Molecular and biochemical study on the potential therapeutic effect of novel composite on the hepatocellular carcinoma-induced rats

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A B S T R A C T

The hepatoprotective effect of basic curcumin and bis (tetrachlorocuprate -lysine) combined with ascorbate against ferric nitrilotriacetate (Fe-NTA) and chloroform induced hepatic carcinogenesis in rats was evaluated. One hundred rats were divided into five equal groups. Normal control group, carcinogenic (Fe-NTA: 9 mg Fe/kg b.wt. i.p.) and chloroform (150 mg/ kg b.wt. orally)-induced group, curcumin group (400 mg/kg. b.wt. orally), tetrachlorocuprate-lysine (25 mg/kg. b. wt. s.c.) and ascorbate (500 mg/kg. b. wt. orally) group and a mixture group (composed of curcumin, tetrachlorocuprate -lysine and ascorbate). Blood samples and liver tissue specimens were collected at the end of experiment (4 months) for determination of the following parameters: activities of serum malondialdehyde (MDA), reduced glutathione (GSH), tissue catalase, myeloperoxidase (MPO), Tumor necrosis factor-α (TNF- α), interleukin-1, nuclear factor kappaB65 (NFkB65) and caspase-3. The obtained results showed that injection of carcinogen caused a significant elevation in the MDA, MPO and immunological markers levels, and significant reduction in the reduced glutathione, and catalase compared to the control group. Compared to carcinogenic group, rats administered with the treated compounds resulted in a significant reduction of MDA, MPO and immunological markers levels, and increased in reduced glutathione and catalase activity. These results concluded that basic curcumin, tetrachlorocuprate-lysine and ascorbate exert chemopreventative effect against hepatocellular carcinoma.

Keywords: Ferric nitrilotriacetate, curcumin, hepatoprotective, antioxidant, carcinogen.

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1. INTRODUCTION

Hepatocellular carcinoma (HCC), also called malignant hepatoma, is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of liver cirrhosis) (Kumar et al., 2003). Ferric nitrilotriacetate (Fe - NTA) is a strong oxidant, which generates highly reactive hydroxyl radical and causes injuries of various organs including the kidney and liver. The formation of 8- hydroxy - 2' - deoxyguanosine (8- OHdG) adducts in the renal DNA is one of the earliest events after treatment with Fe – NTA (Shuyi et al., 2001). Fe-NTA also lead to increase in levels of some inflammatory markers viz NO and MPO and some proinflammatory cytokines viz PGE-2 and TNF-1. Fe-NTA induce renal carcinogenesis and inflammatory response in Wister rat (Muneeb et al., 2012). Chloroform is an organic compound with formula CHCl3. It is one of the four chloromethane (Rossberg et al., 2006). High doses of chloroform induced liver cancer in male and female mice when administered by gavage, chloroform is acting through a nongenotoxic-cytotoxic mode of action (Larson et al., 1996). Curcumin, the principal polyphenolic curcuminoid derived

from the rhizome Curcuma longa, is present in an Indian spice, turmeric. Curcumin possesses antitumor, antioxidant, and anti-inflammatory properties, and has been studied as a cancer chemopreventive agent. Curcumin is extensively studied, evaluated and accepted for its wide range of medicinal properties. The therapeutic activities of curcumin for a wide variety of diseases such as diabetes, allergies, arthritis and other chronic and inflammatory diseases have been known for a long time (Sharmila & Rakesh., 2012). Tetrachlorocuprate, Copper is an essential trace element (i.e., micronutrient) that is required for plant, animal, and human health (Scheiber et al., 2013). Copper complexes are potentially attractive as anticancer agents. Actually, since many years a lot of researches have actively investigated copper compounds based on the assumption proposal that endogenous metals may be less toxic (Marzano et al., 2009). Ascorbic acid is one form of vitamin C (Lachapelle and Drouin., 2010). Vitamin C, at normal physiological concentrations (0.1 mM), is a major water-soluble antioxidant (Geeraert, 2012). tumor angiogenesis is the process of new blood vessel growth toward and into a tumor. It is considered to be critical in tumor growth and metastasis. Reports in the literature suggest that ascorbate's effect on collagen synthesis can act to inhibit formation of new vascular tubules (Ashino, et al., 2003), that ascorbate can inhibit genes necessary for angiogenesis (Belin, et al., 2009). This study was aimed to evaluate the potential therapeutic effect of basic curcumin, tetrachlorocuprate-lysine and ascorbate on hepatocellular carcinogenesis in male rats.

