Protective Effect of Curcumin on Hepatocarcinoma induced byN-Nitrosodiethylamine in Male Rats

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Abstract: Hepatocellular carcinoma (HCC) represents a major worldwide health concern, ranking among the top five most prevalent malignancies**.** Curcumin (Cur)is known to exert an antioxidant and anti-inflammatory effect The present study was designed to investigate the therapeutic effect of Cur on NDEA induced HCC in male rats. Forty adult male Sprague Dawley rats (180-190 g) randomly distributed into 5 groups (8 each). Group 1 control negative group, while the other groups were intoxicated by a single intraperitoneal dose (100 mg/kg) of NDEA injected to rats in the 3rd week, followed by weekly subcutaneous injections of CCl4 from the 4th week till the end of the experiment (6 weeks) for induction of HCC, group 2 (positive) and groups 3, 4 and 5 were orally given Cur in a dose of 20, 40 or 60 mg/kg/day, respectively for 6 weeks. The results revealed that NDEA induced liver damage as evidence by significantly increases in serum indices of liver function enzymes, increase serum levels of alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa beta (NF-κβ), as well as induced significantly high content of hepatic MDA, and low hepatic content of GSH, GPx, SOD and CAT when compared with the normal control group. On the other hand, Cur displayed improvement in a dose dependent manner in all treated parameters. The histopathological examination of the liver of rats received NDEA showed trabecular hepatocellular carcinoma with fat droplets in tumor cells, while liver sections of rats treated with Cur displayed amelioration of hepatocellular architecture in a dose dependent. Given these promising findings, the present study suggests that Cur, which is a potentially safe and inexpensive for clinical use, may be considered as an effective chemopreventive agent against HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer mortality worldwide **(Parkin *et al*., 2005)**. Its occurrence has been increasing steadily over the past decades **(Subbaraj *et al*., 2013)**. The majority of HCC cases are attributable to underlying infections caused by hepatitis B and C viruses **(Schütte *et al*., 2009)**. However, several other risk factors, including obesity, iron overload, environmental pollutants, alcohol consumption, as well as several dietary carcinogens, such as aflatoxins and nitrosamines, have been shown to be involved in its etiology **(Paraskevi and Ronald, 2006)**.

Diethylnitrosamine (DEN) is a well knownhepatocarcinogenic agent present in tobacco smoke, ground water, cured and fried meals, cheddar cheese, alcoholic beverages, agriculture chemicals and pharmaceutical products **(Sivaramkrishnan *et al*., 2008 and Gupta *et al*., 2010)**. N-Nitrosodiethylamine (NDEA) is an N-nitroso alkyl compound depicts as an effective hepatotoxin to experimental animals, producing toxicity after repeated administration. It became metabolically active by the action of cytochrome p450 enzymes to produce reactive electrophiles, which increase oxidative stress level leading to cytotoxicity, mutagenecity and carcinogenicity. Oxidative stress is considered as critical mechanism contributing to NDEA induced hepatotoxicity **(Waris and Ahsan, 2006).** Most drugs currently available for the treatment of cancer have limited potential because they are very toxic, highly inefficient in treating cancer, or highly expensive and thus beyond the reach of the majority, treatments without these disadvantages are needed **(Yates and Kensler, 2007).**

In recent years, natural agents have considerable attention in scientific community for their therapeutic efficacy against various ailments such as cancer prevention and therapy **(Subbaraj *et al*., 2013)**. Curcumin (Cur) is one such agent; a polyphenol derived from turmeric (*Curcumin longa*) **(Anand *et al*., 2007)**. It has long been used as a food, coloring agent and as a healing agent for variety of illnesses. In recent years, a number of studies across the globe have investigated the various biological effects of curcumin **(Aggarwal *et al*., 2007).** Clinical trials investigating curcumin have concluded that, it may be useful in preventing heart failure and effective against a range of diseases, including cancer **(Morimoto *et al*., 2010 and Kanai *et al*., 2012**). In addition to its reported cytoprotective effect, curcumin is known to exert an antioxidant effect by removing free radicals and an anti-inflammatory effect by inhibiting the activation of NF-κB **(Kanai *et al*., 2012)**. Moreover, **Shishodia *et al*. (2007)** and**Strimpakos and Sharma**

(2008) reported that Cu has been shown to suppress transformation, proliferation, and metastasis of tumors. Therefore, the purpose of this study was designed to investigate the therapeutic effect of Cur on NDEA induced HCC in male rats.

