



## CHEMOPREVENTIVE EFFECT OF CURCUMIN ON OXIDATIVE STRESS, ANTIOXIDANT STATUS, DNA FRAGMENTATION AND CASPASE -9 GENE EXPRESSION IN 1, 2-DIMETHYLHYDRAZINE-INDUCED COLON CANCER IN RATS

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### ABSTRACT

The present study was designed to investigate the ameliorative effect of curcumin administration on oxidative stress, antioxidant status, DNA fragmentation and caspase-9 gene expression in colon cancer induced by 1, 2-dimethylhydrazine (DMH) in rats. Seventy male albino rats divided into five groups containing 14 rats each. Group I :( Control group) rats received no drugs. Group II: (colon cancer induced group) rats injected DMH (35 mg/kg b.wt /week, subcutaneously) for ten weeks. Group III: (DMH+curcumin therapeutic group) rats injected DMH and administered curcumin (100 mg/kg b.wt/day, orally) from the 11<sup>th</sup> week until the 16<sup>th</sup> weeks. Group IV: (DMH+curcumin treated group) rats injected DMH and at the same time administered curcumin for 16 weeks (end of experiment). Group V: (control +curcumin group) rats administered curcumin all over the experimental periods. At the end of 16<sup>th</sup> week treatment blood samples and colon tissues were collected for determination of serum lactate dehydrogenase (LDH) and carcino embryonic antigen (CEA) in addition to glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), L-malondialdehyde (L-MDA), nitric oxide (NO), glutathione-S-transferase (GST), Caspase-9 gene and DNA fragmentation in colon tissues. The obtained results revealed that, DMH potentially increased serum LDH activity and CEA level. In addition, CAT, GPx, GST activities, MDA, and NO concentrations in colon tissues of DMH injected rats were significantly increased. However, SOD, GSH, Caspase-9 and DNA fragmentation in colon tissues were significantly decreased. Curcumin treatment to colon cancer rats significantly decreased serum LDH and CEA, CAT and GPx activities and attenuated the increased MDA and NO concentrations in colon tissues. On the other hand, curcumin treatment enhanced the activity of SOD and GST and the level of GSH, caspase-9 and DNA fragmentation in colon tissues. From the obtained results, it could be concluded that, inhibition of peroxidation and oxidative stress markers, enhanced antioxidant status, increased caspase-9 gene expression, and DNA fragmentation in rat colon tissues by curcumin suggest the potential efficacy of curcumin as an addition chemopreventive agent in treatment of colon carcinogenesis.

**KEYWORDS:** Colon cancer, DMH, Curcumin, DNA fragmentation, caspase-9 gene expression, antioxidant enzymes.

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### 1. INTRODUCTION

Colorectal cancer is a malignant tumor with high morbidity and mortality, nearly 639,000 deaths worldwide per year, it is the fourth most

common form of cancer and the third leading cause of cancer-related death worldwide(1). Colorectal cancers develop because of the progressive accumulation of

genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to adenocarcinoma. Indeed, consumption of high levels of red meat and fat together with low levels of fruits, vegetables, and fibers was been suggested to enhance the risk of colorectal cancer (2). Presently available therapies including surgery, radiation and chemotherapeutic drugs, are still limited for the advanced stages of colon carcinogenesis. Chemoprevention remains an effective and promising additional strategy for controlling the incidence of colon cancer (3). 1, 2-Dimethylhydrazine (DMH) is a colon specific carcinogen and an alkylating agent. DMH was been believed to form active intermediates including azoxymethane (AOM) and methylazoxymethanol (MAM) in the liver, which are subsequently transported into the colon through bile. Methylazoxymethanol is decomposed to form methyl diazonium ion, which methylate cellular components and in turn produce tumors in the colon (4). Curcumin (diferuloylmethane), a polyphenolic antioxidant compound of *Curcuma longa* L., is efficient in chemoprevention and cancer therapy where Curcumin inhibits the initiation and promotion stages of chemically induced carcinogenesis in skin, stomach and colon (5). The tumors suppression is due to down-regulation of a variety of transcription factors, enzymes and growth signal transducers such as nuclear factor kappa-B (NF- $\kappa$ B), early growth receptor-1 (EGR1), Cyclooxygenase-2 (COX-2) and Wnt signaling molecules (6). Moreover, curcumin inhibited chemically induced carcinogenesis in the colon when administered at different stages of the cancer process. Laboratory rats, administered curcumin during either initiation or late in the premalignant phase, had a lesser incidence and fewer numbers of invasive malignant colon tumors (7). Accordingly, this study was performed to investigate the protective effect of oral administration of curcumin on biomarkers

of oxidative stress, antioxidant status, DNA fragmentation and caspase-9 gene expression in serum and colon tissues of 1, 2-dimethylhydrazine-induced colon cancer in rats.

