 ppm of DBA and sodium nitrite daily and 50 g of fresh carrot and continued till the end of the experimental period.

During the conditioning period, 2 months, and throughout the trial, food and tap water (containing dibutylamine and sodium nitrite at 2:1 ppm) were provided ad libitum. Body weight and food consumption were recorded periodically.

3-Samples Collection :

(a) **Blood:** At the end of the two months experi-

mental period the animals were fasted overnight and anesthetized with diethyl ether. Incision were made into the abdomen and whole blood samples were collected from the hepatic portal vein into a centrifuge tubes, the samples were left at 37 ° C for 30 minutes, then serum was separated by centrifugation at 4000r.p.m (1790xg), for 15 minutes, and frozen in plastic vials and kept at -20°C for subsequent biochemical analysis. At the same time, the other blood samples were taken into centrifuge tubes containing EDTA, homogenate blood samples were taken for determination of reduced (glutathione) and hematology.

(b) **Tissue:** Liver, kidney and spleen were excised, rinsed in chilled saline solution, then blotted on filter paper, weighed separately to calculate the relative organ weight.

4- Histopathological examination:

One third of liver specimens from different groups were fixed immediately in 10% neutral buffered formalin, dehydrated in different grades of ethyl alcohol, cleared in xylol, embedded in paraffin wax , sectioned at 5 μ thick and stained with Haematoxylin (H) and Eosin (E) according to (Bancraft et al. 1996). The other two third of liver was stored frozen at -20°C until analysis for reduced GSH and lipid peroxides (LPO).

5-Biochemical Analysis:The collected samples

were analyzed for the following biochemical parameters:

Complete blood count (CBC) and blood indices, were processed with a blood counter model KX-21 system (Sysmex coulter, Electronic Kobe, Japan). Serum activities of alanine amino transferase (ALT) and aspartate aminotransferase (AST) according to, Bergmeyer et al., (1976). Serum alkaline phosphatase activity (ALP) according to Recommendation of Scandinavian Society of Clinical Chemistry, (1974). Serum Lactate dehydrogenase activity (LDH) according to, Vassoult et al., (1982). Serum γ -glutamyl transpeptidase activity (GGT) according to, Fischbach and Zawta (1992). Serum total Protein according to, Gornal et al., (1949). Serum Albumin according to, Domas et al., (1971). Serum Globulin = Total serum protein - serum albumin, Albumin/ Globulin ratio (A/G ratio, gm/dL) was calculated by dividing the concentration of serum albumin by the concentration of serum globulin. Serum total and direct bilirubin according to, Malloy and Evelyn, (1937), Indirect Bilirubin= Serum total bilirubin - serum direct bilirubin. GSH and LPO were extracted from liver by the method recommended by El-Seweidy et al., (2002). Quantitative determination of whole blood and liver extraction of glutathione (GSH) according to, Beutler et al., (1963). Quantitative determination of serum and liver extraction levels of malondialdehyde was done according to, Draper and Hadlay (1990).

Statistical Analysis:

SPSS windows version (11.5) was used for analysis of the data. Description of presentative variables in the form of range, mean +SE were done. On way ANOVA (Analysis of variance) was used for comparison of quantitative variables with each other.

P value (Probability)

> 0.05 insignificant

< 0.05 significant

< 0.01 highly significant

< 0.001 very highly significant

RESULTS

It is clear from Table (1) that rats of G3 showed a pronounced significant decrease ($P < 0.001$) in body weight gain and feed intake than did the corresponding G1 and G2 (-28 g, 124 g and 151 g), and (8.1, 15.3 and 14.8 g/day) respectively. But, it was found that group 4 (fed 50 g/day of fresh carrot for 30 d followed by treatment with DBN), had a significant decrease in body weight gain and feed intake than did the corresponding G5 and G6. while there was a significant increase in body weight gain only when compared with G3.

The effect of carrot intake on relative organs weight of liver, kidney and spleen in rats treated with the DBN are shown in Table (2). Generally, it was found that there were significant increase in

the mean value of the relative liver , kidneys and spleen weights (hypertrophy) in group 3 when compared with G1 and G2. Our results revealed that, there was a significant decrease, $P < 0.001$, in the relative liver, kidneys and spleen weights in G4, G5 and G6 when compared to G_3 .

Haematology profiles were done for all groups, and the data were summarized in Table (3) Significant decrease ($P < 0.001$) was noticed in the value of haemoglobin (Hgb) and hematocrit (HCT) value in group 3 (10.9 g/dL,34.9% respectively), when compared with negative control groups 1 and 2 (13.9 ,14 g /dL,43.6 and 43.7% respectively), while when all treated animals compared with DBN positive control group, G3, there was highly significant increase ($P < 0.001$) in all tested groups 4, 5 and 6 . Also, groups 5 and 6 showed significant increase when compared with group 4, while no significant difference was detected between groups 5 and 6 Table (3)

Significant decrease ($p < 0.001$) in the values of red blood cells (RBCs) was noticed in group 3 ($3.63 \times 10^{12}/L$) when compared with group 2 ($4.7 \times 10^{12} /L$). But in another way, there was significant increase in groups 4, 5 and 6 (3.9, 4.3 and $4.5 \times 10^{12} /L$ respectively) when compared with group 3 ($3.63 \times 10^{12}/L$). Also group 5 and 6 showed significant increase in the values of red blood cells when compared with group 4 Table (3).

The effects of fresh carrot containing carotenoids (α and β -carotene) and falcarinol on whole blood and liver glutathione (GSH) and serum and liver lipid peroxides (LPO) in DBN treated rats were shown in Table(4), there was a highly significant decrease, $p < 0.001$, in whole blood GSH level in group 3 (24 mg/dl) when compared with group 2 (39 mg/dL). While, on comparing groups 5 and 6 (37 and 32 mg/dL respectively) with group 3 and group 4 (24 and 28 mg/dL) , there was highly significant increased, $p < 0.001$, in the mean values of whole blood GSH

Whilst, liver GSH exhibited antagonistic results in rats of G_3 where a significant increase in the mean value of liver GSH (42.6 mg/dL) versus low levels in negative control rats which were fed on commercial diet (G1) or commercial diet incorporated with 50 g/day of fresh carrot (G2) (10.6 and 9.5 mg / dL respectively). Table (4). The results revealed that, there was a statistical significant decrease ($p < 0.001$) in the mean values of serum and liver MDA between all treated rats as compared to G_3 .

