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Evaluation of some tumors markers in rats induced with Diethyl nitrosamine and hepatoprotective role of some antioxidants

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Abstract
Hepatocellular carcinoma is one of the most common malignancies worldwide and in Egypt the most common form of primary liver cancer. HCC represent the second most prevalent cancer among men in Egypt. HCC occurs in a number of some conditions that commonly includes hepatitis C and B, alcoholic, schistosomiasis, aflatoxins (AFB) 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 tissues damage of the liver. AFP was found to be a weak diagnostic predictor due to not specific and with low sensitivity.
Objective: To evaluate the early detection of HCC by a novel biomarkers than AFP, 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. Serum lipid profile and some tumors biomarkers were measured for all groups at the end of experiment.
Results: The results showed that the level serum total cholesterol, TG, LDL-C risk ratios revealed a significant increased for them and decreased HDL-C in DEN group when compared with its corresponding level in control group, and when treated with curcumin and vitamin c the results showed a significant decrease when compared with DEN group. Receiver Operator of Characteristics (ROC) curve analysis of AFP revealed that cut off value was >3.62 (ng/ml) leading to 79.7% for sensitivity, 76.0% specificity, 81.2% positive Predictive value, 80.1 negative Predictive val revealed that ue, and 78.4 for accuracy, while (ROC) curve analysis of Alpha L- Fucosidase revealed that cut off value was >285 (ng/ml) leading to 91.2% for sensitivity, 90% specificity, 90.9% positive Predictive value, 94.7 negative Predictive value, and 89.7 for accuracy and (ROC) curve analysis of SCCAg1 had cut off value was >124.8 (ng/ml) leading to 95.5% for sensitivity, 81% specificity, 93.8% positive Predictive value, 90.1 negative Predictive value, and 92.9 for accuracy
Conclusion: AFU and SCCA1 are more accurate marker than AFP for early detection or diagnosis of HCC. AFU and SCCA1 showed higher sensitivity, specificity and correlated more to other indices than AFP. Antioxidants like curcumin and vitamin c administration improved the lipid profile and the parameters of tumors biomarkers.

Keywords: hepatocellular carcinoma, lipid profile, tumor biomarkers, curcumin and Vitamin C

1. Introduction
The liver is the largest, critical and important organ in the body. It performs an array of functions that help support metabolism, immunity, digestion, detoxification, Drugs and chemicals Metabolism, vitamin storage among other functions. The foundation of the lobule is composed of hepatocytes, which have physiologically distinct apical and basolateral membranes. Based on function and perfusion, hepatocytes are divided into 3 zones. (Solomon *et al.*, 2017; Kalra and Tuma, 2018) [1, 2].
Liver tumors are classified into two major categories, the 1st one is primary liver tumors and the 2nd is metastatic liver tumors. The primary tumor originates in the liver, while the metastatic tumor spreads to the liver from other organs of the body, accessing the liver through the portal vein or the hepatic artery (McNally, 2010) [3].
Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and normally develops as a consequence of underling liver disease and often associated with cirrhosis (Li *et al.*, 2013) [4]. Variety of risk factors contribute to the initiation of HCC (Aravalli *et al.*, 2013) [5]. HCC alone accounts for 90% of all cases of primary liver cancer, with nearly 800,000 new cases annually (Llovet *et al.*, 2016) [6]. 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) [7, 8].
In Egypt, the incidence rate of HCC has increased sharply in the last (El-Zayadi *et al.*, 2010) [9]. The development and progression of HCC is a complex process, which involves

the dysregulation of oncogenes and tumor suppressor genes. 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. The bacteria in the mouth chemically reduce nitrate found in many vegetables to nitrite, which in turn can form nitrosating agents. 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) [10]. Diethylnitrosamine (DEN) is a representative chemical of a family of carcinogenic N-nitroso compounds and well-known hepatocarcinogen forming DNA adducts in the liver and inducing HCC (Singer and Crunderger 1984; Fathy *et al.,* 2017) [11, 12].