## 2. MATERIALS AND METHODS

### 2.1. *Animals:*

Seventy white male albino rats of 6- 8 weeks old and weighing 150 - 180 gm housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals fed on constant ration and water was supplied ad-labium.

### 2.2. *Induction of colon cancer:*

Colon cancer was induced by subcutaneously injection of DMH at a dose of (35 mg/kg b.wt /week) for ten weeks (8). DMH have been purchased from Sigma Aldrich Company Co. for Trading Chemicals, Medicines and Medical Appliances.

### 2.3. *Preparation and Dosage of Curcumin:*

Curcumin (purity ~99%) was freshly prepared by dissolved in Dimethylsulfoxide (DMSO) solution, administered orally, and daily at a dose of 100 mg/ kg b.wt (9). It manufactured by Fluka Co for Chemicals and purchased from Elgoumhouria Co for Trading Chemicals Medicines and Medical Appliances, Egypt.

### 2.4. *Experimental design:*

Animals randomly divided into five main equal groups, 14 animals each, placed in individual cages and classified as follow: Group I (control group): Healthy rats not received any drugs and served as control for all experimental groups. Group II (DMH-induced colon cancer group): rats injected DMH (35 mg/kg b.wt /week, subcutaneously) for ten weeks. Group III (DMH +curcumin therapeutic group): rats injected DMH (35 mg/kg b.wt /week, subcutaneously) for ten weeks and administered curcumin (100 mg/kg

b.wt/day, orally) from the beginning of 11<sup>th</sup> week until the end of 16<sup>th</sup> week. Group IV (DMH +Curcumin treated group): rats injected DMH for ten weeks and at the same time co-administered curcumin for 16 weeks (end of experiment). Group V (Normal + curcumin treated group): rats administered curcumin orally at a dose (100mg/kg body weight/day) all over the experimental periods.

#### 2.5. Sampling:

##### *Blood samples:*

At the end of 16<sup>th</sup> week treatment, blood samples and colon tissues were been collected from all animal groups (control and experimental groups). Blood samples for serum separation were been collected by ocular vein puncture in dry, clean, and screw-capped tubes after overnight fasting. Serum was be separated by centrifugation at 4000 r.p.m for 15 minutes. The clean, clear serum separated by Automatic pipette and received in dry sterile samples tube, and kept in a deep freeze at - 20° C until used for subsequent biochemical analysis.

##### *Colon tissues specimens:*

Rats killed by decapitation. The colon specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, colon tissues was minced into small pieces, homogenized with ice cold 0.05 M potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were be centrifuged at 4000 r.p.m for 15 minute at 4°C. The supernatant was used for the determination of L-malonaldehyde (L-MDA), NO, antioxidant enzymes, Caspase-9 and DNA fragmentation.

#### 2.6. Biochemical analysis:

Serum LDH and CEA and colon tissue MDA, SOD, CAT, GSH, GPX, GST, NO,

Caspase-9 gene and DNA fragmentation were analyzed according to the methods described by (10), (11), (12), (13), (14), (15), (16), (17), (18) and (19) respectively.

#### 2.7. Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses performed using the statistical package for social science (SPSS, 13.0 software, 2009) (20). Values of P<0.05 were considered to be significant.

