"Study of chronic E. coli infection as a risk factor for bladder cancer- an experimental study"

A Thesis submitted for the partial fulfillment of Master Degree in Pharmaceutical Sciences (Biochemistry)

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بسم الله الرحمن الرحيم

"رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ"

صدق الله العظيم

سورة النَّمْل آية ١٩

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LIST OF CONTENTS

Publications Related To The Thesis

Publications related to the thesis

INFECTIOUS AGENTS AND CANCER

RESEARCH ARTICLE Open Access

Role of chronic *E. coli* infection in the process of bladder cancer- an experimental study

Hala El-Mosalamy[1*], Tarek M Salman[2], Abeer M Ashmawey[3] and Nada Osama[4]

Abstract

Background: Bladder cancer is a common malignancy in Egypt. A history of urinary tract infection can be considered as a risk factor for bladder cancer. *Escherichia coli (E. coli)* infection is responsible for 70% of urinary tract infection. This study aimed to evaluate the role of chronic *E. coli* infection during bladder carcinogenesis. In order to achieve this aim, we investigated the histopathological changes in bladder tissue and measured the level of nuclear factor kappa p65 (NF-κBp65), Bcl-2 and interleukin 6 (IL-6) in four groups each consisting of 25 male albino rats except of control group consisting of 20 rats. The first group was normal control group, the second group was infected with *E. coli*, the third group was administered nitrosamine precursor, and the forth group was infected with *E. coli* and administered nitrosamine precursor.

Results: The histopathological examination revealed that *E. coli* infected group was able alone to produce some histopathological changes in bladder tissue and that nitrosamine precursor plus *E. coli* group showed highest incidences of urinary bladder lesions than the nitrosamine precursor group. NF-κBp65, Bcl-2 and IL-6 levels were significantly higher in nitrosamine precursor plus *E. coli* group than the other groups.

Conclusion: These findings suggested that urinary bladder infection by *E. coli* may play a major additive and synergistic role during bladder carcinogenesis.

Keywords: Bladder carcinogenesis, *E. coli*, NF-κBp65, Bcl-2, IL-6

Background

Bladder cancer is a common malignancy, worldwide; it is the seventh most prevalent cancer, accounting for 3.2% of all malignancies [1]. Carcinoma of the bladder is the most prevalent cancer in Egypt. At the national cancer institute, Cairo University, it constitutes 30.3% of all cancers [2,3]. Nitrate contamination of drinking water was reported as a risk of bladder cancer. Nitrates are endogenously reduced to nitrites, which through subsequent nitrosation give rise to highly carcinogenic N-nitroso compounds [4]. Other etiological factors implicated in the development and progression of bladder cancer includes urinary tract infections (UTIs) including bacterial, parasitic, fungal, and viral infections; urinary lithiasis and pelvic radiation [5]. Bacteria are the primary cause of UTIs, with the vast majority (70–80%) attributed specifically to infection with *E. coli*. A recurring theme in the link between bacterial infection and carcinogenesis is that of chronic inflammation, which is often a common feature of persistent infection [6,7]. One of the key molecules that link chronic inflammation and cancer is represented by the NF-κB family of transcription factors [7]. NF-κB activation induces the expression of more than 200 genes which have been shown to suppress apoptosis and induce cellular transformation, proliferation, invasion, metastasis, chemo-resistance, radio-resistance, and/or inflammation [8]. Altered expression of genes involved in suppression of apoptosis (i.e. Bcl-2 family members and inhibitor of apoptosis proteins), a key feature of cancer cells, is often due to deregulated NF-κB activity. The expressions of numerous cytokines that are growth factors for tumor cells such as interleukin 1β (IL-1β); tumor necrosis factor (TNF); epidermal growth factor (EGF) and IL-6 are also regulated by NF-κB [9]. IL-6 is a major proinflammatory cytokine that participates in inflammation-associated carcinogenesis [10]. Elevated plasma and urine levels of IL-6 have been demonstrated in cancer

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and inflammatory diseases of the urinary tract [11]. This study aimed to evaluate the possible role of *E. coli* infection during bladder carcinogenesis and the changes in NF-κB pathway and its related products.

Results

All experimental protocols and procedures were approved by the Animal Ethics Committee of Cairo National Cancer Institute.

Histopathological examination

Bladder histopathological changes are presented in Figure 1. The histopathological examination revealed that *E. coli* infected group was able alone to produce some histopathological changes in bladder tissue ranging from inflammation to dysplasia and that nitrosamine precursor plus *E. coli* group showed highest incidences of urinary bladder lesions than the nitrosamine precursor group and *E. coli* group.