2. Material and Methods
Curcumin
Curcumin was purchased from Sigma Chemical Co (St. Louis, MO, USA).Cur was suspended in 0.5% carboxymethyl cellulose solution.

Chemicals and kits
N-nitrosodiethylamine (Synonym: Diethyl-nitrosamine, Product number: N0258, molecular formula: C4H10N2O, NDEA) is a yellow liquid dispensed in 1 ml ampoules. It was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Carbon tetrachloride (CCl4), Serum aspartate aminotransferase, alanine amino-transferase, alkaline phosphatase, antioxidant enzymes glutathione peroxidase, superoxide dismutase, catalase and MDA kit were purchased from Sigma-Aldrich Company. Kits for the determination of alpha fetoprotein (AFP), tumor necrosis factor alpha (TNF-α) and nuclear factor-kappa β (NF-κ β) were purchased from Glory Science Co., Taiwan.

Rats
Forty adult male Sprague Dawley rats weighing 180-190 g body weight and 10-11 weeks old were used in this study. Animals were obtained from Faculty of Pharmacy, King Abdul-Aziz University, Jeddah, Saudi Arabia. Rats were housed in a well ventilated laboratory room under standard conditions of 24°C temperature, 50-52% relative humidity and 12 hr light/12 hr dark cycles. Basal experiment was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC).

Feeding of rats
All rats will be fed on commercial rat pellets obtained from Grain Silos and Flour Mills Organization, Jeddah, KSA. These pellets are consisted of 20% crude protein, 4% fat, 3.5% crude fibers, 6% ash, 1% calcium, 0.6% phosphorus, 0.5% other salts, 20 IU/g vitamin A, 2.2 IU/g vitamin D and 70 IU/g vitamin E. These constituents will be thoroughly mixed and commercially manufactured in the form of pellets.

Induction of hepatocarcinoma
The preneoplastic lesions of hepatocarcinoma were induced by a single intraperitoneal injection of N-nitrosodiethylamine (NDEA) in a dose of 100 mg/kg b.wt dissolved in dimethyl sulfoxide (DMSO) in the 3rd week of experiment period. This was followed by weekly subcutaneous injections (3 injections/week) of CCl4 diluted with liquid paraffin (1:1, V: V) at 2ml/kg b.wt during the 4th week till the 6th week of the experiment period as described by Sundraresanand Subramanian (2003).

Experiment design
The experiment was performed on forty adult Sprague Dawleyrats randomly distributed into 5 groups, of 8 animals each. Group 1 was kept as a normal (negative) control group, received a single intraperitoneal (i.p.) injection of normal saline (2.5ml/kg), while the other 4 groups were intoxicated by a single intraperitoneal dose (100 mg/kg) of NDEA injected to rats in the 3rd week, followed by weekly subcutaneous injections (3injections/week) of CCl4 from the 4th week till the end of the experiment (6 weeks) for induction of hepatocarcinoma. Group 2 was kept intoxicated (positive) control group and groups 3, 4 and 5 were fed on commercial rat pellets and orally givencurcumin in a dose of 20,40 and 60 mg/kg/day, respectively for 6 weeks. At the end of the experiment, the rats were euthanized by prolonged exposure to ether and blood samples were withdrawn for separating the serum by centrifugation at 8000 rpm for 15 min. Serum samples were kept frozen at -70 Ċ till biochemical analyses. Rats were sacrificed and a part of the liver was kept frozen until used for preparing tissue homogenates for biochemical analyses. The other part was preserved in 10% formalin solution till processed for the histopathological examination of liver.

Biochemical analyses
Serum aspartate aminotransferase, alanine aminotransferase (Bergmeyer *et al*., 1978) and alkaline phosphatase (Roy, 1970) were chemically determined using specific kits. Serum alpha fetoprotein (as a traditional tumor marker) levels was estimated as described by Gibbs *et al*. (1987). Serum levels of tumor necrosis factor- alpha (as a proinflammatory cytokine) were quantified as described by Pennica *et al*. (1985) and nuclear factor-kappa beta(as a transcription factor) level was quantified as described by Adams (2009) using ELISA kits (Glory Science Company, Taiwan.) according to the manufacturer's instructions.