### 3. RESULTS

The obtained results demonstrated in (table 1) revealed that, administration of DMH induced colon cancer in rats exhibited a significant increase in serum LDH activity and CEA concentration when compared with normal control group. Treatment with curcumin to DMH-induced colon cancer in rats significantly reduced elevated serum LDH activity and CEA concentration when compared with DMH-induced Colon cancer non-treated group. The obtained data presented in (table 2) revealed that, administration of DMH-induced colon cancer in rats caused significant increase in CAT, GPx and GST activities and MDA and NO concentrations in colon tissues. However, SOD, GSH, Caspase-9 and DNA fragmentation in colon tissues were significantly decreased when compared with normal control group. Curcumin treatment to colon cancer rats significantly decreased CAT and GPx activities and attenuated the increased MDA and NO concentrations in colon tissues. On the other hand, curcumin treatment enhanced the activity of SOD and GST and the level of GSH, caspase-9 gene expression and DNA fragmentation in colon tissues when compared with DMH-induced Colon cancer non-treated group.

Table (1) Effect of curcumin treatment on serum LDH activity and CEA concentration in 1,2 dimethylhydrazine-induced colon cancer in rats and their control.

Animal groups	Group I	Group II	Group III	Group IV	Group V
LDH(U/L)	229.14±15.72 <sup>b</sup>	338.57±28.87 <sup>a</sup>	233.0±22.45 <sup>b</sup>	226.57±14.33 <sup>b</sup>	272.29±12.02 <sup>b</sup>
CEA (ng/ml)	2.96±0.12 <sup>c</sup>	12.6±1.67 <sup>a</sup>	8.63±0.58 <sup>b</sup>	8.80±0.87 <sup>b</sup>	2.46±0.18 <sup>c</sup>

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 ( $P<0.05$ ). Group I:( Control), Group II: (colon cancer), Group III: (DMH+curcumin therapeutic), Group IV: ( DMH+curcumin treated), Group V: (control +curcumin) .

Table (2) Effect of curcumin treatment on biomarkers of oxidative stress, antioxidant enzymes, DNA fragmentation and caspase -9 gene expression in colon tissue of 1,2 dimethylhydrazine-induced colon cancer in rats and their control.

Animal groups	Group I	Group II	Group III	Group IV	Group V
CAT ( U /g tissue)	45.62±1.81 <sup>c</sup>	66.40±1.49 <sup>a</sup>	50.27±0.89 <sup>b,c</sup>	54.53±3.92 <sup>b</sup>	48.73±1.94 <sup>b,c</sup>
SOD (U/g tissue)	58.72±1.55 <sup>a</sup>	48.49±1.24 <sup>b</sup>	51.08±1.50 <sup>b</sup>	56.27±1.76 <sup>a</sup>	51.12±1.62 <sup>b</sup>
GPx (ng/g tissue)	21.09±0.77 <sup>c</sup>	29.40±1.23 <sup>a</sup>	24.46±1.03 <sup>b</sup>	26.71±0.46 <sup>a,b</sup>	24.72±1.35 <sup>b</sup>
GST (ng/g tissue)	0.28±0.04 <sup>b</sup>	0.34±0.02 <sup>b</sup>	0.55±0.02 <sup>a</sup>	0.35±0.04 <sup>b</sup>	0.37±0.02 <sup>b</sup>
GSH (ng/g tissue)	2.91±0.19 <sup>a</sup>	2.26±0.14 <sup>b</sup>	2.68±0.12 <sup>a,b</sup>	2.99±0.24 <sup>a</sup>	2.85±0.12 <sup>a</sup>
L-MDA (mmol/g tissue)	44.75±1.70 <sup>b</sup>	61.97±4.81 <sup>a</sup>	47.24±1.54 <sup>b</sup>	41.73±3.59 <sup>b</sup>	44.73±1.46 <sup>b</sup>
NO( μmol/g tissue)	12.61±0.95 <sup>b</sup>	20.84±1.32 <sup>a</sup>	12.97±0.77 <sup>b</sup>	14.13±1.04 <sup>b</sup>	11.46±1.19 <sup>b</sup>
DNA Frag. (%)	87.26±1.10 <sup>a</sup>	44.44±3.62 <sup>c</sup>	58.95±4.36 <sup>b</sup>	68.41±5.31 <sup>b</sup>	92.96±2.12 <sup>a</sup>
Caspase-9 gene (%)	16.85±1.75 <sup>b</sup>	11.16±0.28 <sup>c</sup>	12.18±0.79 <sup>b</sup>	13.57±0.02 <sup>b,c</sup>	23.63±2.21 <sup>a</sup>

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 ( $P<0.05$ ).