NF-κB p65, Bcl-2 and IL-6 levels

Levels of NF-κB p65, Bcl-2 and IL-6 are presented in Table 1. As indicated in Table 1, at 3 months interval, the mean ± SD of NF-κB p65, Bcl-2 and IL-6 levels were significantly higher in the three groups compared with those obtained in the control group ($P < 0.05$). In addition a significant difference was observed among the three groups, with those of group IV (receiving nitrosamine precursor and infected with *E. coli*) showing the highest values. Regarding NF-κB p65 levels, there was a significant increase in group II (0.92 ± 0.22 ng/ml), group III (0.84 ± 0.17 ng/ml), and group IV (1.19 ± 0.19 ng/ml) compared with the control group (0.57 ± 0.07 ng/ml). The anti-apoptotic protein; Bcl-2 level was significantly increased in groups II (354.74 ± 23.44 U/ml), group III (331.78 ± 11.86 U/ml), and group IV (387.05 ± 8.40 U/ml) compared with the control group level (309. 14 ± 14.55 U/ml). Finally for IL-6 level there was a significant increase in group II (19.76 ± 1.64 pg/ml), group III (19.21 ± 1.56 pg/ml), and group IV (24.80 ± 2.20 pg/ml) compared with the control group (14.09 ± 0.87 pg/ml).

At 6 months interval, the mean ± SD of NF-κB p65, Bcl-2 and IL-6 levels also showed significant increase in the three groups compared with those obtained in the control group. In addition a significant difference was also observed among the three groups, with those of group IV (receiving nitrosamine precursor and infected with *E. coli*) showing the highest values. Regarding NF-κB p65 levels, there was a significant increase in group II (1.11 ± 0.19 ng/ml), group III (1.27 ± 0.20 ng/ml), and group IV (1.52 ± 0.21 ng/ml) compared with the control group (0.61 ± 0.08 ng/ml). The anti-apoptotic protein; Bcl-2 level was significantly increased in groups II (485.36 ± 60.12 U/ml), group III (361.59 ± 11.95 U/ml), and group IV (544.54 ± 37.11 U/ml) compared with the control group level (320.61 ± 7.47 U/ml). Finally for IL-6 level there was a significant increase in group II (23.13 ± 1.46 pg/ml), group III (22.29 ± 1.48 pg/ml), and group IV (30.66 ± 3.20 pg/ml) compared with the control group (14.74 ± 1.23 pg/ml).

At 9 months interval, the mean ± SD of NF-κB p65, Bcl-2 and IL-6 levels were significantly higher in the three groups compared with those obtained in the control group. In addition a significant difference was observed among the three groups, with those of group IV (receiving nitrosamine precursor and infected with *E. coli*) showing the highest values. Regarding NF-κB p65 levels, there was a significant increase in group II (1.30 ± 0.22 ng/ml), group III (1.66 ± 0.27 ng/ml), and group IV (1.72 ± 0.14 ng/ml) compared with the control group (0.70 ± 0.11 ng/ml). The anti-apoptotic protein; Bcl-2 level was significantly increased in groups II (485.58 ± 120.36 U/ml), group III (386.92 ± 19.14 U/ml), and group IV (592.60 ± 75.22 U/ml) compared with the control group level (323.47 ± 14.33 U/ml). Finally for IL-6 level there was a significant increase in group II (26.41 ± 1.89 pg/ml), group III (24.37 ± 1.32 pg/ml), and group IV (40.55 ± 2.69 pg/ml) compared with the control group (15.63 ± 0.89 pg/ml).

Discussion

The involvement of bacteria in the process of carcinogenesis remains controversial [6] because of a lack of agreement on potential molecular mechanisms.

It was proven that urinary tract infection promotes carcinogenesis in the urinary tract of the rat and that infection with live *E. coli* resulted in persistent infection and diffuses urothelial hyperplasia in addition to acute and chronic inflammation [12].

In our study; Group IV (nitrosamine precursor plus *E. coli* group) showed highest incidences of urinary bladder lesions than the nitrosamine precursor group; moreover *E. coli* group alone was able to produce some histopathological changes in bladder tissue. These findings suggested that urinary bladder infection by *E. coli* may play a major additive and synergistic role in bladder carcinogenesis.

