RESEARCH ARTICLE

# Evaluating the Role of Curcum Powder as a Protective Factor against Bladder Cancer - An Experimental Study

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## Abstract

**Throughout human history, plant products have been used for many purposes including as medicines. Herbal products and spices can be used as preventive agents against cancer due to their antimicrobial, antioxidant and antitumorigenic properties. This study was designed to evaluate the potential protective effect of curcum in rats administered nitrosamine precursors; dibutylamine (DBA) and sodium nitrate (NaNO3); and infected with *Escherichia coli* (*E. coli*) and also to monitor changes in nuclear factor the Kappa B p65 (NF-κB p56) pathway and its downstream products, Bcl-2 and interleukin-6 (IL-6), in parallel with nitrosamine precursors, *E. coli* and curcum treatment. Rats were divided into three groups (n=25 each; except of control group, n+20). Group I a normal control group, group II administered DBA/NaNO3 in drinking water and infected with *E. coli* and group III was administered DBA/NaNO3 in drinking water, infected with *E. coli* and receiving standard diet containing 1% curcum powder. Histopathological examination reflected that the curcum treated group featured a lower incidence of urinary bladder lesions,and lower levels of NF-κB, Bcl-2 and IL-6, than the group receiving nitrosamine precursor and infected with *E. coli*. These findings suggested that curcum may have a protective role during the process of bladder carcinogenesis by inhibiting the NF-κB pathway and its downstream products.**

**Keywords:** Bladder carcinogenesis - curcum - *E. coli* - NF-κB p65 - Bcl-2 - IL-6

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## Introduction

Cancer is a leading cause of death worldwide. More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030 (WHO, 2009). Over the last several years the incidence of bladder cancer has been increasing (Jemal et al., 2007). Nitrosamines are considered one of the most important environmental carcinogens (Bartsch and Montesano, 1984). It was found that N-nitrosamines can be formed in bladder in the presence of nitrate reducing bacteria (Dominique, 2007). It is estimated that 20-25% of all human cancers are caused by chronic infection and inflammation (De Marzo et al., 2007). One of the key molecules that link chronic inflammation and cancer is represented by NF-κB family of transcription factors (Karin et al., 2002). NF-κB regulates the transcription of genes for proinflamatory cytokines (e.g. IL-6 and tissue necrosis factor alpha TNFα), adhesion molecules (Barnes and Karin, 1997) and the expression of several pro-survival genes (e.g. Bcl-2) (Calzado et al., 2007).

Herbal medicine and spices can be used as preventive measurement against cancer due to their antimicrobial, antioxidant, and antitumorigenic properties, as well as their direct suppressive effect on carcinogen bioactivation (Kaefer and Milner, 2008). More natural and dietary compounds including curcumin have been recognized as cancer chemopreventive agents due to its non-toxic and anti-carcinogenic properties (Sarkar et al., 2009). Curcumin (diferuloylmethane) is a major constituent of the yellow spice turmeric derived from the rhizomes of Curcuma longa. It is safe and nontoxic and has demonstrable antitumor, antiinflammatory, apoptotic, and antioxidant properties. Curcumin also inhibits tumor metastasis, invasion, and angiogenesis (Kunnumakkara et al., 2007; 2008). The main mechanism of action of curcumin is inhibition of the transcription factor NF- κB (Thangapazham et al., 2006). As curcumin inhibits NF-κB, all of its products will be inhibited.

Curcumin also decrease formation of free radicals, potent carcinogens, and induce liver detoxification enzymes (Thangapazham et al., 2006). This study amid to evaluate the possible protective effect of curcum powder during bladder carcinogenesis and the changes in NF-κB pathway and its related products.

## Materials and Methods

*Experimental Animals and Dosing*

Seventy five male albino rats, weighing 50-60 gm were divided into three groups (n=25 each; except of control group, n+20). Group I was normal control group, group II received nitrosamine precursor; 1000 ppm DBA

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and 2000 ppm NaNO3; in drinking water as previously described by (El Gendy et al., 2010) and infected by 0.1 ml saline containing suspension of *E. coli* in the bladder (approximately $2x10^6$ organisms), as previously described by (Higgy et al., 1987) and group III received DBA/NaNO3 in drinking water, infected with *E. coli* and received standard diet containing 1% curcum powder (obtained from commercial market) mixed in the diet, 2 weeks prior *E. coli* infection and all over the experimental period (Thapliyal et al., 2003).