2. MATERIAL AND METHODS

2.1. Chemical and Reagents

NTA was obtained from Thermo Fisher Scientific Inc- England and Ferric Nitrate from El-Nasr chemical company- Egypt.
Preparation of Fe-NTA solution: Fe-NTA is prepared fresh immediately before its use, to prepare Fe-NTA, ferric nitrate solution is mixed with fourfold molar excess of disodium salt of NTA and the pH is adjusted to 7.4 with sodium bicarbonate solution (Awai et al., 1979). Chloroform was obtained from Thermo Fisher Scientific Inc- England. Ascorbic Acid was obtained from TS laboratory company- Egypt. Curcumin was obtained from Loba Chemie Company – India and mixed with Sodium bicarbonate at a concentration of (1:4) to form basic curcumin. Tetrachloro cupprate and Lysine were obtained from Sigma Aldrich- USA. Preparation of Bis(L-Lysine-Tetrachlorocuprate): L-Lysine HCl and CuCl2 in a 2:1 molar ratio were mixed together and grinded in an agate mortar for about 30 minutes at room temperature to get the best homogeneity.The mixure was gradualy turned to light green indicating the formation of Tetrachlorocuprate complex (Adams et al., 2010).

2.2. Animals and Grouping

This work was carried out on 100 male albino rats, weighting 40-60gm., purchased from the animal house colony of the National Cancer Institute (NCI), Cairo University, Egypt. Animals were housed under normal environmental conditions of standard temperature, humidity and diurnal environment of light and dark and fed a standard diet, which composed of (24% proteins, 5.55% fibers, 5.5% ash) and drink tap water ad libitum. Rats were divided into five main equal groups, 20 rats each and classified as follows: Group (1): Rats were fed standard food and drinking tap water ad-libitum for 4 months and served as control group. Group (2): Rats were given intraperitoneal injection of Fe-NTA (9 mg Fe/kg body weight) (Summya et al., 2013) and chloroform (150 mg/ kg b.wt.) dissolved in corn oil, orally (Afrah et al., 2014) for 4 months. Group (3): Rats were administered orally with basic curcumin (400 mg/kg) (Yumei et al., 2008) half an hour prior to Fe-NTA and chloroform administration and continued till the end of experiment (4 months). Group

(4): Rats were received Bis (tetraclorocuprate- Lysine) dissolved in ethyloacetate (25 mg/kg. b. wt. s.c.) (Frechilla et al., 1990) and ascorbate (500 mg/kg. b. wt.) (Adejuwon and bJoseph., 2008) orally and daily half an hour prior to the administration of Fe-NTA and Chloroform till the end of 4 months. Group (5): Rats treated daily with Bis (L- Lysine) tetrachlorocuprate combined with ascorbate and basic curcumin half an hour prior to the administration of Fe-NTA and chloroform till the end of 4 months.

2.3. Sampling

At the end of the experiment (4 months), all animals were sacrificed, blood samples were collected and serum was separated by centrifugation at 2500 r.p.m for 15 minutes, the clean clear serum was separated by Pasteur pipette and kept in a deep freeze at -20 ºc until used for biochemical analysis. Also, livers tissue specimens of the experimental animals groups will be quickly removed**,** perfuse immediately with ice-cold saline (0.9%w/u) and homogenized (Glas-Col, Terr Hauter, USA) in chilled phosphate buffer (0.1 M, pH 7.4) containing Kcl (1.17%w/u). The homogenate was centrifuged at 3000 rpm for 10 min at 4ºc and clear supernatants were used for biochemical analysis.