Estimation of Liver antioxidant enzymes
One gram of frozen liver tissue was thawed, washed with ice-cooled 0.9% NaCl solution and homogenized in 100 ml of ice-cooled 1.5% potassium chloride solution and 50 mmol potassium phosphate buffer solutions (pH 7.4) to yield 1% homogenate (W/V). Liver homogenates were centrifuged at 9000 rpm for 15 min at 4°C. The supernatants were used for estimation of lipid peroxidation (LPO) as described by Ohkawa *et al.,* (1979). The technique is based on the reaction of thiobarbituric acid with malondialdehyde (MDA) in acidic media at 95°C for 45 min to form thiobarbituric acid reactive substance (TBARS) and

expressed as MDA content. Reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by Bulaj *et al*. (1998). Activities of antioxidant enzymes glutathione peroxidase (Paglia and Valentaine, 1979);

superoxide dismutase (Spitz and Oberley, 1989) and catalase (Sinha, 1972) were colorimetrically determined. The tissue levels of MDA, GSH, GPx, SOD and CAT were chemically assayed using commercial assay kits.

Table (1): Effect of oral administration of curcumin (Cur) on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rats intoxicated with N-nitrosodiethylamine. (n = 8 rats)

Parameters groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group (1) -ve Control	80.2 ± 5.16[d]	93.7 ± 2.21[d]	97.5 ± 5.15[d]
Group (2) + ve Control	145.6 ± 9.33[a]	167.3 ± 8.54[a]	171.4 ± 9.17[a]
Group (3) Cur 20 mg/kg	125.4 ± 5.13[b]	136.23 ± 6.43[b]	153.0 ± 6.56[b]
Group (4) Cur 40 mg/kg	104.9 ± 6.62[c]	115.0 ± 7.61[c]	128.0 ± 8.11[c]
Group (5) Cur 60 mg/kg	85.4 ± 4.42[d]	98.2 ± 5.44[d]	105.0 ± 7.56[d]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant
at P < 0.05 or P < 0.01 using one way ANOVA test.

Table (2): Effect of oral administration of curcumin (Cur) on serum levels of alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF-α) and nuclearfactor-kappa beta (NF-κβ) in rats intoxicated with nitrosodiethylamine. (n=8 rats)

Parameters Groups	AFP (ng/ml)	TNF-α (ng/ ml)	NF-κβ (ng/ml)
Group (1) -ve Control	4.23 ± 0.05[d]	3.85 ± 0.44[d]	134.0 ± 2.54[d]
Group (2) + ve Control	9.34 ± 0.12[a]	6.85 ± 0.07[a]	193.5 ± 4.34[a]
Group (3) Cur 20 mg/kg	7.12 ± 0.04[b]	5.65 ± 0.01[b]	178.4± 3.67[bc]
Group (4) Cur 40 mg/kg	5.87 ± 0.04[c]	4.47 ± 0.02[c]	134.6 ± 6.42[d]
Group (5) Cur 60 mg/kg	4.78 ± 0.22[d]	3.76 ± 0.02[d]	129.89 ± 8.76[d]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant
at P < 0.05 or P < 0.01 using one way ANOVA test.

Histological procedure

The other part of livers of the sacrificed rats was preserved in 10 % neutral formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. Tissue specimens were then cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H&E stain) and examined under the microscope (Carleton, 1976).

Statistical analysis

Results were expressed as a (mean ±SD). Data were analyzed statistically by analysis of variance, for

statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to Snedecor and Cochron (1989). SPSS version 20 was used for these calculations.

3. Results

Single intraperitoneal injection of NDEA by a dose 100mg/kg b.wt in rats markedly elevated serum levels of liver enzymes aspartate aminotransferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) when compared with normal control rats, indicating incidence of liver damage. Oral administration of curcumin in doses of 20, 40 and 60

mg/kg/day significantly lowered the elevated serum AST, ALT and ALP levels when compared to NDEA group as recorded in Table (1).

