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ORIGINAL ARTICLE

Protective Effect of Curcumin Decreases Incidence of Gastric Cancer Induced by *Helicobacter pylori* and *N*-methyl-*N*-nitrosourea in Rats

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ABSTRACT

Aim: To study the effects of curcumin on *Helicobacter pylori* and *N*-methyl-*N*-nitrosourea (MNU)-induced gastric cancer in rats. **Materials and Methods:** Male Wistar rats were divided into three groups: control (CO), *H. pylori* inoculation and 30 ppm MNU in drinking water for 20 weeks (*Hp* + MNU), and *H. pylori* and MNU supplemented with 60 mg/kg curcumin for 30 weeks (*Hp* + MNU + Cur). The stomach was removed to examine nuclear factor kappa B (NF-κB) p65, 8-hydroxy-2′-deoxyguanosine (8-OHdG), cyclin D1, and Ki-67 in gastric epithelial cells by immunohistochemistry. The expression of apoptotic cells was measured by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling reaction and gastric histopathology. **Results:** Two rats in *Hp* + MNU developed adenocarcinoma (ADC) of the glandular stomach (40% incidence, $n = 5$), while in *Hp* + MNU + Cur, no gastric ADC was found. Histopathology of gastric ADC showed the invasion of malignant cuboidal epithelial cells to submucosal layer. The percentages of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 immunoreactive cells in *Hp* + MNU compared with CO were 12.20% ± 1.10% versus 1.86% ± 1.49%, 13.21% ± 0.90% versus 2.84% ± 1.29%, 66.96% ± 5.91% versus 6.06% ± 6.48%, and 42.29% ± 0.08% versus 14.95% ± 0.12%, $P < 0.05$, respectively. The expression of apoptotic cells significantly increased in *Hp* + MNU compared with CO (8.41% ± 0.01% vs. 0.53% ± 0.02%, $P < 0.05$). Curcumin supplementation reduced the gastric cancer incidence compared with *Hp* + MNU. Percentages of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 immunoreactive cells in *Hp* + MNU + Cur compared with *Hp* + MNU were 4.76% ± 3.73% versus 12.20% ± 1.10%, 1.76% ± 0.94% versus 13.21% ± 0.90%, 24.71% ± 4.62% versus 66.96% ± 5.91%, and 24.99% ± 0.05% versus 42.29% ± 0.08, $P < 0.05$, respectively. The apoptosis expression was significantly improved (4.14% ± 0.16% vs. 8.41% ± 0.01%, $P < 0.05$). **Conclusion:** Curcumin can reduce gastric cancer incidence induced by *H. pylori* infection and MNU administration through the suppression of key proteins and apoptosis involved in carcinogenesis.

Key words: Apoptosis, curcumin, gastric cancer, *Helicobacter pylori*, *N*-methyl-*N*-nitrosourea

SUMMARY

- This study reported about curcumin (diferuloymethane) with many biological activities mediated by the efficient modulation of nuclear factor kappa B (NF-κB).

Helicobacter pylori infection and *N*-methyl-*N*-nitrosourea (MNU) administration induce gastric epithelial NF-κB p65, 8-hydroxy-2′-deoxyguanosine, cyclin D1, and Ki-67 activations and apoptosis expression. Hotspots of this study indicate that curcumin treatment may exert its attenuate gastric cancer effect through suppression of key proteins and apoptosis involved in carcinogenesis in the gastric epithelial cells. Curcumin might be a novel therapeutic strategy against gastric cancer induced by *H. pylori* infection and MNU administration.

Parameter	NF-κB p65	8-OHdG	Cyclin D1	Ki-67	Apoptosis	Gastric cancer incidence
Control	normal	normal	normal	normal	normal	0%
Gastric cancer (*Hp* + MNU)	increased	increased	increased	increased	increased	40%
Curcumin+ Gastric cancer (*Hp* + MNU + Cur)	decreased	decreased	decreased	decreased	decreased	0%

Abbreviations used: *H. pylori*: *Helicobacter pylori*; MNU: *N*-methyl-*N*-nitrosourea; MNNG: *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; NF-κB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor-alpha; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma; H_2O_2: Hydrogen peroxide; DMSO: Dimethyl sulfoxide; DAB: Diaminobenzidine; CFU: Colony-forming unit.

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INTRODUCTION

Gastric cancer can develop in any part of the stomach. Poorly detected, gastric cancer causes nearly one million annual deaths worldwide.[1,2] Gastric cancer is closely associated with *Helicobacter pylori* infection and *N*-nitroso compounds, such as *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) or *N*-methyl-*N*-nitrosourea (MNU).

H. pylori is a Gram-negative, spiral-shaped bacterium that has the unique ability of being able to colonize the human gastric mucosa and infects more than half of the world's population. *H. pylori* causes chronic

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gastritis, plays an etiologic role in peptic ulcer disease, and is considered a risk factor in the development of gastric cancer and gastric lymphoma.[3] Virulent strains of *H. pylori*, such as those containing CagA, can activate or induce the production of proinflammatory mediators. These mediators, namely nuclear factor-κB (NF-κB) and interleukin-8, lead to inflammation, epithelial damage, apoptosis, cell proliferation, and tumor progression.[4] *H. pylori* eradication is suboptimal because the current treatment regimens result in adverse side effects, poor compliance, and an increasing prevalence of antibiotic resistance.[5] Therefore, alternative treatments are of interest. MNNG or MNU is a common carcinogenic agent; it acts directly on the gastric and intestinal epithelial. This creates a basic group in the DNA chain due to alkylation and eventually generates mutations. It has been generally acknowledged that MNG can induce experimental gastric cancer.[6] Previous studies reported that the administration of MNU by oral gavage induced gastric cancer in rats and mice.[7] In addition to inducing gastric cancer in animal models, *N*-nitroso compounds are positively associated with stomach cancer risk in human studies.[8] In the north and northeastern part of Thailand, gastric cancer is significantly related to the ingested amount of nitrite, nitrate, and *N*-nitroso dimethylamine.[9]