The effects of DBN as hepatocarcinogenic agent and fresh carrot intake as chemopreventive agent on serum enzyme activities were presented in Table (5). Significant increase, $p < 0.001$, was revealed in serum ALT,AST,ALP,LDH and GGT activity in positive control G_3 as compared with

negative control groups (G₂ and G₁). While there was highly significant decrease ($p < 0.001$) in groups 4, 5 and 6 in serum ALT, AST, ALP, LDH and GGT activity when compared with positive control G₃. There was significant decrease in serum ALT, AST, ALP in groups 5 and 6 when compared to G₄.

Total, direct and indirect serum bilirubin showed that, there was highly significant increase, $P < 0.001$, in group 3 when compared with group 2, and a significant decrease in serum total and indirect bilirubin in groups 4, 5 and 6 when compared with positive control G₃. By comparing group 5 with group 4 and group 6, a significant decrease ($P < 0.01$) in serum total bilirubin, was found Table(6)

The effects of DibutylNitrosamine and carrot intake on serum proteins were presented in Table (7), there was significant decrease in serum total proteins and albumin in positive control G₃ compared to G₁ and G₂ and no significant difference between all treated groups when compared to G₃.

All rats in control groups G₁ and G₂ remained healthy in appearance and performance for the duration of the study. Rats which received DBN in drinking water G₃ showed, lethargy; significant reduction in performance, alopecia, weight loss and increase mortality rate to reach about 50% at the end of the experiment (60 days). Three of

eleven rats died in both G₄ and G₅ during the time of treatment by the hepatocarcinogen DBN, while no mortality was observed in G₆.

Morphological examination of the surface of the liver in rats treated with DBN-induced carcinogenesis (G₃) showed enlargement, hemorrhages, dissolution and appearance of numerous white patches different from the hepatic parenchyma. Rats fed carrot G₄, G₅ and G₆ showed significant reduction in the appearance of these nodules on the surface of the liver or mostly not present, but hemorrhages and enlargement may be present in different degree. In general, G₅ is the nearest group to the normal control G₁ and G₂. Where enlargement was noticed in the kidney and spleen of G₃ when compared to G₁ and G₂.

Microscopical examination of liver from control, untreated rats revealed the normal histological structure of hepatic lobule, which consists of central vein and hepatocytes arranged in hepatic cords (Fig.1). Examined sections from carrot fed rats showed no histopathological alterations. Conversely, liver of dibutylnitrosamine treated rats showed hepatocellular adenocarcinoma, pleomorphism of hepatocytes with hyperchromasia as well as oval cells proliferation and mitotic figures of the cells (Fig. 2) which invaded the hepatic parenchyma.

Liver of rats fed 50g/day of fresh carrot for 30d

then treated with dibutyl nitrosamine showed slight improvement in the histopathological picture described in dibutyl nitrosamine treated group. The liver showed dysplastic hepatocytes, megalocytosis, mitotic figures of hepatocytes, as well as oval cells proliferation (Fig3). Bile duct hyperplasia associated with pericholangitis was also noticed in some examined sections.

Liver sections of rats treated with dibutyl nitrosamine for 30 days followed by feeding fresh carrot (50g/day) revealed no tendency of tumour formation, moderate histopathological changes de-

scribed as Kupffer cell proliferation, focal leucocytic cells aggregation, focal hemorrhage, as well as portal infiltration with mononuclear leucocytic cells. More over, some examined sections showed apparent normal histological structure of hepatic lobule.

Histopathologically, liver of rats fed 50g/day of fresh carrot and treated at the same time with the precursor of DBN for 60 days showed focal hepatic hemorrhage, dysplastic hepatocytes as well as mitotic Figures of some hepatocytes, (Fig 4).

Table (1): Influence of dietary regimen on body weight gain and feed intake in DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
N.S		118.2±3.3	119.7±2.7	119.6±3.5	115±3.5	115.7±3.3	114.8±4.1
Initial weight (g)	% change from G2	---	---	---	-3.9	-3.3	-4.09
	% change from G3	---	---	---	-3.8	-3.2	-4.0
Final weight (g)	% change from G2	1,a 242±8.6	a,2,* 270±5.8	1,2,3 91.5±8.55	1,2,3,4 145±10.4	3,4 250±11.0	*,3,4 245±7.1
	% change from G3	---	---	---	-66.1 -46.3	-7.4	-9.3
Weight gain (g)	% change from G2	1,* 124±8.6	*,2 151±6.2	1,2,3 -28±8.9	1,2,3,4 30±9.2	3,4 134±11.8	3,4 130±7.0
	% change from G3	---	---	-118.5	-80.1	-11.3	-13.9
Feed intake (g/day)	% change from G2	1 15.3±0.05	2 14.8±0.1	1,2,3 8.1±0.47	1,2,4 8.6±0.69	1,2,3,4 13.2±0.2	1,2,3,4 12.8±0.15
	% change from G3	---	---	-45.2	-41.9	-10.8	-13.5
		---	---	---	6.17	62.9	58

* Values are expressed as means ± S.E (Standard error), n = 11

1,2,a,b,..... Mean within the same raw bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.

*(LSD at p < 0.05)

N.S Non Significant

Table (2): Influence of dietary regimen on relative organs weight (g%) in DBN treated rats.*

Diets Relative organs weight	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Liver		1	2,a	1,2,3	1,2,3	1,a,3	1,3
	% change from G2	2.4±0.09	2.9±0.1	5.0±0.37	3.9±0.29	3.7±0.15	3.4±0.19
	% change from G3	--	--	72.4	34.5	27.5	17.2
Kidney		1	2	1,2,3	1,2,3,4*	3,4	3,*
	% change from G2	0.6±0.02	0.6±0.01	1.2±0.12	0.9±0.07	0.66±0.03	0.7±0.02
	% change from G3	---	---	100	50	10	16.6
Spleen		1	2	1,2,3	3	3	3
	% change from G2	0.36±0.03	0.4±0.03	0.6±0.07	0.48±0.02	0.43±0.08	0.39±0.03
	% change from G3	---	---	50	20	7.5	-2.5
	% change from G3	---	---	---	-20	-28.3	-35

* Values are expressed as means ± S.E (Standard error), n = 11

1,2,a,b,.... Mean within the same row bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.