#### 4. DISCUSSION

Colorectal cancer (CRC) is one of the most common and extensively studied gastrointestinal cancers in modernized countries. Despite awareness about risk factors and pathologic mechanisms, it remains the third highest cause of cancer death (21). The goals of cancer chemoprevention are to slow, block or reverse the process of carcinogenesis by natural or synthetic compounds. Some (not all) dietary compounds have the innate ability to modify the deregulated intracellular pathways thereby delaying the process of carcinogenesis (22).

Administration of DMH to normal rats exhibited a significant increase in serum LDH activity. These results are nearly similar to those reported by (23) who showed that, a significant higher value in LDH activity observed in DMH-group compared to control. The marked elevation in the activity of serum LDH were been shown in the current study after DMH injection may be attributed to cancer induction effect or damage in liver cells. As confirmed by (24) who recorded that, DMH is not only a colon carcinogen but also a potent hepatic necrogenic and agent that alkylate hepatocellular DNA. The same results was suggested by (25) who recorded that, serum LDH activity in patients with colon and rectal cancer was significantly higher than the control groups, Who added that, serum LDH activity in patients with colon cancer was higher than patients with rectal cancer. It is well known that glycolysis in cancer tissue increase significantly, consequently, as an important enzyme of the glycolytic pathway; LDH may manifest a higher activity in cancer patients; serum and tissues (26).

Treatment with curcumin to DMH-induced colon cancer in rats significantly reduced elevated serum LDH activity (table 1). Similarly, (27) observed that, curcumin treatment was accompanied by a decrease in serum lipid peroxides, CK and LDH

suggests that, this treatment exerted a membrane stabilizing effect. The decrease in LDH activity may be due to the direct effect of curcumin that reduce and protect cells from carcinogenesis and led to reduce glycolysis and lowered the elevation in LDH or may be due to protect myocardial tissues against destruction(28). The obtained results demonstrated administration of DMH to normal rats exhibited a significant increase in serum CEA concentration when compared with normal control group (table 1). Similarly, (29) suggested that, elevation in CEA concentration was observed in DMH-induced colon cancer in rats compared to control. It varies inversely with tumor grade (well-differentiated tumors secrete more CEA). CEA is elevated more in tumors with lymph node and distant metastasis than in organ-confined tumors (varies directly with tumor stage). In addition, CEA, a tumor-associated antigen, is a widely used serum biomarker for colorectal cancer. Interestingly, in the normal colonic mucosa, CEA expression showed a crypt-surface distribution. CEA expression was strong in surface epithelial cells and goblet cells of the upper crypts, while very weak in the mid crypt and at the base. Cell lines with high expression of CEA showed shuttle-shape morphologic changes with long or dendrite-like cytoplasm processes (30). Furthermore, (31) recorded that, CEA is the best marker in colorectal cancer patients and most thoroughly characterized tumor-associated antigens, in both biochemical and clinical aspects. In addition, data demonstrated in tables (1) revealed that, treatment with curcumin to DMH-induced colon cancer in rats significantly reduced elevated serum CEA concentration when compared with DMH-induced Colon cancer non-treated group. (32) Supported these results by reported that, chemotherapeutic effect of oral curcumin extract administration was described: All patients enrolled exhibited radiological evidence of progressive malignant disease before recruitment.

Levels of the tumor marker CEA (carcinoembryonic antigen) in venous blood were above the normal range in all patients, and those of CA19.9 were abnormal in 80% of patients. In one patient, who received 440 mg of Curcuma extract (equivalent to 36 mg of curcumin) daily, venous blood CEA levels decreased from a pretreatment value of  $310 \pm 15$  to  $175 \pm 9$   $\mu\text{g/liter}$  after 2 months of treatment.