Curcumin

Turmeric (*curcuma longa*), also known as'*curcuma domestica'* is a perennial herbaceous plant of the ginger *family Zingiberaceae* (Priyadarsini, 2014) [13]. 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) [14]. The effects of curcumin, having a polyphenol structure, on certain cytokines, kinases, enzymes, transcription factors, growth factors and receptors have been studied, and it has shown some antimicrobial, antiinflammatory, antiox idant, immunomodulatory, renoprotective, hepatoprotective and hypoglycaemic effects (Kocaadam and Şanlier, 2017) [15].

Vitamin C

Intake of antioxidant vitamins which are widely distributed in fruits could be beneficial in protecting against hepatotoxicity **(**Galati *et al.,* 2005) [16]. Among antioxidants, ascorbic acid (vitamin C) and tocopherol (vitamin E) used as a nutritional supplements, are the essential elements in almost all biological systems (Howard *et al.,* 2000) [17]. It is one of the most widely available and affordable non-enzymatic antioxidant molecules that have been used to mitigate oxidative damage (Naidu, 2003) [18]. It readily scavenges physiological ROS as well as reactive nitrogen species ''RNS" (Carr and Frei, 1999) [19]. 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) [20, 21].

Materials and methods

Chemicals

Diethylnitrosamine

Diethylnitrosamine 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 and reagents were of high analytical grade and were purchased from standard commercial suppliers.

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) **[22, 23]**, crystalline powder practically insoluble in water.

Dose of curcumin and preparation

Curcumin was prepared to 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 was received curcumin in a concentration of 150 mg/kg body weight of rat according to previous studies (Khedr and Khedr 2014) **[24]**.

Vitamin C (Vit. C)

Vitamin C (ascorbic acid) was purchased from El-Nasr Company for pharmaceutical industries, Egypt.

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) [25].

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.

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.

2. Experimental design

A patch of 90 male Wister albino rats weights range (190 ± 10 g) at the beginning of the experiment were divided into 9 main group according to the treatment and requirements of the experiment.

90 male albino rats were randomly divided into nine equal groups and labeled as groups 1,2,3,4,5,6,7,8 and 9 each group contain 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 day.
- **Group (3)**: Rats of this group were administered Curcumin at dose (150 mg/kg) daily via oral gavage tube for 30 day.

- **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 day.
- **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 day.
- **Group (7):** Rats of this group were administered Curcumin at dose (200 mg/kg) daily via oral gavage tube for 30 day, then treated with DEN in drinking water (100 µg/L) for 30 day.
- **Group (8)**: Rats of this group were administered Vitamin C at dose (200 mg/kg) daily via oral gavage tube for 30 day, then treated with DEN in drinking water (100 µg/L) for 30 day.
- **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 day then, treated with DEN in drinking water (100 µg/L) for 30 day.

Blood sampling

At the end of experimental periods, animals were fasted overnight and following diethyl ether anesthesia. blood samples were collected from all animals through retro-orbital venous plexus and one part of blood were transferred into non heparinized tubes, to obtain serum by centrifugation at 3000 r.p.m for 10 minutes; sera were frozen at -20 C° for investigation of some hepatic tumor markers.

Serum tumor markers

Estimation of Squamous cell carcinoma antigen1 (SCCA1) in whish levels were assayed by Sandwich ELISA technique according to (Röijer *et al.*, 2003) [26], Serum gamma-glutamyl transferase (γ-GT) (Rosalki 1971) [27] activity was determined kinetically (Elitech diagnostic Co. France). Serum alpha-L-fucosidase (AFU) (WUHAN EIAAB SCIENCE CO., LTD., CHINA), alpha-fetoprotein (AFP) (LifeSpan BioSciences, Inc. Seattle, WA, USA) and Carcinoembryonic antigen (CEA) in which CEA and sample concentration is determined on Cobas e411 according to (Guder *et al.*, 1996) [28].

Statistical analysis

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

Results

Hepatocellular carcinoma tumor biomarkers

The results of Carcinoembryonic antigen (CEA), Alpha-Fetoprotein (AFP), Alpha-L-fucosidase (FUC), Squamous cell carcinoma-Ag1 (SCCA1), and Gamma-G lutamyl transferase activity (GGT), 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 CEA, AFP, FUC, SCCA1and GGT enzyme activity at the end of experiment when compared to their corresponding values in the control group. In addition to the levels of CEA, AFP, FUC, SCCA1and GGT levels revealed a significant decrease of curcumin, vitamin c and combination between them with DEN when compared to group treated with DEN only (Table 1,2).