Table (3): Effect of oral administration of curcumin (Cur) onhepaticmalondialdehyde (MDA) and reduced glutathione (GSH) contents in ratsintoxicated with N-nitrosodiethylamine. (n= 8 rats)

Parameters Groups	MDA (μmol/gm protein)	GSH (μmol/gm protein)
Group (1) –ve Control	51.36 ± 4.09[d]	12.38 ± 0.83[a]
Group (2) + ve Control	107. 39 ± 12.11[a]	4.12 ± 0.31[d]
Group (3) Cur 20 mg/kg	89.32 ± 0.45[b]	7.89 ± 0.51[c]
Group (4) Cur 40 mg/kg	68.92 ± 0.32[c]	8.79 ± 0.42[c]
Group (5) Cur 60 mg/kg	57.32 ± 0.4[d]	10.52 ± 0.65[b]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ or $P < 0.01$ using one way ANOVA test.

Table (4): Effect of oral administration of curcumin (Cur) on activities of hepatic glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in rats intoxicated with N- nitrosodiethylamine. (n= 8 rats)

Parameters Groups	GPx (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Group (1) -ve Control	22.84±1.65[a]	13.64±0.54[a]	64.66±2.13 [a]
Group (2) + ve Control 0	12.49±0.83 [d]	6.13±0.32[d]	41.74±1.15[d]
Group (3) Cur 20 mg/kg	16.13 ± 3.23c	8.52 ± 0.78[c]	48.156 ± 0.02[bc]
Group (4) Cur 40 mg/kg	19.73±1.28 [b]	10.54 ± 0.64[b]	54.14±1.35 [b]
Group (5) Cur 60 mg/kg	21.95±1.5 [a]	12.73 ± 0.53[a]	60.84±1.74 [a]

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at $P < 0.05$ or $P < 0.01$ using one way ANOVA test.

Intraperitoneal injection of NDEA in rats significantly elevated serum levels of alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa beta (NF-κ β) when compared with normal control rats. Oral administration of curcumin at 20, 40 and 60 mg/kg significantly lowered the elevated serum AFP, TNF-α and NF-κ β levels compared to NDEA group, in concentration – dependent manner as shown in Table (2).

Rats intoxicated by NDEA had significant high content of hepatic malondialdehyde (MDA) and low content of reduced glutathione (GSH) content when compared with the normal control group. Oral administration of curcumin at 20,40 and 60 mg/kg normalized hepatic contents of MDA and GSH when compared to NDEA group, as depicted in Table (3).

Activities of hepatic glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) antioxidant enzymes in NDEA-intoxicated rats were significantly suppressed as compared with the normal control group. Oral administration of curcumin at 20, 40 and 60 mg/kg significantly enhanced the activity of inhibited hepatic SOD, GPx and CAT enzymes when compared with NDEA group as recorded in Table (4).

Histopathological examination of liver sections of normal control rats showed normal histological structure of hepatic lobule with normal hepatocytes, portal vein and sinusoids (Fig.1). Liver sections of rats injected with NEDA showed trabecular hepatocellular carcinoma (long arrow) with fat droplets in tumor cells as demonstrated in Fig. (2),.Liver sections of rats orally given curcumin in a dose of 40 mg/kg showed only mild regression of preneoplastic lesions induced by

NEDA Fig. (3). Liver sections of rats orally given curcumin in a dose of 60 mg/kg displayed amelioration

of hepatocellular architecture with presence of apoptotic cells as shown in Fig. (4).
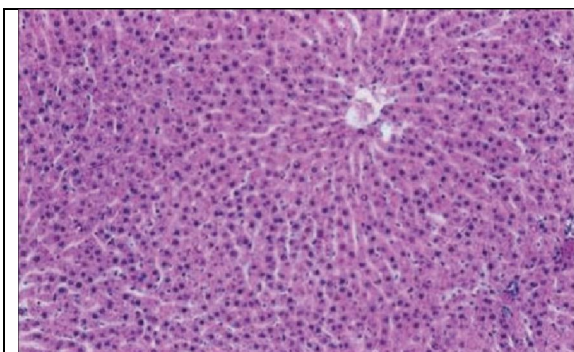