2.4. Biochemical analysis:

Determination of MPO, MDA, GSH and CAT were analyzed according to the methods described by (Joris et al., 2006), (Ohkawa et al., 1979), (Beutler et al., 1963) and (Aebi , 1984 & Fossati et al ., 1980) respectively. Liver TNF-α and IL-1 were determined using ELISA kit according to the methods described by (Chen et al., 1998) and (Liu et al., 1995) respectively. Expression of liver Nuclear Factor K-B and Caspase-3 genes were evaluated by Quantitative Real Time PCR using Moloney murine leukemia virus (Pfaffl., 2001) and (Beyaert and Fiers,1998) respectively.

2.5. Statistical Analysis

All data were expressed as the mean ± SD. Statistical comparison between different groups were done by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison (Graphpad Prism version 5.03, San Diego, CA, USA). $P \leq 0.05$ was considered to be statistically significant.

3. RESULTS

3.1. Effect of basic curcumin, tetra-chlorocuprate-lysine and ascorbic acid on the GSH, MDA, catalase and MPO activities:

Reduced glutathione level of the carcinogen-treated showed a significant decrease compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). However the mean levels of the GSH in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) showed a significant increase compared to the mean levels of the carcinogen-treated group, table (1).

The obtained data in table (1) revealed that, the mean levels of the MDA of the carcinogen-treated group showed a significant increase compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the MDA in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) showed a significant decrease compared to the mean levels of the carcinogen-treated group. The obtained data in table (1) showed that, the mean levels of the catalase of the carcinogen-treated group indicated a significant reduction compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the catalase in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) which

resulted in a significant reduction compared to the mean levels of the carcinogen-treated group. The obtained data in table (1) showed that, the mean levels of the MPO of the carcinogen-treated group indicated a significant elevation compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the MPO in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) which resulted in a significant reduction compared to the mean levels of the carcinogen-treated group.

3.2. Effect of the different compounds on tissue Interleukin-1 and TNF- α activity:

The obtained data in table (2) revealed that, the mean levels of the Interleukin-1 of the carcinogen-treated group showed a significant increase compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the Interleukin-1 in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) showed a significant decrease compared to the mean levels of the carcinogen-treated group. The mean
levels of the TNF- α of the carcinogen-treated group showed a significant increase compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the TNF-α in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) showed a significant decrease compared to the mean levels of the carcinogen-treated group, table (2).

3.3. Effect of the different compounds on tissue *nuclear factor kappa-B65 (NFkB65)* and *caspase-3 activity:*

The obtained data in table (3) showed that, the mean levels of the NFkB65 of the carcinogen-treated group showed a significant increase compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the NFkB65 in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) which showed a significant decrease compared to the mean levels of the carcinogen-treated group. The mean levels of the Casp-3 of the carcinogen-treated group showed a significant increase compared to the mean levels of the control group at the same period of treatment ($p \leq 0.05$). Whereas the mean levels of the Casp-3 in curcumin-treated group, tetrachlorocuprate-lysine+ascorbic acid-treated group and in the mixture-treated group at the same period of treatment ($p \leq 0.05$) which showed a significant decrease compared to the mean levels of the carcinogen-treated group, table (3).