Table 1: Mean values ± S.E of tumor markers (CEA, AFP and Alpha-L-Fucosidase) 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
CEA ng/ml	Mean± S.E	1.15± 0.08^{a}	1.13± 0.07^{a}	0.92± 0.07^{a}	1.26± 0.05^{a}	1.45± 0.02^{a}	4.27± 0.41^{b}	2.34 ±0.12^{c}	2.87± 0.03^{c}	2.99± 0.07^{c}
	% of change	-	-0.14	-0.14	-0.6	-3.	0.80	0. 33	-1.6	-0.14
AFP ng/ml	Mean± S.E	1.38± 0.03^{a}	1.36± 0.04^{a}	1.19± 0.08^{a}	1.29± 0.04^{a}	1.39± 0.07^{a}	36.9± 2.17^{b}	14.2± 0.61^{c}	16.1± 0.29^{c,e}	17.4± 0.36^{e}
	% of change	-	0.25	0.62	0.25	0.57	0.98	0.95	0.89	0.91
Alpha L-Fucosidase ng/ml	Mean± S.E	242± 5.11^{a}	250± 2.96^{a}	244± 2.77^{a}	259± 6.64^{a}	254± 3.44^{a}	632± 14.5^{b}	435± 9.89^{c}	458± 8.56^{e}	483± 3.43^{e}
	% of change	-	-0.72	0.84	0.23	-0.48	0.64	0.48	0.40	-0.48

Table 2: Mean values ± S.E of tumor markers (SCCA1, and GGT) 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
SCCA1 mg/l	Mean ± S.E	85.4 ±1.87^{a}	77.8 ±0.91^{a}	79.3 ±0.66^{a}	86.4 ±1.91^{a}	90.2 ±1.03^{a}	271.7 ±20.9^{b}	166.9 ±4.29^{c}	184.3 ±1.86^{c,d}	192.1± 3.24^{d}
	% of change	-	-1.05	-1.83	0.02	-0.81	0.91	0.56	-0.005	0.42
S. GGT U/l	Mean ± S.E	2.7 ±0.37^{a}	2.5 ±0.29^{a}	2.4 ±0.27^{a}	2.3 ±0.18^{a}	2.7 ±0.34^{a}	8.9 ±1.77^{b}	5.8 ±0.69^{c}	7.2 ±1.03^{d}	7.5 ±0.89^{d,b}
	% of change	-	-0.27	-0.37	-1.05	-0.08	0.79	0.32	0.64	0.58

Receiver operating characteristic (ROC) Curve for DEN group of AFP, Alpha - L- Fucosidase and CCAg1

1. AFP

In DEN group, the Area under ROC curve was 0.84 and cut off value was >3.62 (ng/ml) leading to 79.7% for sensitivity, 76.0% specificity, 81.2% positive Predictive value, 80.1 negative Predictive value, and 78.4 for accuracy (table: (6) ; fig: (1)).

2. Alpha - L- Fucosidase

In DEN group, the Area under ROC curve was 0.92 and cut off value was >285 (ng/ml) leading to 91.2% for sensitivity, 90% specificity, 90.9% positive Predictive value, 94.7 negative Predictive value, and 89.7 for accuracy (table: (6) ; fig: (2)).

3. SCCAg1

In DEN group, the Area under ROC curve was 0.95 and cut off value was >124.8 (ng/ml) leading to 95.5% for sensitivity, 81% specificity, 93.8% positive Predictive value, 90.1 negative Predictive value, and 92.9 for accuracy (table: (3) ; fig: (3)).