These results are consistent with the study of Ashmawey and colleagues [13] who reported that *E. coli* infection in the bladder tissues increases the carcinogenic ability of nitrosamine precursors. Three mechanisms were suggested to account for the tumor enhancing effect of *E. coli* in the experiment. First, *E. coli* infection of bladder tissues increases the carcinogenic ability of nitrosamine precursors and this may be due to increase of nitrite production by the bacteria and continuous production of nitrosoamine by helping in-situ nitrosamine synthesis. Second, *E. coli* infection accelerated urothelial proliferation. This may have

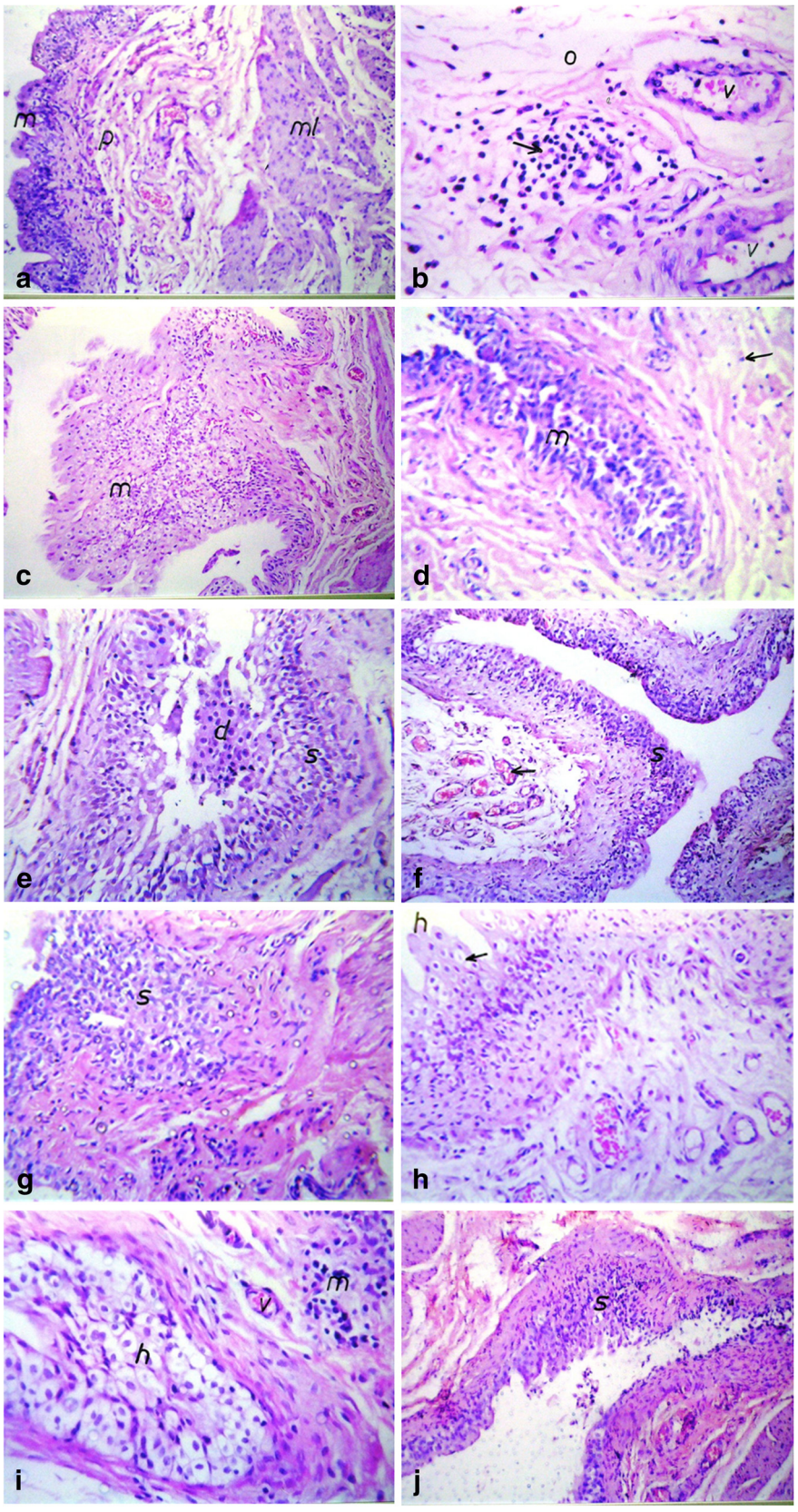

Figure 1 (See legend on next page.)

(See figure on previous page.)