Chemoprevention is promising as a preventive approach for cancer. Curcumin (diferuloymethane), a polyphenol compound, is an active ingredient of turmeric (*Curcuma longa*). Curcumin has chemopreventive properties. Importantly, curcumin is safe for humans and animals.[10] Curcumin shows the beneficial effects in many different types of cancer including colorectal cancer, breast cancer, skin cancer, and oral cancer.[11] Several signaling pathways implicated in carcinogenesis, including nuclear factor kappa B (NF-κB) signaling, have been modulated by curcumin treatment.[12] However, it is still unclear whether curcumin has any effect in gastric cancer and key proteins, NF-κB p65, 8-hydroxy-2'-deoxyguanosine (8-OHdG), cyclin D1, and Ki-67, and apoptosis condition involved in carcinogenesis induced by *H. pylori* inoculation and MNU administration.

The aim of this study was to examine the effect of curcumin on gastric carcinogenesis and key proteins induced by *H. pylori* and MNU in rats.

MATERIALS AND METHODS

Ethics

All experiments and procedures carried out on animals were approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Animal preparation

6-week-old male Wistar rats, weighing 180–220 g, were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. Rats were housed in a temperature controlled room at 25°C ± 1°C under standard conditions (12-h dark-light cycle).

Helicobacter pylori preparation and inoculation

H. pylori was taken from peptic ulcer patients during endoscopy at King Chulalongkorn Memorial Hospital. The collected bacteria were grown in Columbia agar plates supplemented with sheep's blood for 72 h at 37°C in an automatic CO_2-O_2 incubator under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2). On the day of the experiment, *H. pylori* colonies were swabbed into normal saline to form the suspension with concentration of 5×10^8 to 5×10^{10} colony forming unit/mL (CFU/mL).

Chemical preparations

60 mg/kg curcumin (95% purified curcumin, Cayman Chemical, MI, USA) was dissolved in corn oil and given daily to rats by intragastric tube for 30 weeks.

MNU (Sigma-Aldrich, MO, USA) was dissolved in distilled water at a concentration of 30 ppm for 20 weeks. Solution was freshly prepared three times a week and was administered *ad libitum* in a light-shielded bottle as drinking water.

Experimental protocol

All rats were fasted with free access to water *ad libitum*, for 18 h before the experiment, and were randomized into three experimental groups (five rats each) as follows.

- Control rats (CO): Rats were fed distilled water (1 mL/rat) orally twice a week for 20 weeks of the experiment
- *H. pylori* inoculation and 30 ppm MNU in drinking water for 20 weeks (*Hp* + MNU): Rats were inoculated with *H. pylori* suspension and MNU administration according to Sintara *et al.*[13] and Werawatganon.[14] Briefly, *H. pylori* suspension (10^{10} CFU/mL; 1 mL/rat) was inoculated to rats by intragastric tube twice a day at an interval of 4 h for 3 consecutive days. Two weeks after inoculation, rats received MNU in drinking water with a concentration of 30 ppm for 20 weeks
- *H. pylori* + MNU supplemented with 60 mg/kg curcumin (Cur) for 30 weeks (*Hp* + MNU + Cur): Rats were inoculated with *H. pylori* suspension and MNU administration as previously described. 60 mg/kg curcumin was fed daily to rats by intragastric tube for 30 weeks.

After 30 weeks, excision was done under anesthesia using intraperitoneal injection of thiopental (Jagsonpal Pharmaceuticals Ltd., Haryana, India; 60 mg/kg) after overnight fasting. All tissue samples were washed twice with ice-cold phosphate-buffered saline at the a concentration of 0.1 mol/L and pH 7.4, fixed in 4% phosphate-buffer paraformaldehyde, and then embedded in paraffin for histological studies.

Immunohistochemistry

Immunostaining for 8-OHdG or cyclin D1 was performed in paraffin-embedded sections by the following processes. Briefly, the tissue sections were deparaffinized with EZ-Prep™. After that, the sections were retrieved the antigen (8-OHdG or cyclin D1) with sodium chloride sodium citrate pH 6.5–7.5. Next, 1% hydrogen peroxide (H_2O_2, UltraView™ Inhibitor) was used to block endogenous peroxidase activity. Then, the primary antibody used for 8-OHdG (1:400; Japan Institute for the Control of Aging, Japan) or cyclin D1 (1:200; Thermo Scientific, MI, USA) was applied and incubated at 37°C for 60 min or 32 min, respectively. After that, the goat anti-Mouse IgG (UltraView™ HRP Multimer) was used as secondary antibody. Color was developed by UltraView™ diaminobenzidine (DAB) chromogen, UltraView™ H_2O_2, and UltraView™ copper. Then, the slides were counterstained with hematoxylin II and lithium carbonate.