The presented results in (table 2) revealed that, administration of DMH-induced colon cancer in rats exhibited significant increase in CAT, GPx and GST activities in colon tissues. However, SOD and GSH were significantly decreased when compared with normal control group. These results are in agreement with the recorded data of (33) who found that, SOD activity was lower in Azoxymethane compared to control group. Antioxidant enzymes that scavenge intermediates of oxygen reduction provide the primary defense against cytotoxic oxygen radical. It well known that SOD, CAT and GPx play an important role as a protective enzyme against lipid peroxidation in tissues. They are involved in the direct elimination of reactive oxygen metabolites, which is probably one of the most effective defenses of the living body against diseases. GPx, an oxidative stress inducible enzyme plays a significant role in the peroxy scavenging mechanism and in maintaining functional integration of the cell membranes (34). Moreover, (35) demonstrated that, decrease in concentration of GSH was observed in AOM-induced colon cancer rats group compared to control. GSH in conjugation with GPx and GST plays significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging ROS (36). Antioxidants had shown to inhibit initiation and promotion of carcinogenesis and to counteract cell immortalization and transformation (37). SOD catalyzes the dismutation of superoxide anions to form  $\text{H}_2\text{O}_2$ . CAT catalyzes the reduction of  $\text{H}_2\text{O}_2$  and thereby protects the cellular constituents from oxidative damage by the

highly reactive hydroxyl radicals (38). DMH itself can generate  $\text{H}_2\text{O}_2$  in the presence of copper ions (39) In the presence of metal ions such as  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ ;  $\text{H}_2\text{O}_2$  can react with  $\text{O}^{2-}$  to convert it into the more reactive  $\text{OH}^-$  radical. If sufficient amounts of CAT or GPx are not available to decompose  $\text{H}_2\text{O}_2$  (40), the generated OH radicals are capable of attacking DNA. The significantly decreased capacity of a variety of tumors to detoxify  $\text{H}_2\text{O}_2$  linked to the decreased levels of CAT (41). Thus, DMH elicits substantial oxidative stress via the formation of the electrophilic diazonium ion and the generation of  $\text{H}_2\text{O}_2$ . Increased concentrations of  $\text{H}_2\text{O}_2$  implicated in carcinogenesis in two ways: (a) In the form of the  $\text{OH}^-$  radical,  $\text{H}_2\text{O}_2$  could mutate tumor suppressor genes such as p53, and (b) it acts as an intracellular second messenger to activate redox sensitive transcription factors like c-jun, c-fos and NF-KB, resulting in the expression of many growth-promoting genes (42). Reduced glutathione an important non-protein thiol in conjunction with GPx and GST plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging reactive oxygen species (43). In addition, GST and GPx are biotransformation enzymes involved in the detoxification of xenobiotics, carcinogens, free radicals and peroxides by conjugating these toxic substances with GSH, ultimately protecting cells and organs against carcinogen-induced toxicity. Since the reactive ultimate carcinogenic form of DMH is an electrophilic diazonium ion, glutathione-dependent enzymes may play an important role in carcinogen detoxification. These enzymes can also serve as anticarcinogens and as inhibitors at initiation and promotion/transformation stage of carcinogenesis. To put it more clearly, first GSH directly neutralizes the radicals that are crucial to antitumor activity. Second, GST catalyzes the reaction between GSH and the hydrophobic or electrophilic compound (diazonium ion). Third, GPx catalyzes the reduction of

glutathione in presence of NADH; GR then reconverts oxidized GSSG into GSH. Hence, working in concert, the peroxidase/reductase couple counteracts drug mediated oxidative stress. Moreover, antioxidant enzymes such as SOD and CAT are widely distributed in all cells and are present in high amounts in tissues (44). The observed increase in circulating lipid peroxides of DMH-treated animals, in the present study, correlates with the decline in circulatory antioxidants such as GSH and SOD. This may be due to their overutilization to scavenge the products of lipid peroxidation as well as sequestration by tumor cells (45). Curcumin treatment to colon cancer rats significantly decreased CAT and GPx activities in colon tissues. On the other hand, curcumin treatment enhanced the activity of SOD and GST and the level of GSH in colon tissues when compared with DMH non-treated group (table 2). These results are nearly similar to (46) who reported that; curcumin normalized the antioxidant enzymes activities (CAT and SOD) of erythrocyte and liver in db/db mice. Moreover, (29) recorded that, oral administration of curcumin significantly elevated the levels of GSH and enhanced the antioxidant status of SOD activity in the liver, lung and kidney when compared with animals treated with nicotine alone. Glutathione, an important cellular reluctant, offers protection against free radicals, peroxides and toxic compounds (32). The decreased level of tissue GSH in nicotine-treated rats may be due to the enhanced utilization during detoxification of nicotine. Previous study has reported that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by a chemical carcinogen. Curcumin has ability to scavenge free radicals, interacting with oxidative cascade, quenching oxygen, inhibiting oxidative enzymes and chelating metal ions and inhibits lipid peroxidation (47). Reduction of GPX activity after Curcumin administration might play a role in maintaining the balance between these