*(LSD at p < 0.05)

Table (3): Influence of dietary regimen on complete blood count in DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Hb g/dl		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	13.9±0.1	14.0±0.14	10.9±0.45	11.8±0.47	12.6±0.13	12.8±0.17
	% change from G3	--	--	-22.0	-15.7	-10	-8.6
RBCs (x10 ⁶ mm ³)		1	2,*	1,2,3,a	1,2, a, 4	1,2,3,4	*,3,4
	% change from G2	4.64±0.03	4.7±0.03	3.63±0.04	3.9±0.05	4.3±0.09	4.5±0.1
	% change from G3	---	---	-22.7	-17	-8.5	-4.3
HCT (%)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	43.6±0.1	43.7±0.1	34.9±0.36	37.6±0.4	39.4±0.4	40.2±0.51
	% change from G3	---	---	-20.1	-13.9	-9.8	-8.01
MCV NS (FL)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	94±0.3	93±0.6	96±0.3	96±0.3	91±1.0	89±1.3
	% change from G3	--	--	3.2	3.2	-2.2	-4.3
MCH NS Pg		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	30±0.015	29.7±0.2	30±0.0	30±0.0	29±0.28	28.5±0.39
	% change from G3	--	--	1.0	1.0	-2.4	-4.0
MCHC NS (g/dl)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	31.8±0.09	31.8±0.1	31.2±0.1	31±0.09	32±0.0	32±0.0
	% change from G3	--	--	-1.9	-2.5	0.6	0.6
Plat. NS (x10 ³ lmm ³)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	269±7.6	257±25	272±16	272±9.5	272±10.4	256±9.3
	% change from G3	---	---	---	-10.6	-6.6	-7.3
WBCs (x10 ³ lmm ³)		1	2	1,2,3	1,2,3	1,2	1,2
	% change from G2	10.4±0.13	11.2±0.5	15±0.3	13.4±0.6	14±0.4	13.9±0.3
	% change from G3	---	---	33.9	19.6	25	24.1
N.S Neutrophils (%)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	26±2.8	24±2.0	27±2.9	29±1.7	22±1.6	25±1.7
	% change from G3	---	---	---	---	---	---
N.S Lymphocyte (%)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	65±6.2	72±1.2	67±3.4	67±1.8	73±1.5	71±1.7
	% change from G3	---	---	---	---	---	---
N.S Monocyte (%)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	4±0.5	3±0.56	4±0.6	2±0.28	2±0.41	2±0.38
	% change from G3	---	---	---	---	---	---
N.S Eosinophils (%)		1	2	1,2,3	1,2,3,4	1,2,3,4	1,2,3,4
	% change from G2	1±0.27	1±0.3	1±0.37	1±0.14	2±0.53	1±0.26
	% change from G3	---	---	---	---	---	---

* Values are expressed as means ± S.E (Standard error), n = 11

1,2,a,b,.... Mean within the same row bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.

*(LSD at p < 0.05)

N.S Non Significant

Table (4): Influence of dietary regimen on reduced glutathione and lipid peroxidation in liver and blood of DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Blood GSH mg/dl		1 35±0.82	1.2 39±0.93	1,2,3,* 24±0.9	1,2,*4 28±1.25	3,4,5 37±1.09	2,3,4,5 32±1.08
	% change from G2 % change from G3	---	---	-38.5	-28.2	-5.1	-17.9
Liver GSH mg/dl		1 10.6±0.29	2 9.5±0.64	1,2,3,* 42.6±2.3	1,2,3,4 26.9±1.4	1,2,3,4,5 18.8±0.56	1,2,*4,5 38.1±2.09
	% change from G2 % change from G3	---	---	348.4	183.15	97.89	301.1
Serum MDA µmol/L		1 5.02±0.27	2 5.5±0.38	1,2,3 17.3±0.69	1,2,3,4 10.6±0.36	1,2,3,4 7.8±0.41	1,2,3,4 8.8±0.37
	% change from G2 % change from G3	---	---	214.5	92.7	41.8	60
Liver MDA µmol/L		1 5.8±0.13	2 5.4±0.16	1,2,3 10.3±0.32	1,2,3,4 7.4±1.37	3,4 5.2±0.5	3,4 5.8±0.86
	% change from G2 % change from G3	---	---	90.7	37	-3.7	7.4
		---	---	---	-28.15	-49.5	-43.7

* Values are expressed as means ± S.E (Standard error), n = 11
1,2,a,b,.... Mean within the same raw bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different. *(LSD at p < 0.05)

Table (5): Influence of dietary regimen on serum enzyme activities of DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
AST U/L		1 125±1.33	2 127±1.75	1,2,3 197±1.1	1,2,3,4 142±6.4	1,2,3,4,a 66±1.7	1,2,3,4,a 77±2.16
	% change from G2 % change from G3	---	---	55.1	11.8	-48	-39.3
ALT U/L		1 56±1.35	2 57±1.03	1,2,3 74±1.03	3,4 60±1.4	1,2,3,4,5 29±1.0	1,2,3,4,5 42±1.63
	% change from G2 % change from G3	---	---	29.8	5.3	-49.1	-26.3
ALP U/L		1 82±1.48	2 94±2.36	1,2,3 547±9.2	1,2,3,4 274±10.7	1,2,3,4,5 140±7.78	1,2,3,4,5 191±1.29
	% change from G2 % change from G3	---	---	481.9	191.5	-48.9	103.2
LDH U/L		1,a 549±22.7	2,* 545±21.5	1,2,3 1641±48.2	1,2,3,4 696±24.3	3,4 577±16.4	a,*3 634±13.25
	% change from G2 % change from G3	---	---	201.1	27.8	5.87	16.3
GGT mg/dl		1 1.5±0.19	2 1.45±0.19	1,2,3 10±0.45	1,2,3,4 4±0.5	1,2,3,5 3.4±0.2	1,2,3,4,5 5.6±0.45
	% change from G2 % change from G3	---	---	589.6	175.8	134.4	286.2
		---	---	---	-60	-66	-44