The stomach sections were deparaffinized with xylene and gradually dehydrate in ethyl alcohol. Next, antigen retrieval was performed by immersing the sections in citric acid buffer (pH 6.0) in a microwave oven for 13 min. Endogenous peroxidase activity and nonspecific binding were blocked with 3% H_2O_2 (Merck, Hohenbrunn, Germany) for 5 min and 3% normal horse serum (Gibco, Carlsbad, CA, USA) for 20 min, respectively. After that, the sections were incubated with polyclonal antibody against the p65 subunit of NF-κB at a dilution of 1:100 (sc109; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or polyclonal antibody against the Ki-67 (1:300; Thermo

Scientific, MI, USA) in a humidified chamber for 1 h at room temperature. Then, the sections were incubated with biotinylated anti-rabbit immunoglobulin (DAKO, Glostrup, Denmark) in a humidified chamber for 30 min. The reaction was visualized using the substrate DAB (DAKO, Glostrup, Denmark). The sections were then counterstained with hematoxylin.

Under light microscope (Nikon E50i, Nikon Corporation, Japan), immunoreactive cells of NF-κB, 8-OHdG, cyclin D1, and Ki-67 were defined as those with dark-drown-stained nuclei of the gastric epithelial cells. To verify the expressions of these parameters in all animals, digital images were taken in low and high magnification field (×40 and ×100) from each sample using a microscope equipped with a digital camera (Nikon Digital Sight DS-Fi1, Nikon Corporation, Japan). The numbers of dark-brown stained in the nuclei of epithelial cells were counted using ImageScope software program version 10.2.2.2352 (Aperio Technologies, Inc., USA). The data were shown as the percentage (%) of immunoreactive cells.

Gastric apoptosis determination

Apoptosis was measured by the identification of apoptotic nuclei in sections of gastric by fragment end labeling of DNA (Apoptosis Detection Kit, Chemicon, USA). In brief, endogenous peroxidase activity was inactivated by 3% H_2O_2. The DNA fragments were allowed to bind an anti-digoxigenin antibody that was conjugated to a peroxidase. DAB was applied to develop dark-brown color, and then, the slides were counterstained with hematoxylin. The positively stained cells presented dark-brown nuclei under light microscopy. To verify the incidence of apoptosis, the numbers of dark-brown-stained cells were counted. One thousands of gastric epithelial cells were counted for each rat. The data were shown as percentage (%) of apoptotic cells calculating from this equation: the percentage of apoptotic cells (%) = (numbers of positive stained cells × 100)/1000.

Histopathological study

Each stomach was cut along the greater curvature into multiple 5 μm-thick sections and later stained with hematoxylin and eosin. Alterations of the gastric epithelial cells and the incidence of gastric carcinogenesis were determined by a pathologist.

Statistical analysis

NF-κB p65, 8-OHdG, cyclin D1, Ki-67, and apoptotic cell results were shown as mean ± standard deviation and analyzed with one-way analysis of variance and Tukey *post hoc* test. For all comparisons, a $P < 0.05$ was considered to be statistically significant. All the statistical tests were performed using the IBM SPSS Statistics 17 (SPSS Inc., USA) for Windows.

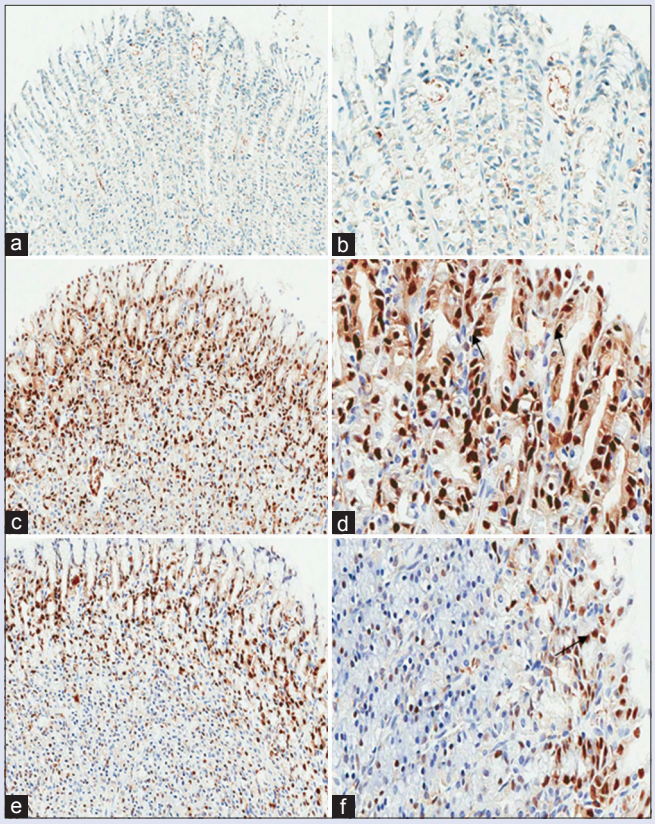

Figure 1: Immunohistochemical staining of nuclear factor kappa B p65 antibody in representative tissue specimens. (a and b) control rats; (c and d) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration group; (e and f) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks. Images were obtained at ×40 (a, c, and e) and ×100 (b, d, and f). Diaminobenzidine staining was used to highlight nuclei in sections (dark-brown stain, arrows)