Fig. (1). Liver of a normal control rat showing normal histological structure of hepatic lobule with normal central vein, hepatocytes and sinusoids. (H&E X 200)
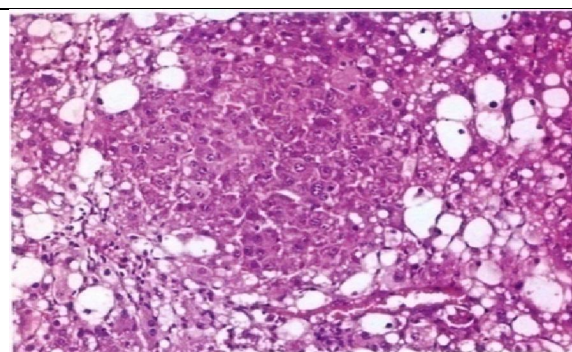

Fig. (2). Liver section of a rat injected with NDEA showing compact hepatocarcinoma with fat droplets in tumor cells and enlarged nuclei. (H&E X 200)
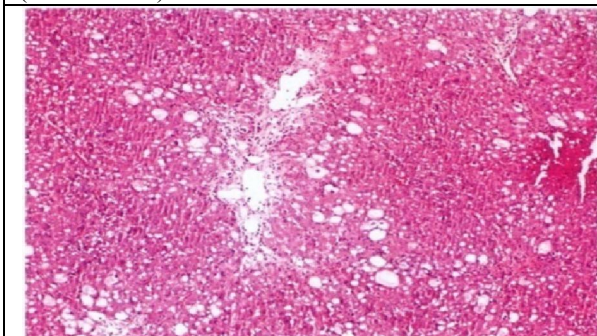

Fig. (3). Liver sections of rats orally given curcumin in a dose of 40 mg/kg showing marked regression of histopathological lesions induced by NEDA and few fat droplets were seen. (H&E X 200)
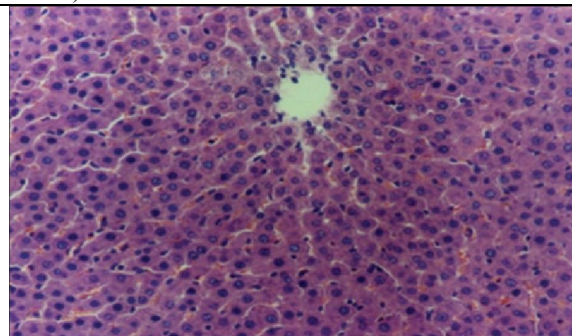

Fig. (4). Liver sections of rats orally given curcumin in a dose of 60 mg/kg showed amelioration of histopathological lesions induced by NDEA and presence of apoptotic cells. (H&E X400)

4. Discussion

Hepatocellular carcinoma (HCC) represents a major worldwide health concern, ranking among the top five most prevalent malignancies (Parkin *et al*., 2001 and Jennifer *et al*., 2008). The incidence of HCC has more than doubled over the last two decades, and accounts for over 500,000 new cases annually (Befeler *et al*., 2002 and Llovet *et al*., 2003). Whereas the incidence of HCC has been increasing steadily, the emergence of new and effective therapeutic agents remains relatively stagnant (Jennifer *et al*., 2008). The limited progress achieved by cancer therapy in the last three decades has increased the interest of researchers in cancer chemoprevention (LÓpez-Lázaro, 2008), especially using nutraceuticals derived from nutritional sources which are naturally multitargeting, less expensive, safer and immediately available (Gupta *et al*., 2011). The most important types of such nutraceuticals are

plant polyphenols derivatives that have been characterized in several cell culture and animal cancer models with antitumor effects (Indap and Barkume, 2003). Curcumin exhibits anticarcinogenic effects on leukemias, lymphomas, multiple myeloma, brain cancer and melanoma as well as skin, cervix, lung, prostate, breast, ovarian, bladder, liver, gastrointestinal tract, pancreatic and colorectal epithelial cancers. It is also a potent tumor-inhibitory agent with chemopreventive properties against intestinal and colon cancers (Sa and Das, 2008, Kwon and Magnuson, 2009 and Mimeault and Batra, 2011).