*Laboratory procedures*

At three, six and nine months, rats in different groups were sacrificed; bladder was removed and an autopsy samples were taken from the urinary bladder of rats in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examination through the electric light microscope (Banchroft et al., 1996). Other specimens of bladder were removed immediately from sacrificed animals, washed with saline, dried, cut into weighed pieces and kept frozen at-80°C then tissue homogenate was prepared according to Tripathi and Jena (2010) for NF-κB p65 determination by ELISA kit (Glory Science Co., Ltd, USA) following the manufacturer instructions. For biochemical investigations, serum was separated by centrifugation and stored at -80ºC until used for of Bcl-2 determination by ELISA kit (the Calbiochem Laboratories, USA, Cat QIA23) and IL-6 determination by ELISA kit (IBL, USA, Cat IB39452) following the manufacturer instructions

*Statistical analysis*

All statistical analyses were performed using GraphPad Prism version 5.01 software package (GraphPad Software, Inc. CA, USA). Data are presented as mean±standard deviation (S.D). To determine differences between groups, analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc analysis was used for multiple comparisons between different groups. The level of statistical significance was set at probability P≤0.05.

## Results

Bladder histopathological changes are presented in Figure 1. Group receiving nitrosamine precursor and infected with *E. coli* (group II) showed the highest incidence of urinary bladder lesions represented by hyperplasia and dysplasia while curcum treated group (group III) showed only minor histopathological changes represented by congestion in the blood capillaries of lamina propra , focal desquamation and focal hemorrhage. Curcum treated group also showed lower level of NF-κB, Bcl-2 and IL-6 than group receiving nitrosamine precursor and infected with *E. coli*.

Level of NF-κB p65, Bcl-2 and IL-6 are presented in Table 1. As indicated in Table 1, the mean±SD of NF-κB p65, Bcl-2 and IL-6 levels were significantly lower in curcum treated group (group III) than nitrosamine

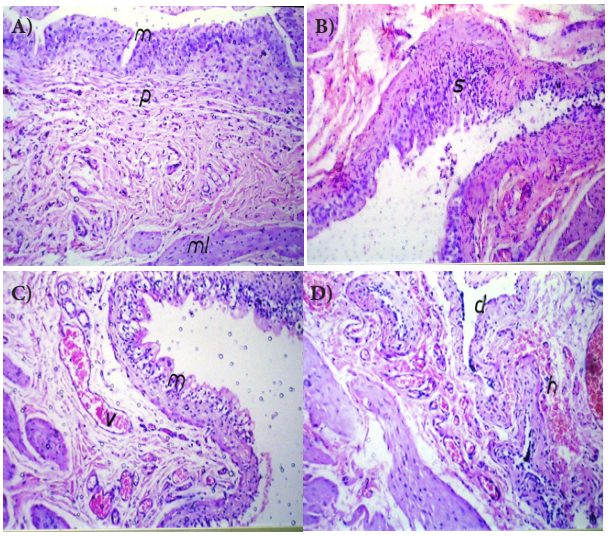


**Figure 1. Histopathological Changes in Different Treated Groups; Control Group (Group I), *E. coli* and Nitrosamine Precursors Group (Group II), and *E. coli*, Nitrosamine Precursors and Curcum Group (III).** 1A) urinary bladder of rat in control group (group I). showing normal histological structure of the lining mucosal epithelium (M), underlining lamina propria (P) and muscularis (Ml). 1B): urinary bladder of rat in *E. coli* and nitrosamine precursors group (group II) showing hyperplasia and dysplasia (s) in mucosal lining epithelium. 1C): urinary bladder of rat in *E. coli*, nitrosamine precursors and curcum group (group III) showing congestion in the blood capillaries of lamina propra. 1D): Urinary bladder of rat in *E. coli*, nitrosamine precursors and curcum group (group III) showing desquamation of mucosal epithelium (d) and focal hemorrhage in the lamina propria