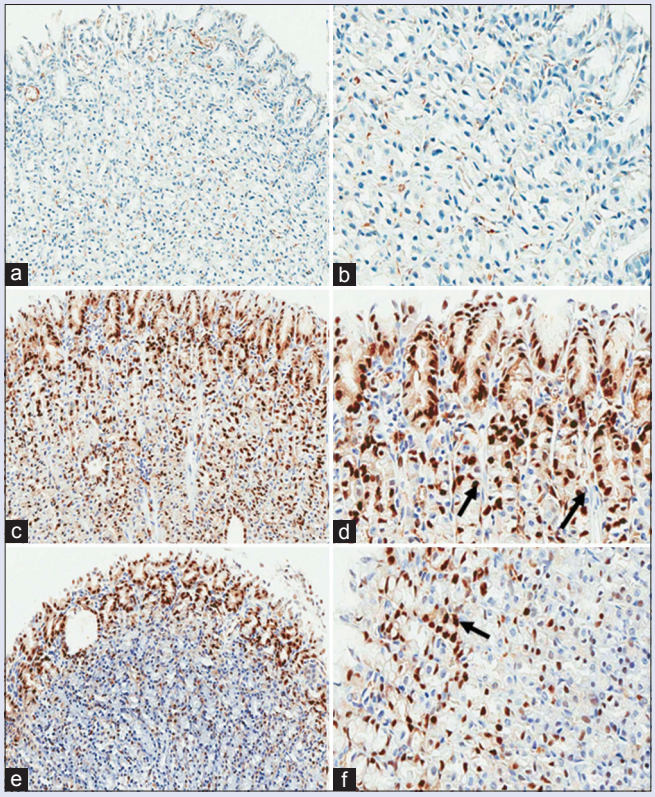

Figure 2: Immunohistochemical staining of 8-hydroxy-2′-deoxyguanosine antibody in representative tissue specimens. (a and b) control rats; (c and d) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration group; (e and f) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks. Images were obtained at ×40 (a, c, and e) and ×100 (b, d, and f). Diaminobenzidine staining was used to highlight nuclei in sections (dark-brown stain, arrows)

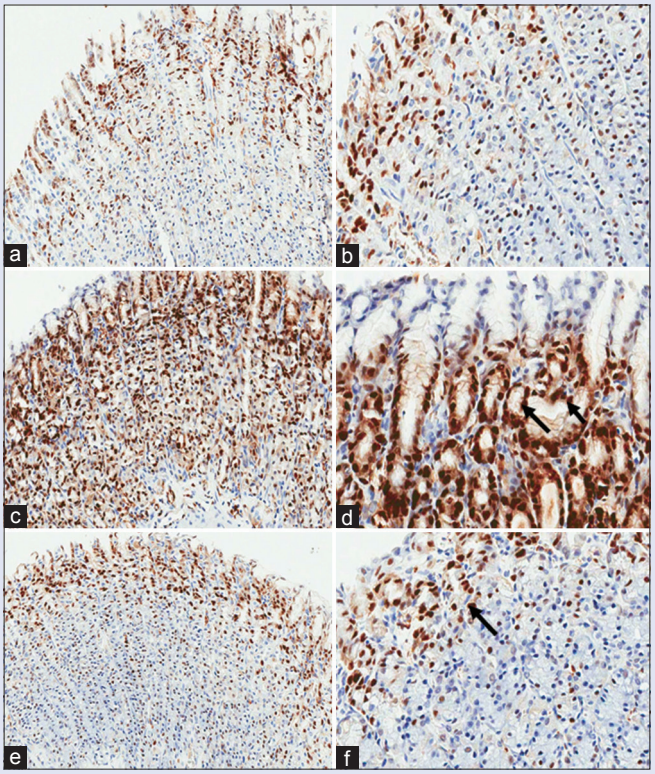

Figure 3: Immunohistochemical staining of cyclin D1 antibody in representative tissue specimens. (a and b) control rats; (c and d) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration group; (e and f) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks. Images were obtained at ×40 (a, c, and e) and ×100 (b, d, and f). Diaminobenzidine staining was used to highlight nuclei in sections (dark-brown stain, arrows)

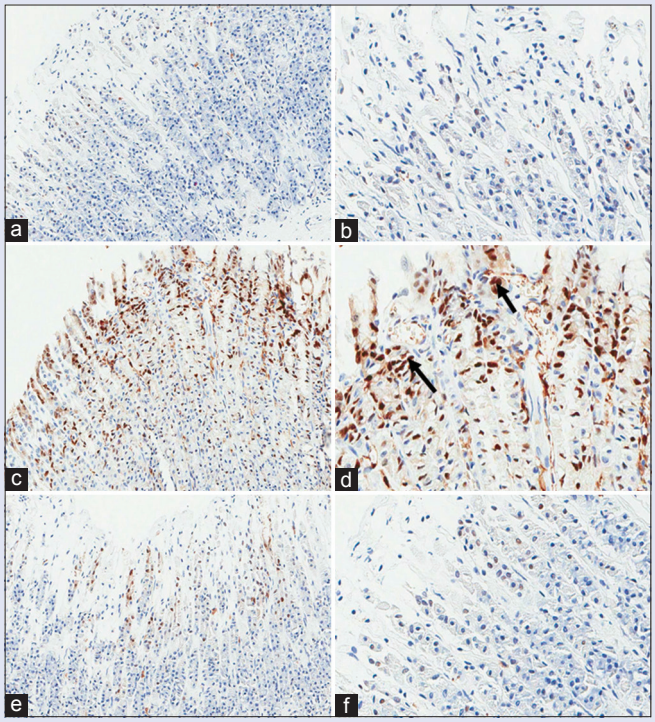

Figure 4: Immunohistochemical staining of Ki-67 antibody in the representative tissue specimens. (a and b) control rats; (c and d) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration group; (e and f) *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks. Images were obtained at ×20 (a, c, and e) and ×40 (b, d, and f). Diaminobenzidine stained immunoreactive cells (dark brown stain in their nuclei, arrows)

