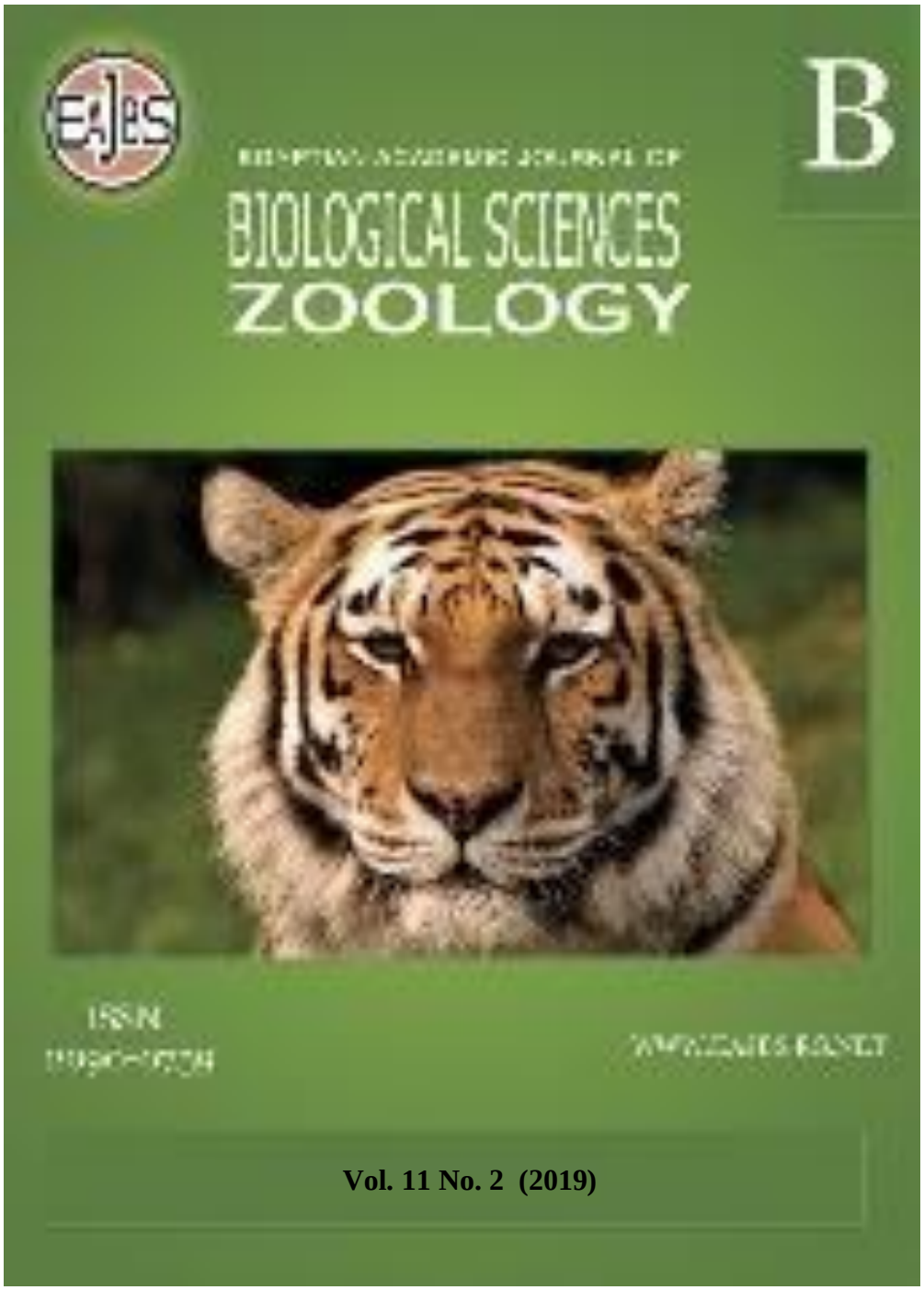

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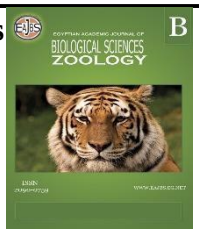

Hematological and Biochemical Changes in Rats Induced with Diethyl Nitrosamine and the Hepatoprotective Role of Some Antioxidants

Samir. A. Zaahkouk[1], Ahmed. B. M. Mehany[1], Sawsan, A. El-Shamy[2]and Salah. M. EL-Sharkawy*[1]

1-Zoology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

2-General Biology, Basic Science Center, Misr University for Science and Technology, 6th of October City, Egypt.