4. DISCUSSION

Lipid peroxidation plays an important role in carcinogenesis (Banakar et al., 2004), is the most studied biologically free radical chain reaction. Lipid peroxidation may lead to the formation of several toxic byproducts such as malondialdehyde (MDE) and 4-hydroxynonenal which can attack cellular targets including DNA and lead to mutagenecity. An excellent model of in vivo free radical induced damage, associated with extensive lipid peroxidation, is the ferricnitrilotriacetate (Fe-NTA) model, administration of Fe-NTA leads to increasing oxidative stress, that starts from the plasma compartment, where Fe-NTA finds the ideal environment to react with oxidizable lipids (Deiana et al., 2007). This may explain the elevated levels of MDA in rats administrated with Fe-NTA. The presence of curcumin significantly decreased lipid peroxidation (Sankar et al., 2012). Similarly, the results of this work demonstrated that, feeding rats with basic

Table.1) Effect of basic curcumin, tetrachlorocuprate-lysine and ascorbic acid on the serum GSH, MAD and tissue CAT and MPO activities in hepatocarcinogenesis induced rats:

Parameter / Groups	GSH (µmol/ L)	MDA (µmol/ L)	CAT (U/g)	MPO (U/g)
Control	29.37± 0.99[a]	2.35 ± 0.19 [a]	122.5±1.76 [a]	0.22± 0.01 [a]
Carcinogen	14.67±1.11	6.22±0.58	70.53 ± 3.119	1.79±0.07
Curcumin+ Carcinogen	24.13±0.7[b]	3.79±0.14 [b]	96.35± 1.8 [b]	0.84±0.06 [bc]
Tetraclorocuprate -lysine+Ascorbic+ Carcinogen	27.88±0.61 [b]	3.46±0.12 [b]	101.0 ± 4.14 [b]	0.82±0.05 [bc]
Mixture+ Carcinogen	29.50±0.60 [b]	3.12±0.02 [b]	111.2± 1.88 [b]	0.37±0.04 [b]

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P < 0.05$).

Table.2) Effect of basic curcumin, tetrachlorocuprate-lysine and ascorbic acid on the tissue TNF- α and IL-1 activities in hepatocarcinogenesis induced rats:

Parameter / Groups	IL-1 (pg/ml)	TNF- α (pg/ml)
Control	30.89± 1.011[a]	33.04±0.97[a]
Carcinogen	113.5±1.21	117.6 ± 2.42
Curcumin+ Carcinogen	86.50±1.97[bc]	69.95 ± 2.29[bc]
Tetraclorocuprate -lysine+Ascorbic+ Carcinogen	77.10±1.41[bc]	69.07 ± 1.18[bc]
Mixture + Carcinogen	61.52±3.93[b]	47.40 ±1.71[b]

Table 3: Effect of basic curcumin, tetrachlorocuprate-lysine and ascorbic acid on the tissue NF-kB65 and IL-1 activities in hepatocarcinogenesis induced rats

Parameter / Groups	NF-kB-65	Caspase-3
Control	1.19±0.042 [a]	1.058±0.02[a]
Carcinogen	9.87± 0.65	8.61±0.69
Curcumin+ Carcinogen	7.64± 0.34[c]	6.90±0.18[c]
Tetraclorocuprate -lysine+Ascorbic+ Carcinogen	6.62± 0.26[bc]	6.09±0.37 [bc]
Mixture+ Carcinogen	5.35± 0.28 [bc]	3.32±0.24 [bc]

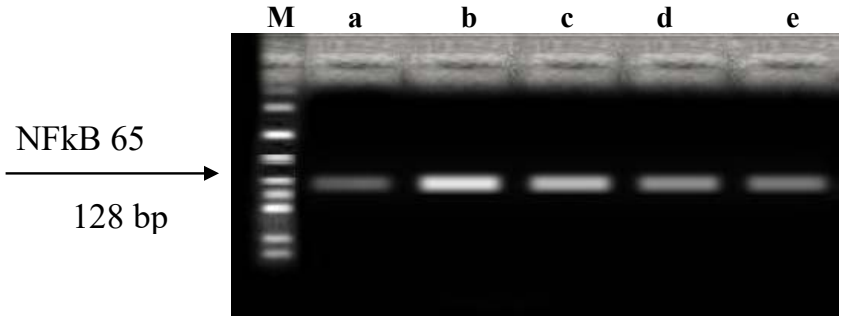

Figure 1: An agarose gel electrophoresis shows PCR products of gene expression of NFkB65 in different studied groups, M: DNA marker lanes (1352-7 base pairs), Lane a: Control band, lane b: Carcinogen band, lane C: Curcumin band, lane d: Ascorbate- tetrachlorocuprate lysine band and lane e: curcumin-Ascorbate and Tetrachlorocuprate lysine band.