The present study showed that NDEA induced liver damage as evidence by significantly increases in serum indices of liver function enzymes compared with normal control rats. The obtained results agree with Ahmed *et al.* (2014) who reported that the untreated HCC group revealed significant elevation in

live enzymes. Such elevations in the transaminases are considered as the most sensitive markers in the diagnosis of hepatocellular damage and loss of functional integrity of the membrane **(Plaa and Hewitt, 1989). Ramakrishnan et al. (2007)** attributed the increases in serum aminotransferase enzyme activities to their intracellular location in the cytosol, so toxicity affecting the liver with subsequent breakdown in membrane architecture of the cells leads to their spillage into serum where their concentration rises. Another key hepatic marker enzyme is ALP. Its elevated activity in serum indicates pathological alterations in bile flow. Studies have shown that dividing cells shed more of ALP as they are located in the bile canalicular plasma membrane **(Frederiks et al., 1990)**. Moreover, aminotransferase enzyme elevation reveals cholestasis and bile duct necrosis, this significant elevation may be attributed to its liberation from the plasma membrane into the circulation as it is located on the outer membrane of the hepatic cells, indicating damage of the cell membrane as a result of carcinogenesis **(Bulle et al., 1990)**. The overproduction of these enzymes in tumor cells may cause increased permeability of the cell membrane which leads to such drastic rise of enzymes in serum **(Revathi and Manju, 2013).**

Curcumin supplementation prevented the increase in such hepatic aminotransferase enzymes in a dose dependent manner, suggesting that Cur may have a potential protective effect against NDEA-induced liver damage. This finding was similar to the results of the previous studies reported by **Ahmed et al. (2014).** The effect of Cur may be attributed to it suppresses the activation of several transcription factors that are implicated in carcinogenesis **(Aggarwal et al., 2003).** Moreover, **Hassan et al. (2014)** found that Cur supplementation prevented the increase in such hepatic enzymes, suggesting the therapeutic effect of Cur against NDEA –induced hepatocellular carcinoma in rats.

Intoxicated by NDEA revealed significantly increase serum levels of alpha fetoprotein (AFP), tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa beta (NF-κβ), as well as induced significantly high content of hepatic MDA, and low hepatic content of GSH, GPx, SOD and CAT when compared with the normal control group. Our results agree with **(Bharti et al., 2003).** Tumor necrosis factor-alpha (TNF-α) is a growth factor for most tumor cells **(Sugarman et al., 1985)**. It has been shown to mediate tumor initiation, promotion and metastasis **(Aggarwal, 2003)**. The pro-inflammatory effects of TNF-α are due primarily to its ability to activate NF- κ β. Almost all cell types, when exposed to TNF-α, activate NF- κβ, leading to expression of inflammatory gene, cell adhesion molecules,

inflammatory cytokines, chemokines, and inducible nitric oxide synthase **(Aggarwal et al., 1996)**.

Nuclear factor-kappa beta (NF-κβ) is a family of five closely related proteins which are found in several dimeric combinations and bind to the κB sites on DNA **(Aggarwal and Shishodia, 2004)**. On activation by free radicals, inflammatory stimuli, cytokines, carcinogens, tumor promoters or endotoxins. The NF-κβ is translocated to the nucleus and induces cellular transformation, proliferation, invasion, metastasis, and/or inflammation. Many of these activated target genes are critical to establishment of the early and late stages of aggressive cancers, including those encoding expression of cyclin D1, apoptosis suppressor proteins, and proteins required for metastasis and angiogenesis, such as matrix metalloproteinases and vascular endothelial growth factor **(Wang et al., 1996).**

On the other hand, our results showed that, Cur suppresses the activation of NF-κβ, AFP and TNF-α and normalized hepatic contents of MDA and GSH, GPx and SOD and CAT in a dose dependent manner when compared to untreated NDEA group. These results agree with **Shishodia et al. (2005)**. The therapeutic effect of Cur may be attributed to it induced down-regulation of NF-κβ through suppression of IκBα kinase (IKK) activation **(Jobin et al., 1999 and Plummer et al., 1999)**, arrested cell growth at the G2/M phase and induced apoptosis in human melanoma cells **(Zheng et al., 2004)**. Moreover, it has the ability to inhibit carcinogen bioactivation*via* suppression of specific cytochrome P450 isozymes, and to induce the activity or expression of phase II carcinogen detoxifying enzymes, which may account for its cancer chemopreventive effects **(Awasthi et al., 1996 and Mukhopadhyay et al., 2002).** Furthermore, Cur led to suppression of TNF-α, which leads to inhibition of NF-κβ and cell proliferation **(Shishodia et al., 2005).** Curcumin also modulates expression of genes involved in cell proliferation, cell invasion, metastasis, angiogenesis, and resistance to chemotherapy **(Aggarwal et al., 2003)**. It has been shown to down-regulate the expression of Bcl-2, *Bcl*XL, cyclooxygenase- 2 (COX-2), matrix metalloproteinase (MMP)-9, tumor necrosis factor (TNF), cyclin D1, and the adhesion molecules **(Shishodia et al., 2005)**. Numerous studies in animals have demonstrated that curcumin has potent chemopreventive activity against a wide variety of tumors.