E.Mail.: salahmontaser10@yahoo.com

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ABSTRACT

Abstract: Hepatocellular carcinoma is one of the most common malignancies worldwide, and in Egypt, it is 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, alcoholism, schistosomiasis, aflatoxins (AFB), hypothyroidism, and cirrhosis. Diethyl nitrosamine (DEN) is a well-known, strong hepatocarcinogenic agent. It is known that DEN induces damage to enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models such as rats. Curcumin is a strong anti-inflammatory agent and has 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 biomembranes from peroxide damage and tissue damage to the liver, as well as 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*) with an average weight of 190 ± 10 g. They were allowed to acclimate in the laboratory and distributed into 9 groups of 10 rats each. Hematological parameters and some hepatic tests were measured for all groups at the end of the experiment. **Results:** The results showed significant alterations in all hematological parameters and hepatic function tests when compared to their corresponding levels in the control group, and when treated with curcumin and vitamin C, the results showed improvement in some parameters when compared to the 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 most important organ in the body. It plays many vital functions in the maintenance, performance and regulation of the homeostasis. 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 produces 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; 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). Although it 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, which has 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 nutritional supplement, are 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, that 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; 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 to the liver (Salah *et al*., 2010; 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.

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. All animals received human care in accordance with the guidelines of the ethical committee of the Faculty of Science, Al- Azhar University. 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 at 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% vitamin mixture), purchased from Meladco Feed Company (Aubor City, Cairo, Egypt) and supplied with water ad libitum throughout the experimental period.

Chemicals:

Curcumin:

Curcumin is a bright 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), a crystalline powder practically insoluble in water.

Curcumin was prepared for supplementation by dissolving 1500 mg of curcumin (powder) in 30 ml of 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 at a concentration of 150 mg/kg body weight, 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 200 mg/kg of vitamin C daily 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 in this group were administered olive oil daily via oral gavage tube for 30 days.
- **Group (3)**: Rats in this group were administered curcumin at a dose 150 mg/kg daily via oral gavage tube for 30 days.
- **Group (4)**: Rats in this group were administered vitamin C at dose (200 mg/kg) daily via oral gavage tube during the period of the experiment.

- **Group (5)**: Rats in this group were administered a mix of curcumin and vitamin C daily via oral gavage tube for 30 days.
- **Group (6):** Rats in this group were used as a positive control for the HCC model in which DEN was administered in drinking water (100 mg/L) for 30 days.
- **Group (7):** Rats in this group were administered curcumin at a dose 150 mg/kg daily via oral gavage tube for 30 days, then treated with DEN in the drinking water (100 mg/L) for 30 days.
- **Group (8)**: Rats in this group were administered vitamin C at a dose 200 mg/kg daily via oral gavage tube for 30 days, then treated with DEN in the drinking water (100 mg/L) for 30 days.
- **Group (9)**: Rats in this group were administered a mixture of curcumin and vitamin C daily via oral gavage tube for 30 days, then treated with DEN in the drinking water (100 mg/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, ASAT and ALAT (Bergmeyer *et al.,* 1986), alkaline phosphatase (ALP) (Carl and David. 2001), 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 using a statistical package for social sciences computer program (SPSS/CP) (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 a combination of curcumin and vitamin C recorded an insignificant change in RBCs, Hb, WBCs, and platelet count when compared to their corresponding values in the control group, while rats induced with DEN revealed a significant decrease in RBCs, Hb, and platelet 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 a combination of them with DEN when compared to the group treated with DEN only, while WBCs count revealed a significant decrease ($p<0.05$) in curcumin or vitamin C or a combination of them when compared to the group treated with DEN only (Table 1).

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

Parameters \ Treatments		Groups								
		30 days						60 days		
		Control	Olive oil	Curcumin	Vitamin C	Curcumin + Vitamin C	DEN	Curcumin then DEN	Vitamin 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	-	-0.88	0.63	3.55	-0.25	-18.6	-9.77	-13.4	-16.7
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	--	-0.71	-0.71	4.25	0	-8.51	-2.12	-3.54	-2.12
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	--	-1.31	-5.26	-1.31	0	94.7	50.0	67.1	72.3
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	--	-1.59	-3.90	5.78	8.24	-31.6	-15.5	-9.69	-22.1

Each value represents a means of 10 records ± S.E.
a, b, c, d, e means comparison between all groups, where the groups with the same letter mean there is no significance difference and those with a different letter mean there is a significance change. %: percent of changes from control values.

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

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

parameters \ Treatments		Groups								
		30 days						60 days		
		Control	Olive oil	Curcumin	Vitamin C	Curcumin + Vitamin C	DEN	Curcumin then DEN	Vitamin 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	-	-0.23	3.71	-1.85	4.17	-7.42	-3.01	-1.85	-6.72
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	-	0.73	1.46	-0.91	-2.5	-6.5	8.59	13.3	12.1
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	-	-1.68	-1.12	1.68	0.56	-1.12	10.1	11.7	12.9
MCHC %	Mean ± S.E	32.6 ± 0.15^{a}	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	-	-7.36	-7.05	-13.8	-9.20	6.13	1.53	-1.22	4.60

Liver function tests

Our data demonstrated that groups treated with olive oil, curcumin, vitamin C and a combination of curcumin and vitamin for 30 days revealed insignificant changes in ALAT, ASAT, total protein, albumin, total bilirubin and alkaline phosphatase levels when compared to their corresponding values in the control group while, the DEN administered groups recorded a significant increase in ALAT, ASAT, total bilirubin and alkaline phosphatase levels and a significant decrease in the levels of total protein and albumin throughout the experimental period when compared to their corresponding value in the control group. In addition to ALAT, ASAT enzyme activity, total bilirubin and alkaline phosphatase values revealed a significant decrease in the groups treated with curcumin or vitamin C or a combination of them then, with DEN when compared to the group treated with DEN only**.** On the other hand, total protein and albumin levels revealed insignificant changes in vitamin C and a combination of them then, DEN when compared to the group treated with DEN, except for the group treated with curcumin with DEN showed 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, and albumin) in adult male albino rats subjected to different treatment conditions for 30 and 60 days.