* Values are expressed as means ± S.E (Standard error), n = 11
1,2,a,b,.... Mean within the same raw bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.
*(LSD at p < 0.05) N.S Non Significant

Table (6): Influence of dietary regimen on serum bilirubin in DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Total Bilirubin mg/dl		1,* 0.37±0.03	2 0.4±0.01	1,2,3,a 0.7±0.05	1,2,a,b 0.6±0.04	*,3,b,c 0.48±0.02	1,2,a,c 0.6±0.04
	% change from G2	---	---	75	50	20	50
	% change from G3	---	---	---	-14.3	-31.4	-14.3
Direct bilirubin mg/dl		1 0.1±0.0	2 0.1±0.01	1,2,3 0.16±0.015	1,2,4 0.16±0.013	3,4,5 0.1±0.006	1,2,5 0.16±0.013
	% change from G2	---	---	60	60	0	60
	% change from G3	---	---	---	0	-37.5	0
Indirect bilirubin mg/dl		1 0.27±0.03	2,a 0.31±0.01	1,2,3,b 0.57±0.05	1,a,b 0.44±0.038	3 0.37±0.02	1,a,b 0.44±0.04
	% change from G2	---	---	83.9	41.9	19.4	41.9
	% change from G3	---	---	---	-22.8	-35.1	-22.8

* Values are expressed as means ± S.E (Standard error), n = 11

1,2,a,b,.... Mean within the same row bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.

*(LSD at p < 0.05)

N.S Non Significant

Table (7): Influence of dietary regimen on serum total proteins in DBN treated rats.*

Diets Parameter	Groups						
		Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Total protein g/dl		1 4.9±0.2	2 5.0±0.17	1,2 4.1±0.17	1,2 4.1±0.18	1,2 4.1±0.16	1,2 3.9±0.18
	% change from G2	---	---	-18	-18	-18	-22
	% change from G3	---	---	---	0	0	-4.9
Albumin g/dl		1 2.77±0.1	2 2.8±0.13	1,2 1.8±0.05	1,2 1.9±0.1	1,2 1.8±0.1	1,2 1.99±0.05
	% change from G2	---	---	-35.7	-32.1	-35.7	-28.9
	% change from G3	---	---	---	5.5	0	10.5
Globulin g/dL		2.1±0.23	2.2±0.17	2.3±0.17	* 2.5±0.27	2.2±0.18	* 1.9±0.14
	% change from G2	---	---	4.54	13.6	0	-13.6
	% change from G3	---	---	---	8.69	-4.34	-17.4
A/G Ratio		1 1.5±0.23	2,a 1.46±0.16	1,2 0.87±0.1	1,2 0.8±0.06	1,a 0.9±0.1	1 1.1±0.1
	% change from G2	---	---	-40.4	-45.2	-38.3	-24.7
	% change from G3	---	---	---	-8.04	3.45	26.43

* Values are expressed as means ± S.E (Standard error), n = 11

1,2,a,b,.... Mean within the same row bearing similar numerical (L.S.D. at P <0.001) or alphabetic (L.S.D. at P <0.01) Subscripts are significantly different.

*(LSD at p < 0.05)

N.S Non Significant

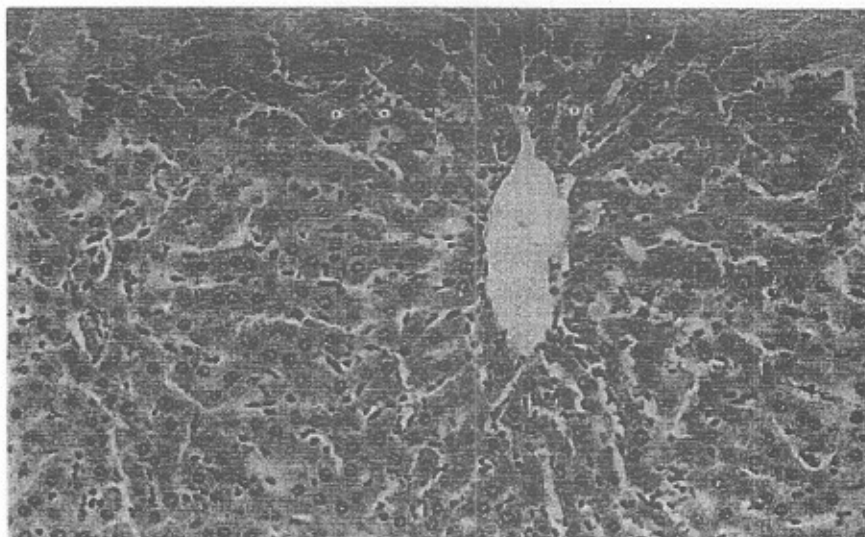


Fig.(1): Liver of control untreated rat showing the normal histological structure (H and E stain X 200).