**Table 1. Tissue Homogenate Level of NF-κBp65 (ng/ml), Serum Level of Bcl-2 (U/ml) and IL-6 (pg/ml) in Different Treated Groups; Control Group (group I), *E. coli* and Nitrosamine Precursor Group (group II), and *E. coli*, Nitrosamine Precursor and Curcum Group (group III)**

| Group | | NF-κBp65 (ng/ml) | | | Bcl-2 (U/ml) | | | IL-6 (pg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 months | 6 months | 9 months | 3 months | 6 months | 9 months | 3 months | 6 months | 9 months |
| I | Range | 0.51-0.67 | 0.51-0.75 | 0.60-0.87 | 293.3-331.9 | 310.7-331.9 | 305.8-348.2 | 12.7-15.2 | 13.6-17 | 14.5-17.0 |
| | Mean±S.D | 0.57±0.07 | 0.61±0.08 | 0.70±0.11 | 309. 1±14.6 | 320.6±7.47 | 323.5±14.3 | 14.1±0.87 | 14.7±1.23 | 15.6±0.89 |
| II | Range | 0.94-1.40 | 1.30-1.87 | 1.58-1.95 | 377.1-399.9 | 484.7-595.5 | 522.1-726.6 | 22.0-27.5 | 26.6-34.8 | 35.9-43.5 |
| | Mean±S.D | 1.19±0.19[a,c] | 1.52±0.21[a,c] | 1.72±0.14[a,c] | 387.1±8.40[a,c] | 544.5±37.1[a,c] | 592.6±75.2[a,c] | 24.8±2.20[a,c] | 30.7±3.20[a,c] | 40.6±2.69[a,c] |
| III | Range | 0.60-0.74 | 0.63-0.71 | 0.63-0.86 | 305.8-337.9 | 312.8-339.9 | 305.8-345.0 | 12.2-15.9 | 13.3-17.9 | 13.2-18.8 |
| | Mean±S.D | 0.66±0.05[b] | 0.68±0.03[b] | 0.73±0.08[b] | 324±13.8[b] | 325.3±8.92[b] | 327.4 ±15.1[b] | 14.1±1.39[b] | 15.3±1.67[b] | 16.0±2.21[b] |

[a]Significantly different from control group (group I) at P<0.05, [b]Significantly different from *E. coli*+nitrosamine precursors group (group II) at P<0.05, [c]Significantly different from *E. coli*+nitrosamine precursors + Curcum group (group III) at P<0.05.

precursor and *E. coli* group (group II) all over the experiment duration. At three months interval, NF-κB p65 level was significantly higher in group II (1.19±0.19 ng/ml) than control group (0.57±0.07 ng/ml) and NF-κB p65 level in curcum treated group (group III) (0.66±0.05 ng/ml) was significantly lower than group II. At six months interval, NF-κB p65 level was significantly higher in group II (1.52±0.21 ng/ml) than control group (0.61±0.08 ng/ml) and NF-κB p65 level in curcum treated group (group III) (0.68±0.03 ng/ml) was significantly lower than group II. At nine months interval, NF-κB p65 level was significantly higher in group II (1.72±0.14 ng/ml) than control group (0.70±0.11 ng/ml) and NF-κB p65 level in curcum treated group (group III) (0.73±0.08 ng/ml) was significantly lower than group II. Regarding the anti-apoptotic protein (Bcl-2); at three months its level was significantly increased in groups II (387.1±8.40 U/ml) compared with the control group level (309. 1±14.6 U/ml) and Bcl-2 level in curcum treated group (group III) (324.0±13.8 U/ml) was significantly lower than group II. At six months interval; Bcl-2 level was significantly higher in group II (544.6±37.1U/ml) compared with the control group level (320.6±7.47 U/ml). On the other hand curcum treated group (group III) (325.3±8.92 ng/ml) was significantly lower than group II. At nine months interval Bcl-2 level was significantly increased in groups II (592.6±75.2 U/ml) compared with the control group level (323.5±14.3 U/ml) and Bcl-2 level in curcum treated group (group III) (327.4±15.1 U/ml) was significantly lower than group II. Finally IL6 level was significantly higher in group II at three months interval (24.8±2.20 pg/ml), six months interval (30.7±3.20 pg/ml) and nine months (40.6±2.69 pg/ml) than control group at three months (14.1±0.87 pg/ml), six months (14.7±1.23 pg/ml) and nine months (15.6±0.89 pg/ml) respectively. IL6 level was significantly lower in group III at three months interval (14.1±1.39 pg/ml), six months interval (15.3±1.67 pg/ml) and nine months (16.0±2.21 pg/ml) than group II at three months, six months and nine months respectivel.