Oxidative stress is associated with damage to a wide range of macromolecular species including lipids, proteins, and nucleic acids thereby producing major interrelated derangements of cellular metabolism including peroxidation of lipids **(Revathi and Manju, 2013)**. It has been reported that the

uncompromised generation of free radicals and reactive oxygen species (ROS) in the liver may be responsible for disturbing the antioxidant status and ultimately leading to oxidative stress and paving the way to carcinogenesis **(Gey, 1993)**. Lipid peroxidation plays an important role in carcinogenesis **(Banakar *et al*., 2004)**, and may lead to the formation of several toxic products, such as MDA and 4-hydroxynonenal, these products can attack cellular targets, thereby inducing carcinogenicity **(de Zwart *et al*., 1999).** The increase in lipid peroxidation was reported during NDEA induced hepatocarcinogenesis **(Revathi and Manju, 2013)**. In our study, group treated with NDEA have a significant increase in the levels of MDA as compared with normal group animals. The inhibition of peroxidation by Cur is mainly attributed to the scavenging of the ROS involved in the peroxidation **(Wright, 2002).** Animals in groups received curcumin exhibited significantly low level of MDA in a dose dependent, when compared with animals untreated. This verifies the anti-lipid peroxidative role of Cur by its ability to scavenge free radicals generation **(Hassan *et al*., 2014)**.

In the present study, the cancer rats showed decreased activities of enzymatic antioxidants (SOD, CAT and GPx) and non-enzymatic antioxidants (GSH) in liver tissues in comparison with normal animals. Our data are consistent with previous findings **(Rajeshkumar and Kuttan, 2000 and Ghosh *et al*. 2012)**. **Pradeep *et al*. (2007)** reported that such subsequent decrease in the antioxidant defense is due to the decreased expression of these antioxidants during hepatocellular damage. On the other hand, there is a significant increase in the enzymatic and non-enzymatic antioxidants guard in the liver of the animals administered Cur in a dose dependent when compared with untreated animals administered NDEA. This increase is due to the ability of Cur to prevent the formation of free radicals, enhance the endogenous antioxidant activity beyond its free radicals scavenging property and the reduction of hepatic lipoperoxide formation **(Bruck *et al*., 2007).**

Changes occurring in the biochemical parameters were confirmed by the histopathological observation. The histopathological examination of the liver of rats received NDEA, showed trabecular hepatocellular carcinoma with fat droplets in tumor cells. On the other hand liver sections of rats treated with Cur displayed amelioration of hepatocellular architecture in a dose dependent. The obtained results in line with **Ahmed *et al*. (2014)** who reported that the liver tissues in HCC group showed typical anaplasia, while treatment with Cur showed remarkable improvement in the histological features of liver tissues. In addition, the study of **Hassan *et al*. (2014)** revealed that HCC

rats showed aberrant hepatocellular phenotype with variation in nuclear size, hyperchromatism, and irregular sinusoids with prominent hyperbasophilicpreneoplastic focal lesions and eosinophilic clear cell foci, while animals received curcumin their hepatocytes, appeared with portal areas and hepatic lobules that were more or less like the control ones. Moreover, **Sreepriya *et al*. (2006)** stated that the presence of atypical nuclei is a marker of hepatocellular carcinoma. This effect of Cur indicating liver regeneration which may be explained by **Ghosh *et al*. (2012)** who proofed that Cur maintains the normal liver cell function.

Conclusion

Curcumin appeared to be an effective free radicals quencher with antioxidant activities, and capable of inhibiting oxidative stress. These effects are mediated through its regulation of various transcription factors, suppress activation of NF-κβ, inflammatory cytokines, protein kinases, and other enzymes. Given these promising findings, the present study suggests that Cur, which is a potentially safe and inexpensive for clinical use, may be considered as an effective chemopreventive agent against HCC.

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