antioxidant enzymes. Furthermore, the decrease in GPX activities, along with the increase in GSH and GST activity in Curcumin-treated animals, could be used to explain the increased hepatic and colonic GSH contents. Curcumin reduces the oxidative stress in animals, by its high ROS scavenging capacity and by protecting the antioxidant enzymes from denaturation.

The obtained data demonstrated in (table 2) revealed that, administration of DMH in rats exhibited significant increase in NO concentration in colon tissues. Similarly, (48) reported that, inducible nitric oxide synthase activity was enhanced by DMH induction compared to control group. Inducible form of nitric oxide (NO) synthase (iNOS) greatly increases the level of NO, leading to a marked increase in the levels of peroxynitrite (49). In the present study, the increase in NO level perhaps up-regulation of iNOS mRNA correlates with the elevation of colonic nitrite and nitrotyrosine accumulation. Over-expression of iNOS and nitrosamine stress may lead to apoptosis resistance, and increase in tumour vasculature and metastatic potential (50). This provides evidence that peroxynitrite accumulates through the cross talk between the superoxide-releasing NADPH oxidase and nitric oxide releasing NO synthase activities by the transmural invading phagocytes (neutrophils and macrophages) into inflammatory colons. These results are similar to (51) who reported that had earlier related curcumin to increased peroxynitrite levels. Inducible form of nitric oxide (NO) synthase (iNOS) greatly increases the level of NO, leading to a marked increase in the levels of peroxynitrite. The elevated peroxynitrite levels cause HPA axis dysfunction and thus cause fatigue symptoms. Curcumin treatment in DMH-induced colon cancer in rats attenuated the increased in NO concentrations in colon tissues.

DMH-induced colon cancer in rats exhibited significant increase in MDA concentration in colon tissues. Similarly,

(52) reported that, the level of MDA and H<sub>2</sub>O<sub>2</sub> content were significantly enhanced in DMH group as compared to control. In addition, (33) reported that, an increase in plasma and colonic MDA and decreased antioxidant potential were observed after DMH injection in rats. MDA, the major product of lipid peroxidation is mutagenic and genotoxic and may contribute to human cancer development. Enhanced concentrations of circulating lipid peroxidation associated with antioxidant depletion were observed in DMH-induced colon tumor bearing animals. DMH, a procarcinogen undergoes metabolism in the liver, resulting in the production of active carcinogenic electrophile (diazonium ion), which is capable of producing toxic effects at sites far from tumor. Document these results accorded by (52) who observed that, the production of reactive oxygen metabolites (ROMs) during the hepatic metabolism of DMH and/or during the process of colon carcinogenesis well. Early reports also suggest that tumor cells produce substantial amount of H<sub>2</sub>O<sub>2</sub> that released into the circulation. In conditions of severe oxidative stress such as carcinogenesis, reactive oxidant species such as superoxide (O<sup>2-</sup>) and hydroxyl radical (OH) are released into circulation resulting in increased susceptibility to lipid peroxidation in DMH-treated rats. Curcumin treatment to colon cancer rats significantly attenuated the increased MDA level (table 2). The obtained results are nearly similar with those of (47) oral administration of curcumin and BDMC-A decreased the levels of plasma (MDA) and hydro peroxides and improved the levels of non-enzymatic antioxidant. Treatment with BDMC-A to DMH-treated animals resulted in substantial reduction in LPO in the liver. Enhanced LPO in the liver of DMH-induced colon tumour bearing rats could be attributed to the DMH-induced oxidative stress and production of reactive oxygen metabolites (ROMs). Administration of BDMC-A to DMH-treated rats decreased the levels of LPO and

enhanced the activities of detoxification enzymes GPx and GST in the liver.

