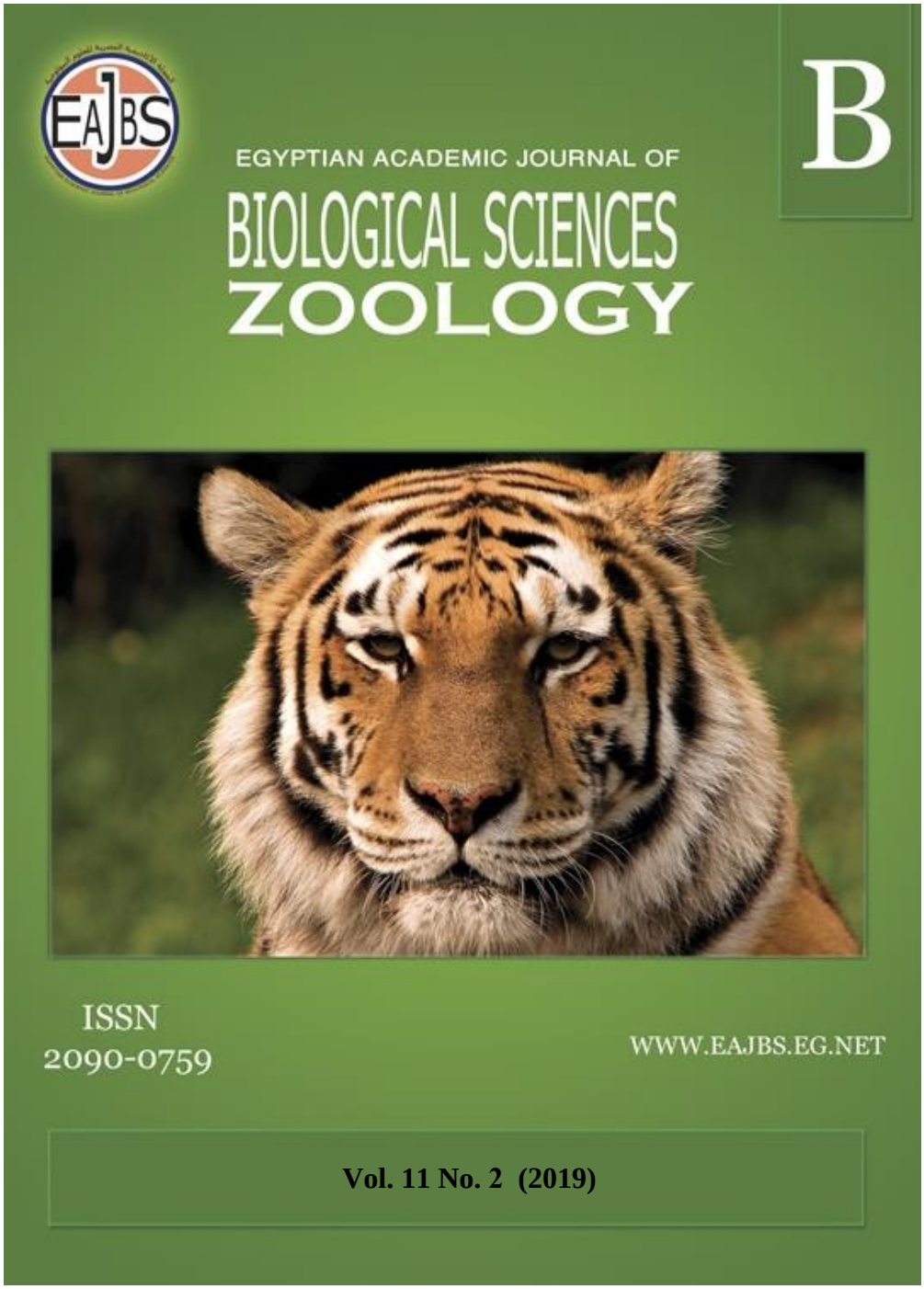

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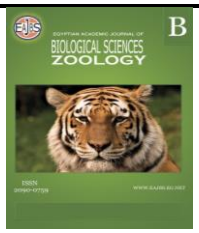

Hematological and Biochemical Changes in Rats Induced With Diethyl Nitrosamine and the Hepatoprotective Role of Some Antioxidants