Table 3: Diagnostic indices of AFP, Alpha L- Fucosidase, and SCCAg1 in DEN groups

	AFP	Alpha L- Fucosidase	SCCAg1
Cut off	>3.62	>285	>124.8
Area under ROC curve	0.845	0.924	0.954
Sensitivity	79.7%	91.2%	95.5%
Specificity	76%	90%	81%
positive Predictive value	81.2%	90.9%	93.8%
negative Predictive value	80.1%	94.7%	90.1%
Accuracy	78.4%	89.7%	92.9%

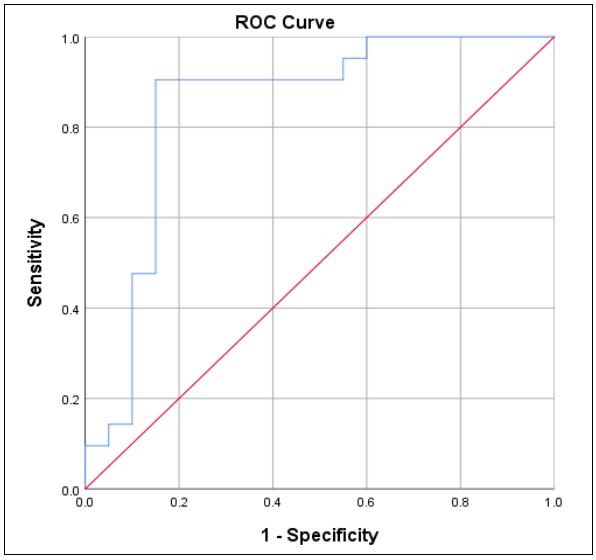

Fig 1: ROC Curve of AFP in DEN group.

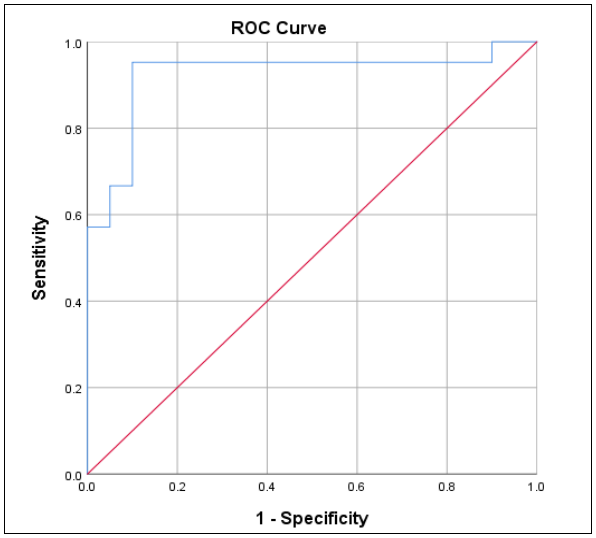

Fig 2: ROC Curve of Alpha - L- Fucosidase in DEN group.

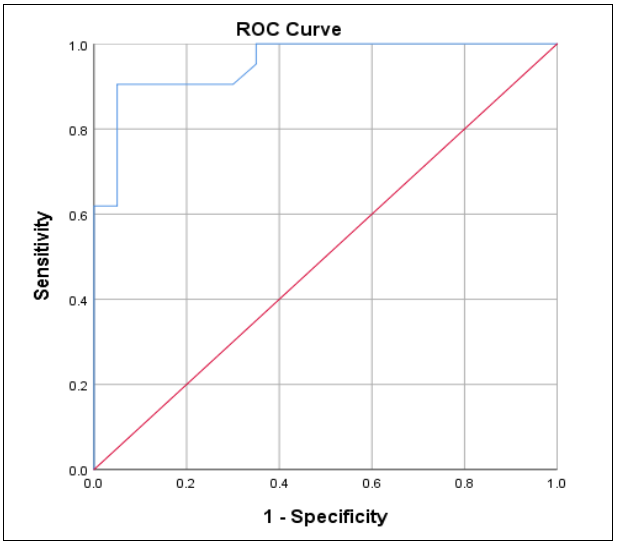

Fig 3: ROC Curve of SCCAg1 in DEN group.

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 occurred in less developed regions of the world (Song *et al.*, 2017; Heimbach *et al.*, 2018) [29, 30].

In view of our data, the identification of a carcinogenic potential for an agent delineates the conditions of exposure (dose, time and duration) under which the agent may induce cancer. Animals are surrogate models of humans since they possess similar physiology and biochemistry. Several factors are important for cancer development, including a loss of normal growth control as recorded in the present study (Hanahan & Weinberg 2011) [31].