RESULTS

Changes in nuclear factor kappa B p65, 8-hydroxy-2′-deoxyguanosine, cyclin D1, and Ki-67 protein expressions

We used immunohistochemistry method to analyze the expression of proteins, NF-κB p65, 8-OHdG, cyclin D1, and Ki-67, expressions and showed as dark-brown stain in their nuclei [Figures 1-4, respectively]. The average percentages of immunoreactive cells of these proteins are shown in Figure 5a-d and Table 1. From the results, the expressions of these proteins significantly increased in *Hp* + MNU group compared with CO group. Rats in *Hp* + MNU group were divided into subgroup no-ADC and ADC groups. The percentages of NF-κB p65, 8-OHdG, and Ki-67 immunoreactive cells in no-ADC group were not significantly different when compared with CO group (4.49% ± 3.65% versus 1.86% ± 1.49%, 3.76% ± 3.43% versus 2.84% ± 1.29%, and 14.93% ± 0.24% versus 14.95% ± 0.12%, $P > 0.05$, respectively). The percentages of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 immunoreactive cells in ADC group were significantly different when compared with CO group (12.2% ± 1.1% vs. 1.86% ± 1.49%, 13.21% ± 0.90% vs. 2.84% ± 1.29%, 66.96% ± 5.91% vs. 6.06% ± 6.48%, and 42.29% ± 0.08% vs. 14.95% ± 0.12%, $P < 0.05$, respectively). Curcumin supplementation for 30 weeks in *Hp* + MNU + Cur group reduced the cancer incidence, resulting in a decrease of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 expressions compared with ADC group. The percentages of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 immunoreactive cells in *Hp* + MNU + Cur group compared with ADC group were 4.76% ± 3.73% versus 12.2% ± 1.1%, 1.76% ± 0.94% versus 13.21% ± 0.90%, 24.71% ± 4.62% versus 66.96% ± 5.91% and 24.99% ± 0.25% versus 42.29% ± 0.08%, $P < 0.05$, respectively.

Table 1: Results of nuclear factor kappa B p65, 8-hydroxy-2′-deoxyguanosine, cyclin D1, Ki-67, and apoptotic immunoreactive cells (%) in all experimental groups

Parameters/ group	CO (*n*=5)	*Hp* + MNU (*n*=5)		*Hp* + MNU + Cur (*n*=5)
		no-ADC (*n*=3)	ADC (*n*=2)	
NF-κB p65	1.86±1.49	4.49±3.65[b]	12.20±1.10[a]	4.76±3.73[b]
8-OHdG	2.84±1.29	3.76±3.43[b]	13.21±0.90[a]	1.76±0.94[b]
Cyclin D1	6.06±6.48	35.78±2.03[a,b]	66.96±5.91[a]	24.71±4.62[a,b]
Ki-67	14.95±0.12	14.93±0.24[b]	42.29±0.08[a]	24.99±0.25[b]
Apoptosis	0.53±0.02	0.57±0.01[b]	8.41±0.01[a]	4.14±0.16[b]

Data were presented as the mean±SD of the percentage of NF-κB p65, 8-OHdG, cyclin D1, Ki-67, and apoptosis immunoreactive cells in all experimental groups. [a]$P<0.05$ versus control rats (CO); [b]$P<0.05$ versus adenocarcinoma rats (ADC). No-ADC: No adenocarcinoma was found; *Hp* + MNU: *Helicobacter pylori* infection and MNU administration; *Hp* + MNU + Cur: *Helicobacter pylori* infection and MNU administration supplemented with 60 mg/kg curcumin for 30 weeks; SD: Standard deviation; MNU: *N*-methyl-*N*-nitrosourea; 8-OHdG: 8-hydroxy-2′-deoxyguanosine