Parameters \ Treatments		Groups								
		30 days						60 days		
		Control	Olive oil	Curcumin	Vitamin C	Curcumin + Vitamin C	DEN	Curcumin then DEN	Vitamin 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 ± 3.83^{c}
	% of change	--	-6.06	9.09	15.1	6.06	115	45.4	57.5	42.4
S. ASAT U/L	Mean ± S.E	70 ± 3.04^{a}	65 ± 2.01^{a}	68 ± 1.99^{a}	75 ± 4.91^{a}	71 ± 4.41^{a}	214 ± 13.97^{b}	85 ± 1.96^{a}	80 ± 2.69^{a}	79 ± 3.83^{a}
	% of change	-	-7.14	-2.85	7.14	1.42	205	21.4	14.2	12.8
T. Protein gm/dl	Mean ± S.E	6.83 ± 0.21^{a}	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	-	0.73	-6.44	-3.51	-8.78	-16.3	-13.3	-18.3	-16.2
Albumin gm/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}	2.86 ± 0.08^{c}	3.01 ± 0.06^{b}	2.99 ± 0.09^{b}
	% of change	-	2.69	4.58	-9.43	-14.5	-26.9	-22.9	-18.8	-19.4

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

Parameters \ Treatments		Groups								
		30 days						60 days		
		Control	Olive oil	Curcumin	Vitamin C	Curcumin + Vitamin C	DEN	Curcumin then DEN	Vitamin 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}	1.01 ± 0.05^{b}	0.63 ± 0.04^{c}	0.67 ± 0.04^{c}	0.71 ± 0.03^{c}
	% of change	-	2.56	17.9	7.69	10.2	158	61.5	71.7	82.1
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	-	-5.15	10.6	11.6	17.7	55.8	29.3	30.2	19.1

Aspartate Aminotransferase to Platelet Ratio Index (APRI)

The results of the aspartate aminotransferase to platelet ratio index (APRI) revealed insignificant changes in groups treated with olive oil, curcumin, vitamin C and a combination of curcumin and vitamin C when compared to their corresponding values in the 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, the level of APRI revealed a significant decrease in curcumin, vitamin c and a combination of 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.

Parameters / Treatments		Groups								
		30 days						60 days		
		Control	Olive oil	Curcumin	Vitamin C	Curcumin + Vitamin C	DEN	Curcumin then DEN	Vitamin 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 d	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 such as surgical resection, radiofrequency ablation, and transplantation, which can lead to amenable aggressive intervention and improved survival in a great number of HCC patients (Goldman *et al.*, 2007; Hashem *et al.*, 2017).

The 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 the prognosis of 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).

In the present study, the data revealed that the mean values of RBCs, Hb, Hct, WBCs and PLT counts and concentrations were significantly decreased in the group treated with DEN when compared with their corresponding levels in the control group ($p< 0.05$) so this case is 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 are in agreement with Carr (2016) ; Selvamani and Thomas (2017) who stated that decreased RBCs and hemoglobin are the 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 are commonly present in the patients with splenomegaly and with a history of bleeding tendencies, also these results are in agreement 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 to the bone marrow and the blood elements are sensitive to the oxidative stress, and their plasma membranes contain high percentage of polyunsaturated fatty acids (Carr, 2016) which 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 such as DEN, and the consumption of foods rich in vitamin C is highly recommended to reduce the damage caused by the toxic compounds in 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 the liver (ASAT, ALAT, 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 ASAT, ALAT, ALP, T. bil, and GGT were generally accepted as an index of liver damage and this propensity was known to be distinguished in rodents. 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, indicating that curcumin has the potential 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 ASAT and ALAT in the present study indicated liver damage and loss of functional integrity of cell membranes (Kolarovic *et al.*, 2010). Thus, proving the hepatocellular damage that 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 the lipid peroxidation of liver cell membranes in which these free radicals initiated the 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. Serum GGT activity was considered one of the best indicators of liver damage. The liberation of this enzyme into the serum indicates damage to the hepatic cells and injury to 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 blood stream, particularly due to 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 curcumin treated groups may be due to the reduction of cellular damage (Zhao *et al.*, 2014; Kadasa *et al.*, 2015) this is in agreement with Hussein *et al.* (2014) who reported that curcumin may be able to ameliorate serum biomarkers of hepatic function, prevent 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 is 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 induce liver failure. On the other side, vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. This is in agreement 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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