Tumor markers parameters

In the present work, the DEN of HCC group recorded a significant alterations in all investigated tumor markers

when compared with their corresponding values in the control groups. In which the DEN-induced group showed a significant increase in the percentage of Carcinoembryonic antigen (CEA), Alpha-Fetoprotein (AFP), Alpha-L-fucosidase (FUC), Squamous cell carcinoma-Ag1 (SCCA1) and Gamma-G lutamyl transferase activity (GGT) when compared with their corresponding values in the control group. In addition to the levels of CEA, AFP, FUC, SCCA1and GGT levels revealed a significant decrease in groups treated with curcumin, vitamin c and combination between them, then with DEN when compared to group treated with DEN only.

HCC diagnosis is always discovered lately. In many patients, HCC is asymptomatic and is diagnosed in an advanced stage. This is why, surveillance is strongly recommended to detect early HCC to increase the chance for curative treatment and limit tumor-related death (El-Serag *et al.,* 2008) [32]. Early detection of HCC is the most critical step in the management process**.** A combination of both pathological features and biochemical markers with high sensitivity and specificity is still main objective in medical practice (Yao *et al.,* 2007) [33].

Alpha-Fetoprotein (AFP)

AFP was the most widely used tumor biomarker available for the early detection of HCC (Debruyne and Delanghe, 2008; Kumar *et al.,* 2016)[34, 35]. It has long been recognized that the exposure of rats to certain carcinogens like DEN caused an increase of the circulating AFP levels that associated with HCC (Song *et al,* 2013; Shahat *et al,* 2015)[36, 37]. The upregulation of the AFP gene expression in DEN-administered rats might be due to the necrosis of hepatocytes that regulate the AFP synthesis on the cellular level (Lazarevich, 2000 and Fathy *et al.,* 2017) [38,17] however, it is not secreted in all cases of HCC or secreted in other cases other than HCC cases as in chronic hepatitis, liver cirrhosis and other liver diseases or gastrointestinal cancer (Toro *et al.,* 2014; Liu *et al.,* 2015) [39, 40] in which about, two thirds of HCC patients with the nodule less than 4 cm have serum AFP levels less than 200 ng/mL and up to 20% HCC patients do not produce AFP (Zhang *et al.,* 2015)[41]. Moreover, it has limited utility of differentiating HCC from benign hepatic disorders for the high false-positive and false-negative rates in addition, AFP is negative in approximately 30-40% patients with early stage HCC. Even in advanced HCC, the level of AFP may be normal in 15-30% of patients (Sherman, 2011 and Abdel Hamid *et al.,* 2014) [42, 43].

Alpha-L-fucosidase (AFU)

Due to defects or disadvantages of AFP**,** That is why the need for other reliable serum markers for HCC has become at the highest priority which may be represented in serum Alpha-L-fucosidase (AFU) that is a lysosomal enzyme, its activity increased in the serum of HCC cases (Mossad *et al.,* 2014) [44]. AFU can reveal HCC earlier than ultrasonographic visualization (Pillai and Fimmel, 2012) [45]. AFU activity in the serum was found to be significantly increased in the HCC as shown in the present work (Chen *et al.,* 2012) [46]. The possible mechanism for increased AFU in HCC cases seemed to be due to the increased synthesis of proteins by the tumor with a consequently increased fucose turnover (Hamza *et al.,* 2015) [47]. AFU characterized by the values of serum concentration were not correlated with the tumor size and were frequent in early HCC cases in contrast to AFP levels in addition to AFU have high specificity and sensitivity than AFP and the Combination of AFP with AFU improve AFP sensitivity and specificity (El-Houseini *et al.,* 2005; Abdel Hamid *et al.,* 2014) [48, 43].

Squamous cell carcinoma antigen (SCCA)