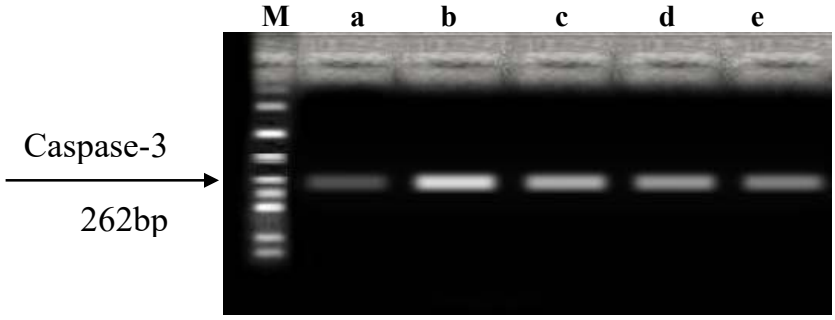

Figure.2: An agarose gel electrophoresis show PCR products of gene expression of Caspase-3 in different studied groups, M: DNA marker lanes (1352-7 base pairs), Lane a: Control band, lane b: Carcinogen band, lane C: Curcumin band, lane d: Ascorbate- tetrachlorocuprate lysine band and lane e: curcumin- Ascorbate and Tetrachlorocuprate lysine band.

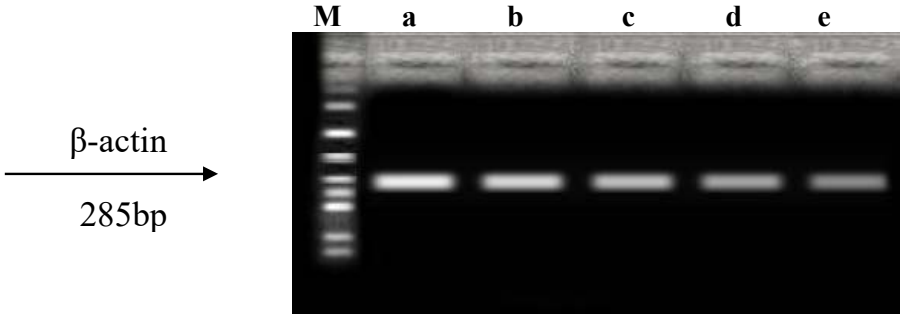

Figure.3: An agarose gel electrophoresis show PCR products of gene expression of β- actin as an endogenous control in different studied groups, M: DNA marker lanes (1352-7 base pairs), Lane a : Control band , lane b: Carcinogen band, lane C: Curcumin band, lane d: Ascorbate- tetrachlorocuprate lysine band and lane e: curcumin- Ascorbate and Tetrachlorocuprate lysine band.

curcumin decreased the lipid peroxidation level compared to the carcinogen group. lipid peroxidation was decreased in the presence of ascorbic acid. This result was in agreement with Elias and Oputiri., (2013) who showed that Vitamin C (ascorbic acid) which is a major water-soluble antioxidant is believed to decrease lipid peroxidation either directly or indirectly by regenerating vitamin E. Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. Malondialdehyde (MDA) concentrations were decreased in plasma and liver tissues, by Cu administration (Jianbo et al., 2011),