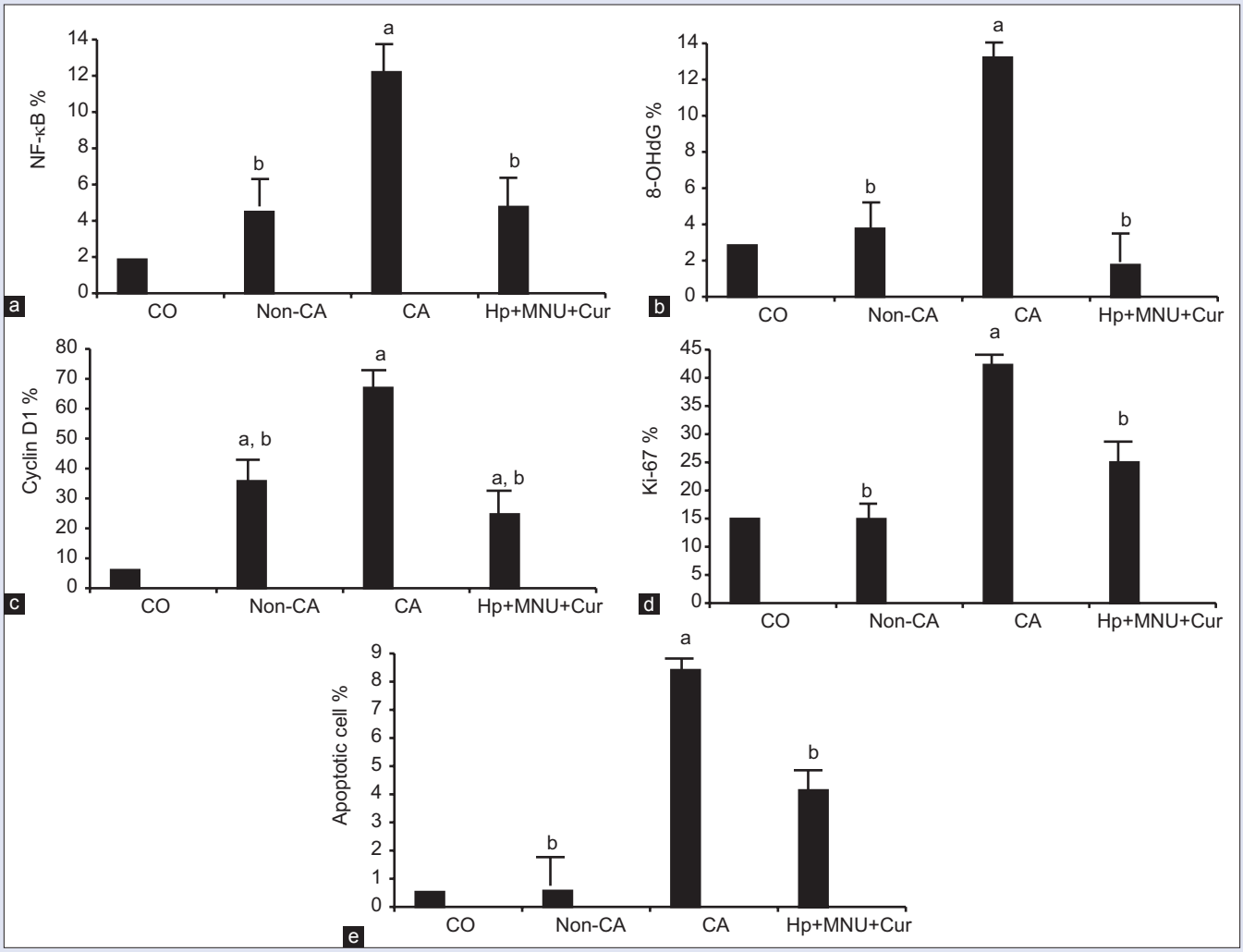

Figure 5: Mean ± standard deviation of the percentage of nuclear factor kappa B p65 (a), 8-OHdG (b), cyclin D1 (c), Ki-67 (d), and apoptotic cell (e) immunoreactive cells in all experimental groups. [a]$P < 0.05$ versus control rats (CO); [b]$P < 0.05$ versus adenocarcinoma rats. No gastric adenocarcinoma was found; *Hp* + MNU + Cur: *H. pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks; CO: Control rats; NF-κB: Nuclear factor kappa B; 8-OHdG: 8-hydroxy-2′-deoxyguanosine

Gastric epithelial cell apoptosis expression

The percentage of apoptotic cells was significantly increased in ADC group when compared with CO group (8.41% ± 0.01% vs. 0.53% ± 0.02, $P < 0.05$, respectively). After treatment with curcumin, the percentage of apoptotic cells was significantly decrease in *Hp* + MNU + Cur group (4.14% ± 0.16% vs. 8.41% ± 0.01%, $P < 0.05$, respectively) compared with ADC group. The average percentages of apoptotic cells of all groups are shown in Figure 5e and Table 1. Figure 6 shows gastric sections processed for apoptosis assay by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling reaction.

Helicobacter pylori and *N*-methyl-*N*-nitrosourea associated with gastric cancer incidence and pathogenesis

Gastric cancer incidence is shown in Table 2. There was no adenocarcinoma (ADC) of glandular stomach in CO [Figure 7a and b] and *Hp* + MNU + Cur groups. In *Hp* + MNU group, ADC was found in the glandular stomach of two rats ($n = 5$). The cancer incidence in this group was 40%. Rats in *Hp* + MNU group was divided into subgroup no-ADC ($n = 3$) and ADC ($n = 2$) groups. In ADC group, gastric ADC was found, while in no-ADC group, no gastric ADC was found in the glandular stomach.

Table 2: Histopathological changes of gastric tissue in all experimental groups

Group	Normal	ADC		Cancer incidence (%)
		No-ADC	ADC	
CO (n=5)	5	-	-	-
Hp + MNU (n=5)	-	3	2	40
Hp + MNU + Cur (n=5)	5	-	-	-

CO: Control rats; *Hp* + MNU: *Helicobacter pylori* infection and MNU administration; *Hp* + MNU + Cur: *H. pylori* infection and MNU administration supplemented with 60 mg/kg curcumin for 30 weeks; No-ADC: No adenocarcinoma was found; ADC: Adenocarcinoma was found; MNU: *N*-methyl-*N*-nitrosourea

Histopathology of ADC showed the invasion of malignant cuboidal epithelial cells to submucosal layer [Figure 7c and d], inflamed lamina propria, disorganized of gland and dysplastic gland [Figure 7e and f] and ADC of the gastric mucosa [Figure 7g and h]. Curcumin