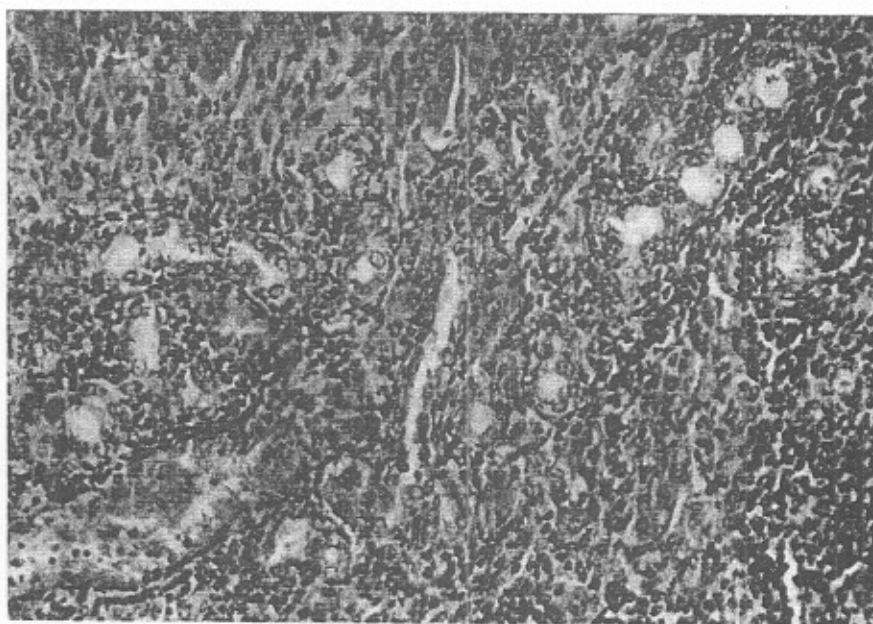


Fig.(2): Liver of DBN treated rats showing hepatocellular adenocarcinoma as well as oval cells proliferation (H and E stain X 200).

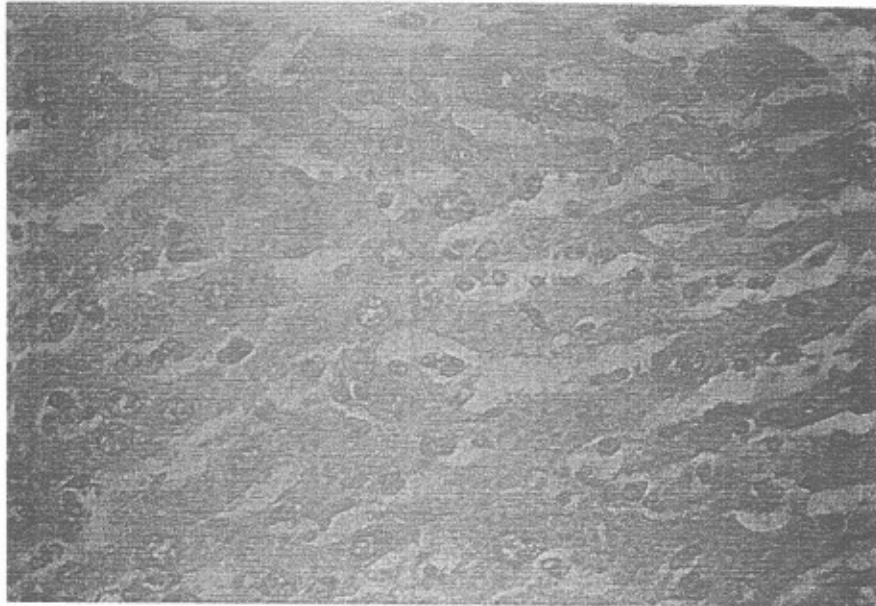


Fig.(3): Liver of rats fed 50g/day of fresh carrot for 30 day then treated with DBN (G4) showing dysplasia, hepatocytes megalocytosis as well as mitotic Figure (H and E stain X 400).

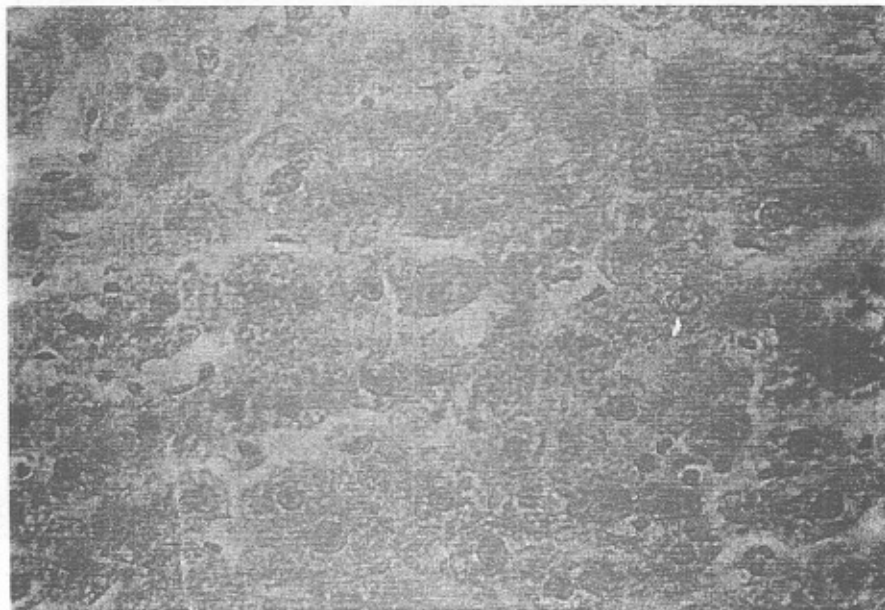


Fig.(4): Liver of rat fed on fresh carrot (50g/day)and treated with DBN for 60 days showing dysplastic hepatocytes as well as mitotic Figures (H and E stain X 400).

DISCUSSION

Results of this study indicated that, there were a highly significant decrease in body weight gain and feed intake in rats treated with dibutyl nitrosamine (DBN) in drinking water in G₃ when compared to G₁ and G₂. This decrease may be due to the highly toxic effect of both sodium nitrite and dibutylamine which take place mainly in the stomach of treated rats, Sen et al., (2001).

This lower body-weight gain has been attributed to induction of the microsomal oxidizing system, increased sympathetic tone and associated thermogenesis and/or enhanced ATP breakdown, Yang et al., (2004).

Feeding carrot for 30 days before administration of water containing DBN (G₄) may have significant improvement on body weight gain than positive control G₃. This can be explained as, the increase in body weight gain and feed intake in the first 30 days is attributed to the beneficial effect of carrot intake, then followed by sudden decrease in body weight gain and feed intake due to the deleterious effect of the carcinogen mixture taken in drinking water.