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ABSTRACT

Abstract: Hepatocellular carcinoma is one of the most common malignancies worldwide and in Egypt the most common form of primary liver cancer. HCC represents the second most prevalent cancer among men in Egypt. The important risk factors for HCC include hepatitis C and B, alcoholic, schistosomiasis, aflatoxins (AFB), hypothirodism and cirrhosis. Diethyl nitrosamine (DEN) is a well-known strong hepatocarcinogenic agent. It is known that DEN induces damage in enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models as rats. Curcumin is a strong anti-inflammatory agent and anti-cancer effects with strong therapeutic potential against a variety of cancers. Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio membranes from peroxide damage and tissue damage of the liver and in HCC cases. **Objective:** To evaluate the hematological parameters and some liver function tests in the early stage of HCC and the hepatoprotective role of curcumin and vitamin C.**Materials and methods:** This study was conducted on a patch of 90 adult mature healthy male albino rats (*Rattus rattus*) averaged weight (190 ± 10 g) were allowed to acclimatize in the laboratory and distributed into 9 groups 10 rats for each. Hematological parameters and some hepatic tests were measured and histopathology of the liver, for all groups at the end of the experiment.**Results:** The results showed that significant alterations in all hematological parameters and hepatic function tests when compared with its corresponding level in the control group, and when treated with curcumin and vitamin c the results showed improvement for some parameters when compared with DEN group. **Conclusion:** Antioxidants like curcumin and vitamin C administration improved the hematological parameters and liver function tests.

INTRODUCTION

The liver is the largest and important organ in the body. It plays many vital functions in maintenance, performance and regulating homeostasis of the body. The important function of the liver is to filter toxic substances from the body. Hepatic damage may occur when the accumulation of toxins is faster than the liver's

metabolizing ability (Bigoniya *et al.,* 2009; Bhakuni et *al.,* 2016). Liver disorders lead to various pathological changes like fatty liver, increase in ROS or oxidative stress, necrosis of liver cells, hepatitis, steatosis, cholestasis, vascular lesions, and granuloma and veno-occlusive diseases, increase in the level of inflammatory markers, hepatocellular carcinoma which further produce portal hypertension and hepatic failure(Buzzetti *et al.,* 2016). Liver tumors are classified into two major categories, primary liver tumors and metastatic liver tumors (McNally, 2010; Lozano *et al.,* 2012; Quaglia, 2018). Hepatocellular carcinoma (HCC) represents the fifth most common cancer in the world, and the second most frequent oncological cause of death among Egyptian men(Jiang *et al, 2017).*

Unlike other cancers, the main risk factors associated with HCC are well defined and include viral hepatitis (B and/or C), alcohol abuse, and nonalcoholic fatty liver disease in patients with metabolic syndrome and diabetes. Other cofactors of HCC development, such as aflatoxin B1(AFB1), Pesticides, Oral contraceptives (OCs), Obesity, Iron overload, Alpha1 antitrypsin deficiency, tyrosinemia, and tobacco, increase the incidence of the disease if other common risk factors are present (Chuang *et al.,* 2009 and Omar *et al.,* 2013).

Nitrosamines are compounds formed by the combination of amines and nitrates or nitrites. Studies have shown that nitrosamines can be formed in the human stomach by a process commonly referred to as endogenous nitrosation. Many foods that contain amines can react with these nitrosating agents in the acidic environment of the stomach to form nitrosamines (Jakszyn and Gonzalez 2006; Fathy *et al.,* 2017).

Turmeric (*curcuma longa*), also known as '*curcuma domestica'* is a perennial herbaceous plant of the ginger family (Zingiberaceae) (Priyadarsini, 2014). Althoughit has more than 300 active components; a substance obtained from its root that has the feature of being a yellow or orange pigment is the main biologically active component constituting the basis for the medicinal properties of this plant (Gupta *et al.,* 2013). This substance called curcumin is also the main component of curry powder commonly used in Asian cuisine. It is also used as a food colorant with the code E100 (Lüer *et al.,* 2014). The effects of curcumin, having a polyphenol structure, on certain cytokines, kinases, enzymes, transcription factors, growth factors and receptors have been studied**.**

Vitamin C:

Antioxidants, ascorbic acid (vitamin C) and tocopherol (vitamin E) used as a nutritional supplement, are the essential elements in almost all biological systems (Howard *et al.,* 2000). It is one of the most widely available and affordable non-enzymatic antioxidant molecules that have been used to mitigate oxidative damage (Naidu, 2003). It readily scavenges physiological ROS as well as reactive nitrogen species ''RNS" (Carr and Frei, 1999). L-Ascorbic acid (Vit. C) is a well-known antioxidant, which can protect the body from damage caused by ROS that can be generated during normal metabolism as well as through exposure to toxins and carcinogens (Halliwell, 1996 and Banerjee *et al*., 2009). Vitamin C or ascorbic acid (AA) is involved in a number of metabolic processes in the human body, including those that are important for the optimal functioning of the oxygen energy system. In addition, AA is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio membranes from peroxide damage and tissues damage of the liver (2008; Salah *et al*., 2010 and Adikwu & Deo, 2013).

MATERIALS AND METHODS

Diethylnitrosamine(DEN) was purchased from Sigma-Aldrich (St. Louis, MO, USA). DEN was given to rats in drinking water (100 mg/L). The DEN solution was prepared as a fresh solution every other day and administered to rats in dark bottles. Other chemicals

Experimental Animals:

A patch of **90** adult mature healthy male albino rats (*Rattus rattus*), obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA, Giza, Egypt) averaged weight (190 ± 10 g) were allowed to acclimatize in the laboratory and distributed into 9 groups of 10 rats each. Rats were maintained under standard laboratory conditions at the animal house, Faculty of Science, Al-Azhar University, Cairo, Egypt. They were kept in a temperature-controlled environment (20-25°C) and under good ventilation 45%–55% relative humidity with an alternating 12 h light-dark cycle. They all received a standard laboratory diet (60% ground corn meal, 10% bran, 15% ground beans, 10% corn oil, 3% casein, 1% mineral mixture and 1% vitamins mixture), purchased from Meladco Feed Company (Aubor City, Cairo, Egypt) and supplied with water ad libitum throughout the experimental period.

Materials:

Curcumin:

Curcumin is a brightly yellow color and may be used as a food coloring. As a food additive, its E number is E100 (Akram *et al.,* 2010; Momtazi *et al.,* 2016; Kocaadam *et al.,* 2017), crystalline powder practically insoluble in water.

Curcumin was prepared for supplementation by dissolving 1500 mg curcumin (powder) in 30 ml olive oil at a concentration of 50 mg/ml just before experimental use. This suspension was given to rats by oral gavage.

Every rat has received curcumin in a concentration of 150 mg/kg body weight of rat according to previous studies (Khedr and Khedr 2014).

Vitamin C (Vit. C):

Vitamin C (ascorbic acid) was purchased from El-Nasr Company for pharmaceutical industries, Egypt. Vitamin C, also known as ascorbic acid and L-ascorbic acid.

Preparation of the Dose:

Rats were orally dosed with daily 200 mg/kg of vitamin C for 30 days and these doses were chosen according to (Adeneye and Olagunju, 2008) and other reagents were of high analytical grade and were purchased from standard commercial suppliers.

Collection of Samples:

At the end of the experiment, blood samples were collected from each animal from the retro-orbital venous plexus puncture. One part of the blood was collected in EDTA tubes for hematological study and another part of the blood was left to clot at room temperature for 15 minutes. Sera were separated by centrifugation at 3000 rpm at 20°C for 15 minutes where the clear serum was obtained and kept frozen at -80°C for various biochemical analyses.

Experimental Design:

A patch of 90 male albino rats was randomly divided into nine equal groups and labeled as groups 1,2,3,4,5,6,7,8 and 9 each group contains 10 rats**:**

- **Group (1)**: Control rats.
- **Group (2)**: Rats of this group were administered Olive oil at dose (200 mg/kg) daily via oral gavage tube for 30 days.