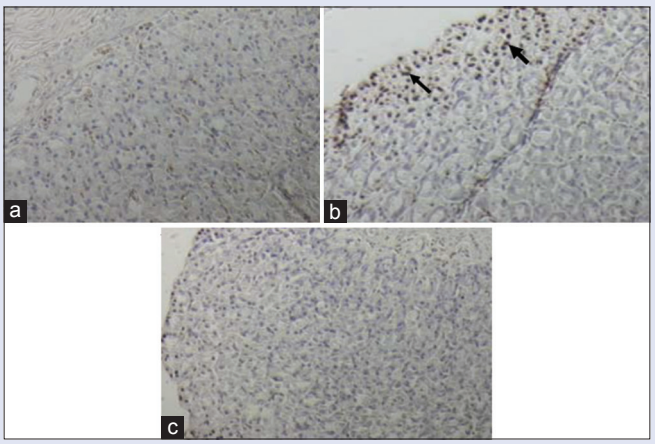

Figure 6: Representative gastric sections processed for the apoptosis assay by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling reaction (×20). (a) CO group; (b) *Hp* + MNU group; (c) *Hp* + MNU + Cur group showed a decrease in gastric epithelium apoptosis. The arrows indicate terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive gastric epithelial cell apoptosis. CO: Control rats; *Hp* + MNU: *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration; *Hp* + MNU + Cur: *H. pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks

supplementation in *Hp* + MNU + Cur group, no gastric ADC was found and essentially normal [Figure 7i and j].

DISCUSSION

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-associated mortality worldwide, with an estimated 952,000 new cases diagnosed and 723,000 deaths registered in 2012.[15] The previous study showed that the gastric histopathology in the *H. pylori*-infected revealed mild-to-moderate *H. pylori* colonization, inflammation, reactive oxygen species (ROS) production, and gastric epithelial cell apoptosis.[16] Furthermore, our results showed that *H. pylori* CagA, VacA strains can induce epithelial cell apoptosis in rats. The CagA gene or expression of VacA might be involved in gastroduodenal diseases by affecting apoptosis.[16] *H. pylori* induces a host inflammatory response including production of cytokines, resulting in mucosal damage. In several models of gastric cancer, *H. pylori* could enhance the carcinogenic effect of *N*-nitroso compound. Sintara *et al.*[13] and Werawatganon[14] found that *H. pylori* infection and/or MNU administration increased the incidence of ADC in rats, compared to another group. Shimizu *et al.* found that the incidence of gastric ADC in Mongolian gerbils treated with *H. pylori* and MNU was significantly higher than those treated with MNU alone.[17] *H. pylori* and MNU worked synergistically to promote gastric carcinogenesis. *H. pylori* infection led to inflammation and epithelial cell destruction, which made gastric tissues more prone to chemical carcinogen exposure. In 2006, Prabjone *et al.* investigated the effects chronic *H. pylori* infection on inflammatory response in rats and found that the increase in inflammatory cytokine in the *H. pylori*-infected rats.[18] *H. pylori* is associated with an increased risk for the development of both peptic ulcer and gastric cancer diseases.

Several previous investigations have shown the chemoprevention of curcumin. Curcumin, which is commonly called diferuloylmethane, is derived from *C. longa*, a plant of the ginger family.[19-21] Curcumin is able to suppress the proliferation and survival of cancer cells by directly or indirectly binding to various targets, including transcription factors,