Furthermore, an increase in body weight gain and feed intake, in group of rats treated with (DBN for 30 days followed by 50g/day of fresh carrot, G₅)

and the rats which fed (50g/day of fresh carrot and treated with DBN in the same time for 60 days, G₆). The increment was significant as compared to G₃ (rats treated with DBN for 60 days) which may be due to the effect of the carcinogen during treatment in reducing cell viability and increasing oxidative stress. However, β -carotene in carrot can improve cell viability and antioxidant status, while falcarinol stimulated growth of epithelial cells and inhibited cancerous lesions, Zidorn et al., (2005).

Our study revealed that, relative organs weight (liver, kidney and spleen) showed a highly significant increase in positive control rats (G₃) as compared with G₁ and G₂. This increase may be due to hyperplasia induced by carcinogenic action of dibutyl nitrosamine. The increase in liver relative weight refer to liver necrosis that cause collapse in the liver and in turn lead to increase collagen formation which accumulated in the liver and fat deposition, Vandenberghe, (1996). Also, the relative kidney weight of G₃ showed a significant increase when compared with normal controls G₁ and G₂. The initial changes were characterized by prominent increase in kidney weight and microcyst formation.

It is evident from this study that, there was a significant increase in the spleen weights shown in the positive control (G₃) only, when compared with negative control groups G₁ and G₂. These

results are in accordance with El-Aaser et al., (1986) who found that, spleen weight increased after DBA treatment, due to spleen congestion which resulted from the toxic effect of the carcinogen.

The present study demonstrated that, there was a significant decrease in red blood cell values, haemoglobin concentration and hematocrite values in rats treated with DBN in drinking water (G₃) compared to the normal control groups G₁ and G₂.

Versus to positive control group (G₃), the present data revealed an increase in RBCs count, HB concentration and HCT value when rats fed carrot 30 days before hepatocarcinogenic treatment (G₄), this may be due to the higher concentration of retinoids in fat-storing cell which decreased the severity of cell damaged and reduced haemolysis. But these results still lower than G₅ and G₆ in which rats fed carrot after or during carcinogenic treatment respectively.

Our results revealed that, remarkable depletion of blood glutathione was shown in rats drunk water containing dibutyl nitrosamine (DBN) (G₃) when compared to negative control groups G₁ and G₂. The same results has been also explained by Andre and Felley-Bosco (2003) who found that, GSH conjugates with nitric oxide (NO) form an S-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release (GSH) and

(NO). An increase in (NO) production by cytotoxicity of macrophage and tumor-induced immunosuppression are conducted by inducible NOs (nitric oxide synthases) cause γ -glutamyl cysteine synthetase (GCs) (a cytosolic enzyme help in GSH synthesis) inhibition and GSH depletion. In this regard carotenoids which inhibit the expression of inducible (NO) synthase and (NO) production, may prevent or attenuate GSH depletion in cells.

While, liver glutathione in G₃ showed highly significant increase. Such high increase may be ascribed to the cytotoxic effect of the carcinogen (DBN) agreeing with Sarker et al., (1995) who found that, hepatic GSH was increased after 48hr of the administration of hepatocarcinogenic material.

On the other hand, treatment with carrot before, after or during hepatocarcinogenesis (G₄, G₅ and G₆ respectively) compared to positive control group (G₃) improved the previous changes in either liver or blood GSH. The free radical scavenging nature of carotenoids specially β -carotene present in carrot and its immediate involvement in trapping singlet oxygen (O₂) providing an overall increased reducing environment in the hepatic tissues which may in turn reduce liver GSH. As oxidative stress is declined and hemolysis is reduced, this leads to increase erythrocyte GSH, Sarkar et al, (1995).

Chattopadhyay et al., (2004) explained the mechanism of β -carotene in oxygen consumption by the cell. They found that the continuous treatment with β -carotene to rats given DEN resulted in a significant increase in oxygen consumption by mitochondrial cells compared with diethylnitrosamine (DEN) control group. These findings regarding oxygen uptake in mitochondrial cells suggest there re-establishment of aerobic metabolism in the treated group with β -carotene compared with DEN control group. This re-establishment of aerobic metabolism not only limits progression of uncontrolled division and growth of cancer cells but also, if held in this state long enough, induces programmed cell death. If the cancer cells restart aerobic metabolism, they revert the cell back to being quasi-normal cells again. However, genetic damage is not corrected, but if the cells are held in a normal state of aerobic metabolism with time, they will go through the normal process of programmed cell death and cancer cell will be permanently eliminated.

Generally, carrot treatment caused a significant decrease in lipid peroxidation as seemed in group 5, which took carrot after the carcinogenesis causing materials, then in group 6 which fed carrot at the same time with DBN treatment and finally in group 4 whose rats fed carrot 30 days before starting treatment with the carcinogen.

The present results demonstrated that, rats treated

with dibutyl nitrosamine (DBN) in drinking water (G_3) showed a high significant increase ($P < 0.001$) in serum AST and ALT activities when compared to negative control groups (G_1 and G_2) which mainly due to liver cell injury leading to release of tissue-specific enzymes into the circulation Burtis and Ashwood, (1996).

Reduction in the activities of AST and ALT was recorded in G_4 as compared with positive control group G_3 . While AST and ALT activities exhibited pronounced reduction when rats treated with fresh carrot after (G_5) and during (G_6) carcinogenesis as compared with the same group of rats G_3 .

Similar results were also recorded by Seifert et al., (1995) who reported that, pre-treatment with β -carotene cannot prevent liver fibrogenesis but can decrease the severity of liver fibrosis in spite of parenchymal cell damage, as reflected by high AST and ALT blood levels. The decreased severity of liver fibrosis may be due to maintenance of higher concentration of retinoids in fat-storing cells postponing their activation to fibroblast-like cells. There is evidence that, 40-60% of β -carotene is transformed in the liver into vitamin A, this agrees with the results of Olson (1989). This amount may be insufficient for complete protection in the liver. The significant decrease showed in G_4 in ALT and AST can be due to beneficial effects of vitamin on lowering these serum

enzymes activities as they acts as free radical scavengers, Wu, (1997).