this explain the decreased levels of MDA in rats administrated with tetrachlorocuprate. Glutathione (GSH) plays an important role in a multitude of cellular processes, including cell differentiation, proliferation, and apoptosis, and disturbances in GSH homeostasis are involved in the etiology and progression of many human diseases including cancer (Nicola et al., 2013). Compared with the control, GSH concentrations in treated rats significantly decreased after exposure to Fe-NTA in agreement with the reports of (Athar & Iqbal., 1998) who showed the decreased levels of GSH in rats administrated with Fe-NTA. The present study showed that administration of basic curcumin increased the GSH levels compared to the carcinogen non treated group. Similarly, Chiagoziem et al., (2014)
demonstrated that administration of curcumin improved the GSH. It was clear from the present study that a significant increase in serum GSH levels was induced after administration of tetrachlorocuprate compared with that received Fe-NTA and Chloroform. This improvement in the glutathione levels may be referred to supplementation with copper increase GSH levels (Derouiche et al., 2013) and presence of ascorbic acid which is important in the production of glutathione (Duke and Atchley, 1984). Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). In the present study, a decrease in liver catalase activity was observed in rats injected with Fe-NTA compared to control group. These results were in agreement with Athar and Iqbal (1998) who showed that the catalase activity was significantly decreased. In our study, the mean values of catalase activity in rats administrated with basic curcumin showed a significant elevation as compared to carcinogen non treated group in agreement with the reports of Prabhakar et al., (2007). It was clear from the present study that a significant increase in serum catalase activity was induced after administration of ascorbic acid which combined with tetrachlorocuprate and in agreement with the reports of Garg and mahajan., (1993). Myeloperoxidase (MPO) is a peroxidase enzyme, most abundantly expressed in neutrophil granulocytes and produces hypohalous acids to carry out their antimicrobial activity (Klebanoff, 2005). It was clear from
the present study that a significant increase in liver myeloperoxidase activity was induced after administration of Fe-NTA was agreement with the reports of Muneeb et al., (2012). The present study also showed that, administration of basic curcumin significantly decreased the MPO levels compared to the carcinogen group. Also, Zhe et al., (2014) demonstrated that, treatment with curcumin significantly reduced the expression of MPO. It was shown that administration of ascorbic acid caused a decrease in MPO activity (Murat et al., 2008).

Interleukins are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by leukocytes (Brocker et al., 2010). Interleukin-1 alpha and interleukin-1 beta (IL-1α and IL-1ß) are cytokines that participate in the regulation of immune responses, inflammatory reactions, and hematopoiesis (Barthelmes et al., 2011). It is obvious from the present study that Interleukin-1 activity in rats administrated with Fe-NTA and Chloroform was significantly elevated compared with its corresponding values in normal group and in agreement with the reports of Ahmad et al., (2005). The results of this work demonstrated that, feeding rats with basic curcumin decreased the Interleukin-1 activity compared to the carcinogen group. Moreover, Gaddipati et al., (2003) reported that, oral administration of curcumin resulted in significant restoration of the cytokines included interleukin-1 to depleted levels. It was clear from the present study

that, a significant decrease in liver interleukin-1 levels was observed after administration of ascorbic acid when compared to carcinogen group. The obtained results were nearly similar to Tomofuji et al., (2009) who demonstrated that, gene expression encoding inflammation, including interleukin-1 alpha and interleukin-1 beta, was more than two fold down-regulated by vitamin C intake.

Tumor necrosis factor (TNF) is a multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death. As a pro-inflammatory cytokine, TNF is secreted by inflammatory cells, which may be involved in inflammation-associated carcinogenesis. Recent studies have focused on sensitizing cancer cells to TNF-induced apoptosis through inhibiting survival signals such as NF-κB, by combined therapy (Xia & Yong., 2008). In addition to apoptosis, TNF can also induce necrotic cell death. Reactive oxygen species (ROS) play a critical role in mediating necrotic cell death because ROS scavenger butylated hydroxyanisole (BHA) can effectively block this pathway (Lin et al., 2004). In the present work, significant decrease in liver TNF-α levels was seen in rats administrated with ascorbic acid compared with carcinogen group in agreement with the reports of Masoumeh et al., (2015) who revealed that ascorbic acid administration could reduce the levels of TNF-α. Also, Senturk et al., (2004) demonstrated that ascorbic acid administration significantly decreased the concentrations of serum tumor necrosis factor- α. It was clear from the present study that a significant decrease in liver TNF-α levels was induced by administration of cupper-lysine in agreement with Jianbo et al., (2008), who demonstrated that, the reduction in backfat depth may be due to copper from Cu-lysine altering TNF-α metabolism in lambs.