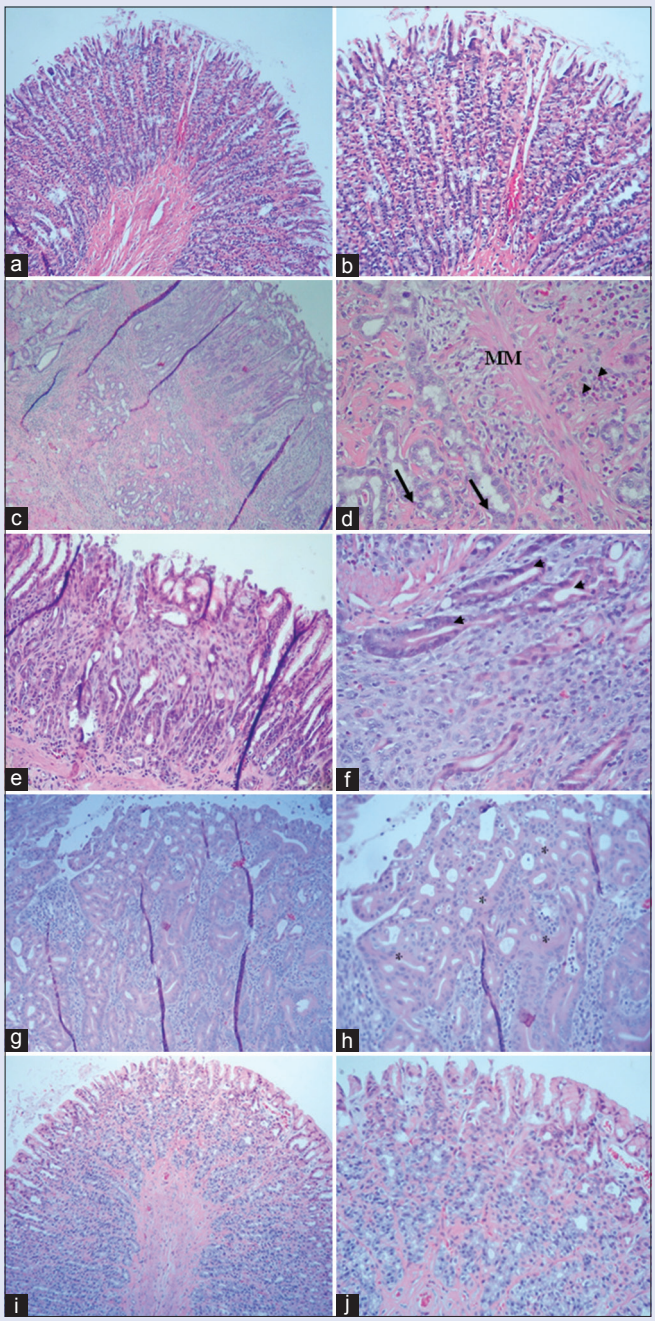

Figure 7: Histopathological changes of rat gastric tissue in all experimental groups. (a and b) CO group; (c-h) *Hp* + MNU group; (i and j) *Hp* + MNU + Cur group (H and E, ×40, ×100). Arrows denote the invasion of malignant cuboidal epithelial cells to submucosal layer. Arrowheads denote dysplastic gland. Asterisks denote ADC of gastric mucosa. MM: Muscularis mucosal layer; CO: Control rats; *Hp* + MNU: *Helicobacter pylori* infection and *N*-methyl-*N*-nitrosourea administration; *Hp* + MNU + Cur: *H. pylori* infection and *N*-methyl-*N*-nitrosourea administration supplemented with 60 mg/kg curcumin for 30 weeks; ADC: Adenocarcinoma

growth factors, and several proteins that are involved in cell signal transduction pathways.[22] Administration of 0.5% and 2.0% of commercial grade curcumin reduced the number of mice with forestomach tumors.[23] Administration of 1 and 2 g/mL of curcumae extract solution during MNNG administration for 40 weeks showed the reduction of tumor incidences in MNNG and 10% NaCl-induced gastric cancer in rats.[6] In

addition, oral gavage of MNU and NaCl induced a 100% cancer incidence in rats. The histological appearance showed that curcumin could attenuate the gastric carcinogenesis induced by MNU and NaCl in rats.[13] The present study demonstrated that inoculation of *H. pylori* suspension and 30 ppm MNU in drinking water for 20 weeks induced a 40% cancer incidence in rats. The histological results showed that curcumin could attenuate the gastric carcinogenesis induced by *H. pylori* and MNU in rats. This is in agreement with previous studies.[6,23] Activation of NF-κB expression plays a major role in carcinogenesis.[24,25] Our study showed that expression of NF-κB was associated with gastric cancer in rats. This alteration is in good agreement with other reports.[26,27] Activation of NF-κB appeared to play a major role of keratinocyte transformation into squamous cell carcinoma (SCC) in mice.[26] Patients with 49% prostate ADC showed NF-κB overexpression that correlated with advanced tumor stage.[27] Curcumin has a chemopreventive property, resulting in suppressing NF-κB activation. Previous study confirmed that curcumin supplementations for 3 and 20 weeks significantly decreased IkappaBα phosphorylations in benign tumor-bearing rats.[13] Our results showed that curcumin supplementation for 30 weeks prevented carcinogenesis by declining NF-κB expression. 8-OHdG is a potent biomarker of oxidative DNA damage and a factor of initiation and promotion of carcinogenesis.[28] In the present study, we showed that 8-OHdG expression significantly increased in the *H. pylori* and MNU group. From the many previous reported, 8-OHdG expression is increased in various types of cancers in patients. It is likely that 8-OHdG expression might participate in carcinogen-induced forestomach SCC. Curcumin showed a potent scavenger of ROS.[29] The reduction of ROS prevents the formation of 8-OHdG. This study showed that 60 mg/kg curcumin supplementation for 30 weeks diminished 8-OHdG expression in *H. pylori* and MNU-induced carcinogenesis. The present study also demonstrated that the cyclin D1 expression, a positive cell cycle regulator, which significantly increased in *H. pylori* and MNU group. Previous studies showed that immunoreactive cells of cyclin D1 correlated with cell proliferation of gastric cancer patients.[30] *H. pylori* induces gastric epithelial cell apoptosis both *in vitro* and *in vivo*.[31,32] Extensive research over the last half century has revealed the therapeutic potential of curcumin in tumor progression, including inducing apoptosis.[19] In 2006, Cabral *et al*. showed that the expression of pro-apoptotic proteins such as Bax and Bak was higher than anti-apoptotic proteins including Bcl-2 and Bcl-XL in patients with *H. pylori* gastritis.[33] The studies have shown that the *H. pylori* colonized stomach contains more apoptotic epithelial cells than normal control. Moreover, the increased numbers of apoptotic epithelial cells decrease to normal after eradication of *H. pylori*.[32] Gerdes *et al*.[34] showed that Ki-67 antigen expression may reflect the proliferative activity of the tumor cells. In addition, it is highly correlated with the development, metastasis, and prognosis of malignant tumors. The present study showed that 60 mg/kg curcumin supplementation for 30 weeks attenuated Ki-67 and apoptotic cell expressions in *H. pylori* and MNU-induced carcinogenesis.

CONCLUSION

Curcumin can reduce cancer incidence induced by *Hp* + MNU. Pathogenesis of gastric cancer is associated with the activation of NF-κB p65, 8-OHdG, cyclin D1, and Ki-67 and apoptotic cell expressions. Curcumin might be a novel therapeutic strategy against gastric cancer induced by *H. pylori* infection and MNU administration.

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Conflicts of interest

There are no conflicts of interest.

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