While, a pronounced reduction in AST and ALT was observed in G5. This decrease may be due to the highly protective effect of fresh carrot on the damaged liver cells which are the main source of β -carotene, α -carotene (equivalent to 1.12 mg retinol) and falcarinol. These compounds can block formation and activation of carcinogens, induce detoxifying enzymes, and suppress tumor promotion, Yu et al., (1995).

Previous study, Seifert et al.,(1995) recorded that, when β -carotene was given during the CCL₄ treatment, inhibition of fibrosis was observed and the paranchymal liver damage was also less than in rats pre-treated with β -carotene, as reflected by the decrease activities of AST and ALT levels.

Versus to negative control groups (G₁ and G₂), the present results indicated that treatment with DBN (G₃) lead to significant increase in LDH activity.

Comparing to positive control group (G₃), highly significant reduction in LDH activity were observed in all groups treated with carrot before, after or during drinking water mixture containing dibutyl nitrosamine (DBN) (G₄, G₅ and G₆). These results may be explained as, LDH is an enzyme that exists in many tissues and organs when

those tissues or organs are damaged LDH is released into the blood from cells, Yang et al., (2004). So that when hepatocyte cells damaged by the carcinogen, LDH activity is significantly increased in the blood. Fresh carrot administration decrease LDH activity by the action of β -carotene in reducing reactive oxygen species(ROS) production Lawlor and O'Brien, (1997) and by the effect of either β -carotene and falcarinol present in carrot in cell viability.

Comparing to the negative control group (G₂) there was a highly significant increase in serum ALP and GGT activities after treatment with DBN (G₃). This marked elevation may be due to tissue damage by the effects of the carcinogen that lead to release of ALP and GGT into the plasma, VanHoof et al., (1997). Groups of rats which were feed carrot as G₄, G₅ and G₆ revealed a highly significant reduction in both ALP and GGT than did the corresponding rats in G₃.

It is evident from the study that, there was a highly significant increase in total, direct and indirect bilirubin in G₃ when compared to G₁ and G₂. This increase may be due to the toxic effect of the carcinogen on hepatocytes and sinusoidal cells which cause the reticulin network surrounding the central vein to collapse and produce hemorrhage. This leads to increase degeneration of haemoglobin, and thus increase bilirubin formation, Marx, (1996).

Feeding fresh carrot (groups 4, 5 and 6) induced highly protective effect on hepatocyte by the action of both β - and α -carotene (equivalent to 1.12 mg retinol) and falcarinol in cell proliferation. These effects were observed mainly in rats fed carrot for 30 days after stopping drinking water mixture containing the hepato-carcinogen (G_5) in which total, direct and indirect bilirubin were significantly reduced compared to G_3 . While the other two groups (G_4 and G_6) showed only a slight reduction in direct bilirubin level. These results were in agreement with Bishayee et al., (1995). Wherein, Wang et al., (2001) found that, falcarinol works as inhibitor for inducible nitric oxide synthase which inturn reduces the oxidative stress and decreases hemolysis.

Our findings indicated that, total serum protein, albumin and A/G ratio levels were significantly reduced in rats drinking water containing dibutyl-nitrosamine (DBN) (G_3), while serum globulin not significantly changed when compared to G_2 and G_1 . Total protein was probably low due to the impaired synthetic liver function and small amount of essential amino acids which are predominantly distributed in the muscles instead of liver cells. Arneil and Metcoff, (1995). The significant decrease in feed intake may also lead to insufficient protein intake and marked loss of protein.

Vandenberghe (1996) found that, hypoalbuminemia may also occur as a result of disorders of the kidney by the toxic effect of N-nitrosamine which lead to increase of albumin loss. He also revealed that, disorder of the liver are accompanied by hypoalbuminemia due to the reduced synthesis and turn over, which results in a prolonged half-life. Hyperglobulinemia is found in hepatocellular disorders and arises as an inflammatory reaction of reticulo-endothelial system.

Treatment with carrot did not significantly improve protein damage caused by the carcinogenic effect of dibutyl-nitrosamine. That is because oxidative damage to protein caused loss of protein functions which may affect on the activity of enzymes, receptors and membrane transporters. Moreover, oxidatively modified proteins may contain very reactive chemical species that could contribute to secondary damage to other biomolecules, Halliwell, (1996).

As a result, many changes can occur in proteins including amino acids modifications, fragmentation, aggregation, change in absorption and decrease or loss of biological functions, Dean et al., (1997). In our study carrot consumption cannot rebuild these changes and failed to improve this damage. So that the protein content still unchanged either by carrot treatment or not. In G_4 , G_5 and G_6 when compared to G_3 .

In this study, rats which received dibutyl nitrosamine (DBN) in drinking water G₃ showed, lethargy and increased mortality rate to reach 50% at the end of the experiment. This finding was explained by Althoff et al., (1974) who observed that, dibutyl nitrosamine administered hamsters can cause death by acute liver dystrophy or circumscribed hemorrhage in the organs of the abdominal and thoracic cavities as well as in the brain. The effect of DBN in this hamster species showed a dose-response relationship related to survival, tumor incidence and latency, and weight in both male and female.

Morphological examination of the surface of liver in rats treated with DBN showed hemorrhages, dissolution and appearance of white nodules, whereas rats fed carrot G₄, G₅ and G₆ showed significant reduction in these lesions but in different degree. The effect of carrot on reducing the hepatocarcinogenic lesions have been described by several authors, Moreno et al., (1995) who reported that, beta-carotene administered rats can reflect a better resistance to the aggression elicited by the application of the hepato-carcinogenesis model, and to the smaller number of hepatocyte nodules present in their liver. Moreover, the majority of the hepatocyte nodule (87%), showed to have less than 1mm, in the carotenoid administered group.

Our study showed that, liver of rats treated with

dibutyl nitrosamine (DBN) in drinking water (G₃) showed hepatocellular adenocarcinoma. Pleomorphism of hepatocytes with hyperchromasia as well as oval cell proliferation and mitotic figures of the cell. Marx, (1996) attributed these results to the toxic effect of dimethylnitrosamine on hepatocytes by causing collapse in the central vein and produce hemorrhages.