- **Group (3)**: Rats of this group were administered Curcumin at dose (150 mg/kg) daily via oral gavage tube for 30 days.
- **Group (4)**: Rats of this group were administered vitamin C at dose (200 mg/kg) daily via oral gavage tube along the period of the experiment.
- **Group (5)**: Rats of this group were administered mix Curcumin and Vitamin C at dose (200 mg/kg) daily via oral gavage tube for 30 days.
- **Group (6):** Rats of this group as a positive control for HCC model in which administered DEN in drinking water (100 μg/L) for 30 days.
- **Group (7):** Rats of this group were administered Curcumin at dose (200 mg/kg) daily via oral gavage tube for 30 days, then treated with DEN in drinking water (100 μg/L) for 30 days.
- **Group (8)**: Rats of this group were administered Vitamin C at dose (200 mg/kg) daily via oral gavage tube for 30 days, then treated with DEN in drinking water (100 μg/L) for 30 days.
- **Group (9)**: Rats of this group were administered mixing between Curcumin and Vitamin C at dose (200 mg/kg) daily via oral gavage tube for 30 days, then treated with DEN in drinking water (100 μg/L) for 30 days.

1-Hematological Parameters:

The erythrocyte count, total and differential leukocyte count, platelet count, hematocrite percentage, and hemoglobin concentration were estimated in the blood by using a CBC analyzer (Sino thinker. sk9000, U.S) and confirmed by (Sysmex KX-21N) automated counter cell, Hematology Analyzer**.**

2-Biochemical Study:

The serum levels of transaminases, AST and ALT (Bergmeyer *et al.*, 1986), alkaline phosphatase (ALP) (the German Society for Clinical Chemistry 1972), total protein (TP) (Gornal *et al.*, 1949), albumin (Doumas *et al.*, 1971), and total bilirubin (TBIL) (Scherwin and Thompson 2003) were estimated using kits from Elitech diagnostic Co., France.

3-Aspartate Aminotransferase-To-Platelet Ratio Index (APRI):

Recently, several serum markers that can be used as noninvasive tools have been identified in human cancers, including HCC **(Lin *et al.*, 2017)** among them, there has been great interest in the aspartate aminotransferase-to-platelet ratio index (APRI) because it is an inexpensive and feasible test that can be used for daily oncologic practice. It has been reported that APRI might be a candidate as a prognostic biomarker in HCC and in cases of cirrhosis pre-HCC (Peng *et al.*, 2016).

Statistical Analysis:

The statistical analysis of the results was performed by using a statistical package for social sciences SPSS/CP computer program (version 20). All values were expressed as mean ± SE and the results were analyzed using a one-way analysis of variance (ANOVA) test followed by the least significant difference (LSD) test for multiple comparisons. Differences were considered statistically significant at $p<0.05$.

RESULTS

Hematological Parameters:

The data revealed that rats treated with Olive oil, curcumin, Vitamin C and combination between curcumin and vitamin C recorded insignificant change in RBCs, Hb, WBCs and Platelets count when compared to their corresponding values in control group, while the rats induced with DEN revealed a significant decrease in RBCs, Hb and Platelets count and showed a significant increase in WBCs count when compared

to their corresponding value in the control group. In addition to RBCs, Hb and PLTs count revealed a significant increase ($p<0.05$) in the groups treated with curcumin or vitamin c or combination between them with DEN when compared to group treated with DEN only, while WBCs count revealed a significant decrease ($p<0.05$) on curcumin or vitamin c or combination between them when compared to group treated with DEN only (Table 1).

Table (1): Mean values ± S.E of hematological profile (RBCs, Hb, WBCs and PLTs) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