Caspase-9 and DNA fragmentation in colon tissues were significantly decreased in DMH-induced colon cancer in rats when compared with normal control group. Similarly, (53) demonstrated that, Caspase-9 was shown to be down regulated in colon cancer specimens in comparison with normal mucosa tissues. Also, (52) reported that, decreased caspase-3 positive cells were evident in DMH induced group as compared to control group. Immunohistochemical analysis reveals that the expression of caspase-9 is variable in the healthy enterocytes. However, in the enterocytic component of 12 among 26 cancer samples analyzed, a decrease in caspase-9 immunostaining intensity has been observed: a profile similar, but to a smaller extent, to that observed for caspase 7. DMH is an alkylating agent which damages cellular DNA by forming an adduct. Moreover, DMH-induced colorectal rodent tumors exhibit k-ras mutations following constitutive activation of PI3K/Akt pathway. Upon activation, Akt inactivates several downstream targets including Bcl-2 family members, caspase-9 thereby blocking apoptosis (54). On contrary, (52) reported that, increased DNA fragmentation cells were evident in DMH induced cancer group. Defects in the cascade of apoptosis-related events during neoplastic development could well affect the execution of apoptotic death and disrupt homeostasis regulation of the colonic tissue.

On the other hand, the obtained results in table (2) revealed that, curcumin treatment enhanced the value of caspase-9 gene expression and DNA fragmentation in colon tissues when compared with DMH-induced Colon cancer non-treated group. The same results suggested by (53) who reported that, induction of apoptosis in curcumin-fed young rats were mediated by activation of caspase-9. However, this relationship was not observed in either middle-aged or old rats. On the other hand,

(55) reported that, curcumin induces apoptosis in HT-29 colon adenocarcinoma cells by up regulating the serine phosphorylation level of p53 and the level of Bax, while down regulating the levels of Bcl-2, pro-caspase-3, and pro-caspase- 9. These findings suggest a mechanism of curcumin action on HT-29 cells and should further establish its use as a valid chemo preventive and chemotherapeutic agent in colon cancer (56). Additionally, (57) showed that, curcumin-induced release of AIF, others have shown the involvement of AIF mediated large-scale DNA fragmentation in response to curcumin. However, (58) declared that, CMN inhibits the generation of ROS that are responsible for the DNA damage. In addition, (59) recorded that, CMN attenuated DNA fragmentation due to the elevation of GSH, Who indicates the importance of CMN in protecting animals against LCT-induced hepatotoxicity through attenuating lipid peroxidation, increasing the activities of antioxidant enzymes and alleviating DNA fragmentation. The increase in apoptotic cells suggests in curcumin treated DMH group could be attributed to curcumin inhibited ACF formation and destroyed the pre-existing neoplastic lesions. The pro-apoptotic effect of curcumin has been already described in a variety of colon tumour cells (55). Curcumin decreases the expression of anti-apoptotic members of the Bcl-2 family and elevates the expression of pro-apoptotic mediators such as p53, Bax and procaspases 3, 8, and 9. The total loss of goblet cells differentiation in dysplastic ACF, 10 weeks post-DMH injection is considered as a specific feature of malignant preneoplastic lesions. These results indicate that, curcumin does not induced differentiation of preneoplastic cells, but rather enhances their apoptosis. The pro-apoptotic effect of curcumin is well established in a variety of cancer cell lines (59). Curcumin was shown to activate caspases 9, 3, and 8 in the colon cancer cell lines SW480 and SW620 (60). In the presence of heat shock proteins a reduction

in the activation of both caspases 9 and 3, but not 8 in SW480 or SW620 cells was noted. Curcumin mediated the release of cytochrome c, the partial blocking of apoptosis inducing factor (AIF), and second mitochondria derived activator of caspase (Smac) was not blocked by heat shock proteins. Lovo cells and HCT-116 cells treated with curcumin were largely accumulated in G2/M phase, which prevented cells from entering the next cell cycle (61).

### **Conclusion:**

The findings of the present study demonstrated that treatment with curcumin provided an effective protection against colon cancer induced by DMH in rats, since curcumin were able to prevent the lipid peroxidation. Also, increase the apoptotic program via increasing caspase-9 gene and DNA fragmentation. The obtained results suggest the curcumin as an addition chemopreventive agent in treatment of colon carcinogenesis. So we recommended that, supplementation of curcumin is very important for protection of different body organs from cancer and for cancer treatment particularly colon cancer.