Livers of rats fed carrot then treated with carcinogen in drinking water (G₄) showed slight improvement in the histopathological picture compared to the positive control group (G₃). The liver showed dysplastic hepatocytes, megalocytosis, mitotic figures of hepatocytes as well as oval cells proliferation. This indicates that pre-treatment with carrot cannot prevent liver fibrogenesis but can decrease the severity of liver fibrosis in spite of parenchymal cell damage this may be due to high concentration of retinoids in fat-storing cells postponing their activation to fibroblast like cells, Seifert et al., (1995).

On the other hand, liver sections of rats treated with water dibutyl nitrosamine (DBN) then with carrot (G₅) revealed no tendency of tumour formation, moderates histopathological changes described as Kupffer cell proliferation, focal leucocytic cell aggregation, focal hemorrhage. Moreover, some examined sections showed apparent normal histopathological structure.

Histopathological appearance of liver in rats treated with carrot together with drinking water containing the carcinogen (G₆) showed focal hepatic hemorrhage, dysplastic hepatocytes as well as mitotic figures of some hepatocytes, in which this results were less severe compared to the positive control group (G₃).

Our results confirmed the results of, Moreno et al., (1995) who found that, H&E stained liver sections revealed a greater number of clear cell foci in β -carotene treated rats, and a tendency to present more mixed cell foci in vitamin A group. While DEN administered rats showed predominantly acidophilic, and morphologically more aggressive γ -glutamyl transpeptidase (γ -GT) foci. β -carotene treated group also presented less fibrosis and oval cell development than control.

Also, Gerster, (1995) demonstrated that, β -carotene had an inhibitory effect on preneoplastic lesions specifically when administered during the early promotion phase of hepatocarcinogenesis, and support experimental evidence that the carotenoids may be an effective cancer chemopreventive agent. In addition, Rizzi et al., (1997) showed that, oral administration of β -carotene for 5 consecutive weeks during the early promotion phase of RH-model of hepatocarcinogenesis reduced the incidence and total number of hepatocyte nodules, as well as the total number γ -GT positive preneoplastic lesions (PNL). Moreover,

Moreno et al., (2002) found that, β -carotene strongly inhibited cell proliferation in hepatic neoplastic lesions during the progression phase of RH model.

Conclusion

Depending on biochemical and pathological results, it was concluded that carrot administration revealed a highly protective and preventative effects on hepatocyte after limited exposure period for hepatocarcinogen. While mild improvement could be detected if carrot was taken before hepatocarcinogenesis. On the other hand, carrot may reduce the severity of carcinogenesis if it was taken at the same time with the hepatocarcinogen.

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تقييم نشاط بروفيتامين (أ) تجاه تسرطن الكبد فى الفئران

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يهدف هذا البحث إلى دراسة التأثير الوقائى للجزر الطازج المتناول يومياً فى حالات الإصابة بسرطان الكبد بدائى بيوتيل نيترو زأمين .

إشتملت هذه الدراسة البيولوجية على ٦٦ فأراً من الذكور من النوع الالبينو تم تقسيمهم إلى ٦ مجموعات ثلاث مجموعات ضابطة كالتالى (المجموعة الأولى الضابطة غذيت على الوجبة التجارية . المجموعة الثانية الضابطة السلبية غذيت على الوجبة التجارية بالإضافة إلى ٥٠ جم من الجزر الطازج يومياً المجموعة الثالثة الضابطة الإيجابية غذيت على الوجبة التجارية بالإضافة إلى المعالجة بدائى بيوتيل نيترو زأمين فى مياه الشرب خلال فترة التجربة وكذلك ثلاثة مجموعات معالجة بدائى بيوتيل نيترو زأمين فى مياه الشرب وغذيت على الوجبة التجارية كالأئى المجموعة الرابعة . تغذيت على ٥٠ جم من الجزر الطازج خلال فترة الـ ٣٠ يوم الأولى من التجربة فقط . المجموعة الخامسة تغذيت على ٥٠ جم من الجزر خلال فترة التجربة كاملة (١٠ يوم) .

بعد ستون يوماً تم سحب عينات دم وتحليلها : الجلوتاثو بالدم والكبد . المألوندايديهيد بالمصل والكبد صورة الدم كاملة . وظائف كبد والتحليل الباثولوجى للكبد .

ولقد أوضحت النتائج أن تناول الجزر أدى إلى حماية خلايا كبد الفئران وذلك متمثلاً فى النقص المعنوى فى نشاط أنزيمات الكبد فى السيرم (الإنزيمات الناقلة للأمين . الإلكلين فوسفات تيز . أنزيم جاما جلوتاميل ترانسفيريز . إنزيم اللاكتات دى هيدرو جيناز) وكذلك وكذلك البيليروبين الكلى ولم يوجد تحسن معنوى ملحوظ فى البروتينات الكلية والألبومين حيث أن نسبة التحسن فى المجموعات يمكن أن ترتب على النحو أن المجموعة الخامسة أفضلهم يليها المجموعة السادسة ثم الرابعة كما وجد زيادة معنوية فى جلوتاثيون الدم ونقص معنوى فى جلوتاثيون الكبد وكذلك فى مألوندايديهيد فى السيرم والكبد .

ولقد أظهر الفحص الباثولوجى تحسن ملحوظ فى . لايا الكبد للفئران المغذاة على الجزر (أثناء وقيل وبعد التعرض للمادة المسببة للسرطان) إذا ما قورنت بالمجموعة الضابطة الإيجابية .

الخلاصة أن النتائج أظهرت أن الجزر عامل غذائى مهم فى منع الإصابة بسرطان الكبد عندما يؤخذ يومياً بعد فترة قصيرة من التعرض للمواد المسببة للسرطان ولكن هذا التحسن كان ضعيفاً عند تناول الجزر قبل يومياً وأثناء التعرض للمواد المسببة للسرطان والتي أظهرت نقص فى شدة المرض .