		Groups								
		30 day						60 day		
		Control	Olive oil	Cur	Vit. C	Cur + Vit. C	DEN	Cur then DEN	Vit. C then DEN	Mix then DEN
RBCs x (10^6c/mm^3)	Mean ± S.E	7.88 ± 0.19 ^{a}	7.81 ± 0.18 ^{a}	7.93 ± 0.11 ^{a}	8.16 ± 0.26 ^{a}	7.86 ± 0.09 ^{a}	6.41 ± 0.21 ^{b}	7.11 ± 0.05 ^{c}	6.82 ± 0.16 ^{c,d}	6.56 ± 0.09 ^{b,d}
	% of change	-	-0.05	-0.72	0.26	-1.11	0.09	-2.8	-0.18	-1.11
Hb (gm)	Mean ± S.E	14.1 ± 0.24 ^{a}	14.0 ± 0.21 ^{a}	14.0 ± 0.21 ^{a}	14.7 ± 0.29 ^{a}	14.1 ± 0.16 ^{a}	12.9 ± 0.25 ^{b}	13.8 ± 0.11 ^{a}	13.6 ± 0.22 ^{a}	13.8 ± 0.12 ^{a}
	% of change	-	-0.14	-0.14	0.17	-0.5	0.04	-1.18	-0.09	-1.0
WBCs x (10^3c/mm^3)	Mean ± S.E	7.6 ± 0.43 ^{a}	7.5 ± 0.40 ^{a}	7.2 ± 0.38 ^{a}	7.5 ± 0.41 ^{a}	7.6 ± 0.39 ^{a}	14.8 ± 1.01 ^{b}	11.4 ± 0.57 ^{c}	12.7 ± 0.28 ^{c,d}	13.1 ± 0.41 ^{d}
	% of change	-	-0.07	-0.13	-0.04	-0.10	0.57	0.24	-0.53	-0.04
PLTs x (10^3c/mm^3)	Mean ± S.E	691 ± 22.5 ^{a}	680 ± 22.8 ^{a}	664 ± 16.4 ^{a}	731 ± 15.4 ^{a}	748 ± 9.2 ^{a}	472 ± 31.2 ^{b}	584 ± 24.7 ^{c}	624 ± 25.1 ^{d,c}	538 ± 22.9 ^{e,c}
	% of change	-	0.01	-2.65	-0.46	-1.44	0.27	0.08	0.10	0.01

The results showed that rats treated with Olive oil, curcumin, Vitamin C and combination between curcumin and vitamin for 30 days revealed insignificant changes in Hct, MCV, MCH and MCHC when compared to their corresponding values in control group, while the DEN administered groups recorded insignificant change in blood indices except for the Hct that is revealed a significant decrease when compared to their corresponding values in control group. In addition to Hct, MCV and MCH values revealed a significant increase in the groups treated with curcumin or vitamin c or combination between them, then with DEN when compared to group treated with DEN only(Table 2).

Table (2): Mean values ± S.E of hematological profile (Hct, MCV, MCH & MCHC) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

		Groups								
		30 day						30 day		
		Control	Olive oil	Cur	Vit. C	Cur + Vit. C	DEN	Cur then DEN	Vit. C then DEN	Mix then DEN
Hct (%)	Mean ± S.E	43.1 ± 0.67^a	43.0 ± 0.61^a	44.7 ± 0.58^a	42.3 ± 1.12^a	44.9 ± 4.93^a	39.9 ± 1.21^b	41.8 ± 0.66^c	42.3 ± 0.82^c	40.2 ± 0.81^{a,c}
	% of change	-	-0.09	-0.15	0.40	0.86	0.44	-0.01	0.18	0.17
MCV (fl)	Mean ± S.E	54.7 ± 0.79^a	55.1 ± 0.63^a	55.5 ± 0.94^a	54.2 ± 1.16^a	53.3 ± 5.73^a	51.1 ± 0.76^a	59.4 ± 0.84^b	62.0 ± 1.16^b	61.3 ± 0.69^b
	% of change	-	-0.25	0.15	0.31	0.86	-0.03	0.05	0.31	-0.14
MCH (Pg)	Mean ± S.E	17.8 ± 0.26^a	17.5 ± 0.19^a	17.6 ± 0.12^a	18.1 ± 0.38^a	17.9 ± 0.12^a	17.6 ± 0.07^a	19.6 ± 0.21^b	19.9 ± 0.37^b	20.1 ± 0.21^b
	% of change	-	-0.36	-1.16	0.31	-1.16	-2.71	-0.23	0.29	-0.23
MCHC (%)	Mean ± S.E	32.6 ± 0.15^{a,c}	30.2 ± 0.16^a	30.3 ± 0.45^a	28.1 ± 0.08^a	29.6 ± 1.12^a	34.6 ± 0.41^c	33.1 ± 0.36^{a,c}	32.2 ± 0.74^a	34.1 ± 0.55^a
	% of change	-	0.06	0.66	-0.87	0.86	0.63	0.58	0.79	0.72

Liver function tests

Our data demonstrated that groups treated with Olive oil, curcumin, Vitamin C and combination between curcumin and vitamin for 30 days revealed insignificant changes in ALAT, ASAL, total protein, albumin, total bilirubin and alkaline phosphatase levels when compared to their corresponding values in control group while, the DEN administered groups recorded a significant increase in ALAT, ASAL, total bilirubin and alkaline phosphatase levels and a significant decrease in the levels of total protein, albumin throughout the experimental period when compared to their corresponding value in the control group. In addition to ALAT, ASAL enzyme activity, total bilirubin and alkaline phosphatase values revealed a significant decrease in the groups treated with curcumin or vitamin c or combination between them then, with DEN when compared to group treated with DEN only**,** on the other hand total protein and albumin levels revealed insignificant changes of vitamin c and combination between them then, DEN when compared to group treated with DEN, except for the group treated with curcumin then, with DEN showed that a significant increase in the level of total protein and albumin when compared to the group treated with DEN. (Tables 3&4).