Squamous cell carcinoma antigen SCCA a member of the high-molecular-weight family of serin protease inhibitors named Serpins (Sun *et al.,* 2017) [49] was recently described to be increased in primary HCC and liver cancer tissue and chronically damaged hepatocytes (Turato *et al.,* 2010 and Liu *et al.,* 2015) [50, 40]. SCCA is Physiologically expressed in the stratified squamous epithelium of tongue, tonsil, oesophagus, uterine cervix, vagina, thymus, skin and in the pseudo-stratified columnar epithelium of the conducting airways (Guarino *et al.,* 2017) [51]. There are two isoforms of SCCA were separated by isoelectric focusing into a neutral form SCCA1 and an acidic form SCCA2 in which normal hepatocytes do not express SCCA; however, the antigen levels increase with liver inflammation (Sun *et al.,* 2017)[49]. In addition, SCCA has been found more expressed in high-grade dysplastic nodules and HCC suggesting a role in hepatocarcinogenesis. Guido *et al*., (2008) [52] found that the expression of SCCA in HCC represented about 93% and100% in dysplastic nodule and increased in the primary stages of HCC formation. SCCA-1 isoform (SerpinB3) renders cells more resistant to apoptotic cell death (Guido *et al.,* 2008; Martini *et al.,* 2015)[52,53] and inhibits migration of NK cells, recent study in a mouse model of acute liver failure has demonstrated that the presence of Serpin B3 determines an enhanced inflammatory background (Villano *et al.,* 2012) [54].

γ-Glutamyltranspeptidase (GGT)

The serum GGT was considered as an important marker for diagnosis of HCC. GGT was the only known enzyme to break the y-glutamyl bond of glutathione and recycled the glutathione and the metabolism of glutathione xenobiotic conjugates (Rocchi *et al.,* 1997; Ma *et al.,* 2014)[55, 56]. The increased activity of GGT might lead to the accumulation of glycine and cysteine in addition to the enhancement of the peroxidative damage of the cell membranes (Khan and Sultana, 2005) [57]. As there was a close connection between GGT and carcinogenesis, the increased GGT could be correlated with a high HCC nodule incidence (Shaarawy *et al.,* 2009; Elsadek *et al.,* 2017) [58, 59]. It has been reported that HCC in both rats and humans expresses GGT enzymes with unique carbohydrate moieties compared with normal liver enzymes. GGT developed with cases of HCC (Carr and Guerra, 2013; Yang *et al.,* 2015) [60, 61].

In the present study, curcumin administration improved the parameters of tumor markers and this in agree with (Shukla and Arora 2003; El-Amir and Hassanein, 2013) [62, 63] who said that curcumin decreased the number and size of pre-neoplastic foci induced by NDEA and CCl4 and altered hepatic foci in rat liver. CUR treatment has an ameliorative effect on tumor markers this is in agree with (Arrieta *et al.,* 2007; Ahmed *et al.,* 2014) [46, 65] who reported that cur treatment has an ameliorative effect on tumor parameters in addition to, this explain the anti-inflammatory activity and anti-cancer of curcumin this is also in agree with (Mageid *et al.,* 2018) [66]. This effect can be produced by the ability of CUR to modulate a variety of molecules that have an

important role in cancer progression such as cytokines (Fujioka *et al.,* 2003; Kadasa *et al.,* 2015) [67, 68] and acting as anti-proliferative, and apoptotic for HCC (Vietri *et al.,* 2003)[69]. The mechanism of action of CUR is based on the secretion of various cytokines that function via the following pathway: Stimulation of antitumor immune responses, followed by induction of tumor cell apoptosis, inhibition of the uncontrolled proliferation of cancer cells and suppression of angiogenesis (Yoysungnoen *et al.,* 2006) [70]. This is in agree with (El-Amir and Hassanein, 2013; Qiu *et al.,*2017) [63, 71] which demonstrated that curcumin relieved DEN-induced hepatocarcinogenesis, drawn back parts of abnormal anti-oxidant enzymes, routine liver function and lipid index, and tumor markers levels to near routine levels and weakened metabolic disorders.

It is known that vitamin C is a water soluble antioxidant, has been reported to ameliorate the free radical induced damage, and it can work both inside and outside the cells to fight free radical damage. (Costa *et al.,* 2016; Jaiswal *et al.,* 2017) [72, 73]. From the other hand, deficiency of ascorbic acid effect on hemostasis, scurvy and changes of vessels structure, and act as anticancer agent and decreasing the risk of diabetes mellitus (Aguirre and May, 2008 ; Son *et al.,* 2018 and Sugiura and Sugiura, 2018) [74, 75, 76].

Bastway *et al.,* (2010) [77] demonstrates that carcinogenic substances that induced liver damages in rats can be ameliorated by administration of extraction of date flesh and ascorbic acid.

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