Nuclear factor-kB (NF-kB) is a transcriptional regulator of genes involved in immunity, inflammatory response, cell fate, and function. Recent attention has focused on the pathophysiological role of NF-kB in the diseased liver (Elsharkawy & Derek., 2007). Studies in experimental models have shown that liver injury is associated with activation of NF-kB with the response being significantly influenced by sex (increased in females), age (reduced in older animals), and fat content (increased in mice fed a high-fat diet) of the liver. Studies on hepatic NF-kB in diseased human liver are rare. However, at least one report has revealed remarkably elevated hepatic NF-kB activity in alcoholic liver disease, which correlates with the degree of inflammation and fibrosis (Ribeiro et al., 2004). Liver NF-kB in this work showed significant elevation in rats administrated with Fe-NTA compared to their respective mean values in the control group in agreement with the reports of Firoz et al., (2015). In the present work, basic curcumin had a pronounced protective effect against liver carcinoma induced by Fe-NTA and chloroform. These results indicated that the levels of NF-kB were significantly reduced in rats administrated with basic curcumin and were confirmed by Simone et al., (2009) who demonstrated that curcumin has been shown to inhibit nuclear factor κB (NF-κB) activation at several steps in the NF-κB signaling pathways and thereby controls numerous NF-κB-regulated genes involved in various diseases. It was clear from the present study that a significant increase in liver NF-kB levels were induced by administration of ascorbic acid in agreement with the reports of Prathibha et al., (2013), presence of copper in comination with improved the liver NF-kB levels in disagreement with the reports of Persichini et al., (2006) who demonstrated that copper activates the transcription factor NF-kappaB n the liver and lung tissues of rats, and that this effect is mediated by oxidative stress.

Caspase-3 is a downstream effector cysteine protease in the apoptotic pathway. It is ubiquitously expressed in normal human tissues including the liver.

Overexpression and loss of expression of caspase-3 has been reported in diverse human malignancies (Persad et al., 2004). It was obvious from the present study that administration of Fe-NTA and Chloroform caused a significant increase in the liver casp-3 levels compared to their mean value in the control group. These results were consistent with previous studies of Sakurai and Cederbaum., (1998) who suggested that lipid peroxidation in the presence of iron, and the ensuing prooxidative state damages mitochondria, releasing factors that activate caspase 3, leading to a loss in cell viability and DNA fragmentation. In the present work, significant improvement in liver capase-3 levels were seen in rats treated with basic curcumin compared with carcinogen group in agreement with the reports of Chin-Cheng et al., (2006) who demonstrated that curcumin can induce apoptosis in cancer cells and that these pathways depend on mitochondria or caspase-3 activation. Comination of ascorbic acid with lysine resulted in significant improvement in mean value of liver capase-3 levels in rats compared with carcinogen group in agreement with the reports of Hsieh et al., (2010) who suggested that, combined arginine and ascorbic acid treatment induces apoptosis; caspase-9 and caspase-3 were activated. Arnal et al., (2014) reported that, when Cu was administered, a significant increase in the activities caspase-3 also was observed.

5. CONCLUSION:

Basic curcumin has potent chemo-preventative activity against a wide variety of tumors and has great potential in the prevention and treatment of cancer, in addition, Tetrachlorocuprate- lysine in combination with ascorbic acid exert chemopreventative activity against hepatocellular-carcinoma, which have antioxidant and free radicals scavenging activity and trapping of activated metabolites of carcinogen.

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