Table (3): Mean values ± S.E of liver function tests (ALAT, ASAT, T. Protein & albumin) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

		Groups								
		30 day						60 day		
	---	Control	Olive oil	Cur	Vit. C	Cur + Vit. C	DEN	Cur then DEN	Vit. C then DEN	Mix Then DEN
S. ALAT U/l	Mean ± S.E	33 ± 1.72^{a}	31 ± 1.66^{a}	36 ± 1.58^{a}	38 ± 0.61^{a}	35 ± 2.14^{a}	71 ± 3.44^{b}	48 ± 1.31^{c}	52 ± 2.58^{c}	47 ± 3.81^{c}
	% of change	--	-0.03	-0.08	-1.81	0.19	0.5	-0.31	0.33	0.55
S. ASAT U/l	Mean ± S.E	70 ± 3.04a	65 ± 2.01^{a}	68 ± 1.99^{a}	75 ± 4.91^{a}	71 ± 4.41^{a}	214 ± 13.97^{b}	85 ± 1.96^{a}	81 ± 2.69^{a}	79 ± 3.83^{a}
	% of change	-	-0.51	-0.52	0.39	0.31	0.78	-0.55	-0.13	0.20
T. Protein g/dl	Mean ± S.E	6.83 ± 0.21a	6.88 ± 0.23^{a}	6.39 ± 0.11^{a}	6.59 ± 0.15^{a}	6.23 ± 0.04^{a}	5.71 ± 0.09^{b}	5.92 ± 0.07^{c}	5.58 ± 0.14^{b}	5.72 ± 0.16^{b}
	% of change	-	0.08	-0.90	-0.4	-4.25	-1.3	-2.	-0.5	-0.31
S. Albumin g/dl	Mean ± S.E	3.71 ± 0.17^{a}	3.81 ± 0.15^{a}	3.88 ± 0.15^{a}	3.36 ± 0.08^{a}	3.17 ± 0.03^{a}	2.71 ± 0.11^{b}	3.01 ± 0.08^{c}	2.86 ± 0.06^{b}	2.99 ± 0.09^{b}

Table (4): Mean values ± S.E of liver function tests (T. Bilirubin & alkaline phosphatase) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

		Groups								
		30 day						60 day		
	---	Control	Olive oil	Cur	Vit. C	Cur + Vit. C	DEN	Cur then DEN	Vit. C then DEN	Mix then DEN
T. Bil mg/dl	Mean ± S.E	0.39 ± 0.03^{a}	0.40 ± 0.03^{a}	0.46 ± 0.01^{a}	0.42 ± 0.01^{a}	0.43 ± 0.01^{a}	1.78 ± 0.05^{b}	0.63 ± 0.04^{c}	0.67 ± 0.04^{c}	0.71 ± 0.03^{c}
	% of change	-	0	-2.	-2.	-2.	0.4	0.25	0.25	0
ALP U/l	Mean ± S.E	137.6 ± 9.51^{a}	130.5 ± 7.14^{a}	152.2 ± 5.48^{a}	153.6 ± 4.38^{a}	162.0 ± 3.52^{a}	214.4 ± 11.61^{b}	178.0 ± 6.21^{c}	179.2 ± 5.49^{c}	164.0 ± 10.72^{c}
	% of change	-	-0.33	-0.73	-1.17	-1.7	0.18	-0.53	-0.73	0.11

Aspartate aminotransferase to platelet ratio index (APRI)

The results of aspartate aminotransferase to platelet ratio index (APRI) revealed insignificant changes in groups treated with Olive oil, curcumin, Vitamin C and combination between curcumin and vitamin C when compared to its corresponding value in control group, while the DEN administered group recorded a significant increase in the levels of APRI, at the end of the experiment when compared to their corresponding values in the control group. In addition to the level of APRI revealed a significant decrease of curcumin, vitamin c and a combination between them with DEN when compared to the group treated with DEN only (Table 5).