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كلية معتمدة 2013

## التأثير الكيميائي الوقائي للكرميين على الاجهاد التأكسدي وحالة مضادات الاكسدة وتجزئة الحامض النووي دي. إن. أيه والتعبير الجيني للكسباس-9 في سرطان القولون المحدث بثنائي داي ميثيل الهيدرازين في الفئران

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### الملخص العربي

يعتبر سرطان القولون واحد من اكثر السرطانات شيوعا حيث يحتل المركز الرابع بين انواع السرطانات خاصة في دول أوروبا والأميركتين ويكون نادرا في اسيا وقليل في افريقيا وذلك لتأثره بطبيعة الغذاء وسلوكيات الشعوب. ونظرا لمضاعفات العلاج الكيميائي والإشعاعي في علاج السرطانات فكانت الحاجة لاتجاه الباحثون لإيجاد بدائل لمثل هذه العلاجات ولما تتميز به الاعشاب من مميزات كمضادات اكسدة وكمواد وقائية فتم استخدامها على نطاق واسع في الابحاث ومن بين هذه الاعشاب كان الكرميين ومن هنا جاءت الفكرة لاستخدام الكرميين لمعرفة مدى تأثيره في علاج سرطان القولون والتخلص من الخلايا السرطانية في القولون المحدثه تجريبيا بواسطة مادة الداى ميثيل هيدرازين والتي تعتبر اكثر المواد شيوعا في احداث سرطان القولون في الفئران. وقد أجريت هذه الدراسة على عدد 70 من ذكور الفئران البيضاء وتتراوح أعمارها بين ستة إلى ثمانية أسابيع وأوزانها بين 180 -150 جرام ، تم احضار هذه الفئران من مركز بحوث حيوانات التجارب بكلية الطب البيطري/جامعة بنها. وقد تم وضع هذه الفئران في أقفاص معدنية منفصلة بحجرة خاصة لرعاية حيوانات التجارب بقسم الكيمياء الحيوية بالكلية. وقد تم تقسيم الفئران الى خمسة مجموعات وهى: المجموعة الاولى (الضابطة) والمجموعة الثانية وهى المجموعة المحدث بها سرطان قولون عن طريق حقن مادة الداى ميثيل هيدرازين بتركيز 35مجم/كجم لمدة 10 اسابيع والمجموعة الثالثة وهى المجموعة التي تم علاجها بالكرميين بتركيز 100مجم/كجم يوميا لمدة ستة اسابيع بعد 10 اسابيع حقن بالمادة المسرطنة والمجموعة الرابعة وهى تلك المجموعة التي تناولت كلا من المادة المسرطنة والكرميين واخير المجموعة الخامسة وهى تلك التي تناولت الكرميين فقط طوال فترة التجربة وهى 16 اسبوع. بعد نهاية الاسبوع السادس عشر ب 24 ساعة صيام قد تم ذبح الفئران بعد اخذ عينات الدم منها لعمل تحاليل اللاكتيت دي هيدروجينز والكارسينو امبريونيك انتيجن كدلالات اورام خاصة بسرطان القولون وتم ايضا استخلاص نسيج القولون لعمل بعض التحاليل مثل الاكسدة الفوقية للدهون ونشاط انزيم الجلوتاتايون بيروكسيديز ومعدل الجلوتاتايون المختزل ومعدل الجلوتاتايون اس ترانسفيريز ومعدل اكسيد النيتريك وكذلك تركيز الكاسباس 9 وجين ومعدل تجزئة الحامض النووي. وقد اظهرت النتائج كما هي موضحة بالجدول 2, 1 ارتفاع نسب المتغيرات الكيميائية الحيوية بعد حقن المادة المسرطنة ثم قل الارتفاع بعد العلاج بالكرميين. لذلك توصى الدراسة بضرورة استغلال تلك المزايا الهائلة للكرميين كماد طبيعية وقائية مضادة للسرطان وإدخالها كماد فعالة في صناعة العقاقير الطبية المستخدمة في وقاية وعلاج سرطان القولون.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):125-138, ديسمبر 2013)