## Discussion

In our study curcum treated group showed lower incidences of urinary bladder lesions, lower level of NF-κB, Bcl-2 and IL-6 than group receiving nitrosamine precursor and infected with *E. coli*. These results indicate that curcumin has a strong protective effect during the process of bladder carcinogenesis. Accumulating evidence suggests that curcumin has a diverse range of molecular targets, which supports the notion that curcumin influences numerous biochemical and molecular cascades. Among its molecular targets are transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis (Goel et al., 2008).

Activation of NF-kappaB occurs mainly via I-kappaB kinase (IKK)–mediated phosphorylation of inhibitory molecules (Viatour et al., 2005). Curcumin blocks the NF-kappaB signaling and inhibits IKK activation, thereby suppressing proliferation of head and neck squamous cell carcinoma (Aggarwal et al., 2006).

Our result is in agreement with Kamat et al. (2009) who reported that curcumin was able to inhibit NF-κB and NF-κB regulated gene products including Bcl-2 both in vitro and in vivo.

Our result is also in agreement with Wang et al. (2011) who reported that curcumin was able to induce apoptosis in bladder cancer cell line and this occurred via blockage of Phosphatidylionositol-3-kinase (PI3K)/ AKT signaling pathway. Akt is a serine/threonine kinase that promotes cell growth and blocks apoptosis. Wang et al. (2011) reported that blockage of PI3K/Akt signaling pathway led to altered balance between pro-apoptotic (increase in Bax level) and anti-apoptotic members (decrease in Bcl-2 level) of Bcl-2 family.

Moreover, LoTempio et al. (2005) and Wang et al. (2008) reported that curcumin treatment of several head and neck squamous cell carcinoma cell lines resulted in growth suppressive effect which was mainly mediated via the effects of curcumin on the NF-κB pathway. In addition, the expression levels of multiple NF-κB regulated gene products (including COX-2, Bcl-2, IL-6, and IL-8) were reduced.

*E. coli* enhancing effect on the carcinogenicity of nitrosamine precursor can be explained by the ability of the bacteria to increase nitrite level which through subsequent nitrosation giving rise to highly carcinogenic N-nitroso compounds (Janković and Radosavljević, 2007). The ability of *E. coli* infection to increase nitrite level can be explained by several mechanisms. First *E. coli* is capable of reducing nitrate to nitrite by a membrane-bound nitrate reductase enzyme (Nishimura et al., 2008). Second, it was proven that *E. coli* lipopolysaccharide (LPS); a major cell wall component of *E. coli*; when instilled intravesically or intraperitoneally is capable of production of inducible nitric oxide synthase (iNOS) (Wheeler et al., 2001; Chen et al., 2006) which is an endogenous source of nitrite (Smith et al., 1994). In our study curcum could have led to decrease in nitrosamines synthesis and decreasing the carcinogenic ability of nitrosamines precursor by the reduction in iNOS level as Thangapazham et al. (2006) reported that curcumin treatment showed antitumorigenic potential by significantly reducing the levels of iNOS.

In conclusion curcum treatment was able to reduce the incidence of bladder lesions and this was accompanied by reduced level of NF-κB, Bcl-2 and IL-6 suggesting that curcum can prevent the deleterious effect of nitrosamine precursor plus *E. coli* by inhibiting NF-κB pathway and its products.

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