Table (5): Mean values ± S.E of Aspartate aminotransferase to platelet ratio index (APRI) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

	Groups									
		30 day						60 day		
	---	Control	Olive oil	CURC	Vit. C	CURC + Vit. C	DEN	CURC then DEN	Vit. C then DEN	Mix Then DEN
APRI	Mean ± S.E	0.15 ± 0.03 ^{a}	0.14 ± 0.06 ^{a}	0.13 ± 0.06 ^{a}	0.14 ± 0.05 ^{a}	0.13 ± 0.009 ^{a}	0.91 ± 0.31 ^{b}	0.31 ± 0.08 ^{c}	0.39 ± 0.08 ^{c}	0.44 ± 0.21 ^{d}

DISCUSSION

According to the World Health Organization, HCC is the fifth most common tumor worldwide and the second most common cause of cancer-related death. Male-to-female predominance is greater than 2:1 with liver cancer, and approximately 83% of the estimated 782,000 new HCC cases in 2012 (Song *et al.*, 2017; Heimbach *et al.*, 2018). In Egypt, HCC represents the second most frequent cancer in men with more than 8000 new cases predicted by the year 2012. Early detection of HCC opens doors for various treatments as surgical resection, radiofrequency ablation, and transplantation, which can lead to amenable to aggressive intervention and improved survival in a great number of HCC patients (Goldman *et al.*, 2007; Hashem *et al.*, 2017).

Hematopoietic system is a very sensitive system to detect the dangerous effects of drugs and toxic substances on our health in addition, hematological parameters and markers of the systemic inflammatory response have been correlated with prognosis in several malignancies. So any kind of severe disease or abnormality has a direct impact on blood parameters so it is necessary to study the changes in hematological parameters in liver cancer patients, at regular intervals during treatment (Ali, 2014; Shrivastava, *et al.*, 2016; Mokh *et al.*, 2019).

The present study, the data revealed that the mean values of RBCs, Hb, Hct, WBCs and PLATS counts and concentration were significantly decreased in group treated with DEN when compared with their corresponding levels in the control group ($p< 0.05$) so this case called pancytopenia while there was no statistically significant difference in the mean values of blood indices in rats treated with DEN when compared with their corresponding levels in the control group. These results in agreed with(Carr, 2016 ; Selvamani and Thomas, 2017) who stated that decreased RBCs and hemoglobin most common anemia in primary HCC patients and often its type is normochromic normocytic anemia as inferred from the study leucopenia and thrombocytopenia are present in most patients and is commonly present in the patients with splenomegaly and with the history of bleeding tendencies, also these results agreed with (Solomon *et al.*, 2017) who showed the Study on hematological

abnormalities in chronic liver diseases and this might be due to the direct damage of the bone marrow and the blood elements are sensitive to the oxidative stress and their plasma membranes contain a highly percentage of polyunsaturated fatty acids(Carr, 2016) and this increases lipid peroxidation products. On the other hand (Nilakanth and Balachandran, 2019) revealed that curcumin treatment could decrease or mitigate the toxic effects of anemia and leukocytosis against DEN induced toxicity. On the other hand vitamin C has potent antioxidant activity against toxic substances as DEN and the consumption of foods rich in vitamin C is highly recommended to reduce the damage caused by the toxic compounds as DEN (Fahmy *et al.,* 2017).

The liver function tests of the DEN group in the present study recorded significant alterations in all investigated liver function parameters when compared with their corresponding values in the control group in which the liver injury caused by DEN generally reflected the instability of liver metabolism and characteristic changes in the serum enzyme activities (Bulle *et al.,* 1990; Zhao *et al.,* 2014; Fathy *et al.,* 2017). The specific enzymes for liver (AST, ALT, GGT, and ALP) are activated at the hepatocellular damage and give rise to abnormal levels for liver function tests (Ansari *et al,* 1991; Ganeshkumar *et al.,* 2016). The changes in the concentrations of AST, ALT, ALP, T. Bil and GGT were generally accepted as an index of liver damage and this propensity was known to be distinguished in rodents and an increase in the level of these enzymes in both serum represents the extent of hepatocellular damage (Injac *et al,* 2008; Kolarovic *et al,* 2010; Elsadek *et al,* 2017; Marslin et al., 2018). A reduction in the level of these enzymes compared to DEN administered animals was observed in animals treated with curcumin in which curcumin the potential role to protect from liver damage has. The increase in transaminases (ALAT and ASAT) was the clearest indication of cellular leakage and loss of functional integrity of the cell membrane (Saraswat *et al.,* 1993; Al-Rejaie *et al.,* 2009). The significant increase of AST and ALT in the present study indicated the liver damage and loss of functional integrity of cell membranes (Kolarovic *et al.,* 2010). Thus proving the hepatocellular damage which might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupturing of the plasma membrane (Gupta *et al.,* 2004). In addition, the increased liver enzymes in the serum are a reflection of lipid peroxidation of liver cell membranes in which this free radicals initiated lipid peroxidation process and protein carbonylation, leading to abnormal structural changes of the bio-membranes and loss of liver integrity and decreased metabolic activity (Azab *et al,* 2011; Fathy *et al.,* 2017).

The GGT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canalicular domain. The serum GGT activity was considered as one of the best indicators of liver damage. The liberation of this enzyme into the serum indicates the damage of the hepatic cells and injury of the liver (Bulle *et al.,* 1990; Yao *et al.*, 2004; Kadasa *et al.,* 2015) on the other hand the elevation in ALP activity and T. BIL in the DEN group could be attributed to the large bile duct obstruction, intrahepatic cholestasis, infiltrative diseases of the liver, or the enzyme release from the tissues to the bloodstream, particularly due to the defects in cell membrane permeability (Carl and David, 2001; Zhao *et al.,* 2014), while the decrease of ALAT, ASAT, ALP Bil and GGT serum levels in CUR treated groups may be due to the reduction of cellular damage (Zhao *et al.,* 2014; Kadasa *et al.,* 2015)this is in agree with (Hussein *et al.,* 2014) who reported that curcumin may be able to ameliorate serum biomarkers of hepatic function, prevent the lipid peroxidation and oxidative stress, This has been enhanced by (Abou Zaid *et al.,* 2016; Zhong *et al.,* 2016; Qiu *et al.,* 2017; Li *et al.,* 2018) who said that treatment with curcumin able to back parts of enzymes to near

normal levels, decreased metabolic disorders by possibly preserving the functional integrity of the hepatocytes, has potent chemo-preventative activity against a wide variety of tumors, has great potential in the prevention and treatment of hepato-carcinogenesis, showing its defense action against DEN induced hepatotoxicity and may serve as a promising candidate to inhibit inflammation and apoptosis signaling for the treatment of endotoxins which may be induced liver failure. On the other side, Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio-membranes from peroxide damage. This is in agree with (Abou Zaid *et al.*, 2016; Lv *et al.*, 2018) who demonstrated that Vitamin C has chemo-preventative effect against hepatocellular-carcinoma via its considers as a strong antioxidant and free radicals scavenging activity and can effectively kill and erase liver cancer cells (CSCs) and used as a novel therapeutic agent for HCC treatment. on the other hand, (Mohammed, 2018) showed that the use of high dose intravenous vitamin C and Helixor injections can improve the liver functions and overall physical performance in a patient with HCC and in case of impairment liver function tests.

Aspartate Aminotransferase to Platelet Ratio Index (APRI):

Aspartate aminotransferase to platelet ratio index (APRI) is a widely investigated indirect marker in assessing liver diseases as fibrosis and cirrhosis .APRI was originally developed for predicting fibrosis and cirrhosis in patients with chronic hepatitis C infection, and most of the investigations that followed were focused on HCV-related fibrosis evaluation (Baranova *et al.,* 2011). A few studies have also reported that APRI might be helpful in the assessment of liver fibrosis in HBV patients. A recent study suggested that APRI was also associated with postoperative prognosis in early stages of HCC patients (Hann *et al.,* 2015).

Ji *et al., (*2016) reported that the combination of neutrophil/ lymphocyte ratio (NLR) and APRI may be a useful prognostic tool to determine survival in patients with HCC after resection, and to further guide their follow-up and postoperative treatment.

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