Figure 1 Histopathological changes in different studied groups; control group (group I), *E. coli* group (group II), nitrosamine precursors group (group III), and *E. coli* and nitrosamine precursors group (IV). (a): 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). **(b)**: Urinary bladder of rat in *E. coli* group (group II) at 3 months showing focal inflammatory cell infiltration (arrow) with sever dilatation of blood vessels in the lamina propria **(c)**: Urinary bladder of rat in *E. coli* group (group II) at 6 months showing focal hyperplasia and stratification in the mucosal epithelium (m). **(d)**: Urinary bladder of rat in *E. coli* group (group II) at 9 months showing dysplasia in the mucosal lining epithelium (m) with inflammatory cells infiltration in lamina propia (arrow). **(e)**: Urinary bladder of rat in nitrosamine precursors group (group III) at 3 months showing dysplasia in the mucosal lining epithelium **(d)** with degenerative changes (s). **(f)**: Urinary bladder of rat in nitrosamine precursors group (group III) at 6 months showing dysplasia in the mucosal epithelium (s) with congestion of newly formed blood capillaries in underlining lamina propria (arrow). **(g)**: Urinary bladder of rat in nitrosamine precursors group (group III) at 9 months showing dysplasia in the lining mucosal epithelium (s). **(h)**: Urinary bladder of rat in *E. coli* and nitrosamine precursors group (group IV) at 3 months showing hyperplasia with polyp formation (h) and degeneration (arrow) in mucosal lining epithelium. **(i)**: Urinary bladder of rat in *E. coli* and nitrosamine precursors group (group IV) at 6 months showing degeneration and dysplasia **(h)** in mucosal lining epithelium with inflammatory cells infiltration (m) in underlying lamina propria. **(j)**: Urinary bladder of rat in *E. coli* and nitrosamine precursors group (group IV) at 9 months showing hyperplasia and dysplasia (s) in mucosal lining epithelium.

augmented the mutagenic effect of the carcinogen. Third, prolonged oxidative and nitrosative stresses which results in DNA damage and mutation [13].

Our results showed the highest level of NF-κBp65 in *E. coli* plus nitrosamine precursor group. In the absence of inflammatory stimuli, NF-κB p65 is retained in an inactive complex in the cytoplasm by the chaperone-like protein inhibitor of Kappa B alpha (I-κBα). With exposure to pro-inflammatory stimuli, such as Toll-like receptors-4 agonist (TLR4) or pro-inflammatory cytokines, phosphorylation of I-κB occurs, leading to its degradation and subsequent release of NF-κB p65 to translocate to the nucleus, driving inflammatory gene expression [14]. The highest level of NF-κBp65 in *E. coli* plus nitrosamine precursor group, observed in this study is in agreement with [15,16] who reported that *E. coli* lipopolysaccharide (LPS), a major cell wall component of *E. coli*, treatment induced IκB phosphorylation, IκB degradation, and NF-κB translocation. Also Saban and colleagues [17] during a study of gene expression changes occurring in the early stages of genitourinary inflammation mediated by LPS reported that NF-κB pathway genes were up-regulated by LPS stimulation. This can be explained by the fact that induction of inflammation by LPS or *E. coli* in bladder uroepithelial cells involves the TLRs and CD14. These activate signaling pathways, including NF-κB and p38 mitogen- activated protein kinase (p38 MAPKs) [18].

Our results showed highest level of Bcl-2 in *E. coli* plus nitrosamine precursor group followed by *E. coli* group and nitrosamine precursor group respectively. These results can be explained by the ability of bacterial pathogens to inhibit apoptosis in eukaryotic cells during infection as prevention of apoptosis provides a survival advantage because it enables the bacteria to replicate inside host cells [19].

Many pathogens rely on NF-κB activation to inhibit apoptosis. The Gram-negative bacteria cell surface component LPS activates the NF-κB pathway during infection [20]. Because NF-κB activation has many pro-survival effects on the host cell, activation of the NF-κB pathway by LPS might be a simple explanation of how most bacteria inhibit apoptosis [19].

The increase in Bcl-2 level in nitrosamine precursor group observed in our results is in consistent with El Gendy and colleagues [21] who reported that the levels of Bcl-2 protein significantly increased over all the periods of treatment (12 months) in rats receiving nitrosamine precursors compared with the corresponding level of normal control rats fed with standard diet.

Our results showed highest level of IL-6 in *E. coli* plus nitrosamine precursor group followed by *E. coli* group and nitrosamine precursor group respectively. *E. coli* enhancing effect on IL-6 production clearly observed in our study is in agreement with Feng and colleagues [15] who reported that LPS treatment caused a marked increase in IL-6 production in macrophages.

Neuhaus and colleagues [22] had also shown that IL-6 and IL-6 receptor expression was found in urothelium, lamina propria and detrusor cells isolated from bladder biopsies of tumor patients; these researchers further found that LPS stimulation evoked a time-dependent synthesis and/or release of IL-6, IL-6 receptor, and transcription factor signal transducer and activator of transcription 3 (Stat3) in cultured human detrusor smooth muscle cells. The ability of *E. coli* to increase IL-6 level can be explained by activating several signaling pathways, including NF-κB and p38MAPKs [18] with subsequent production of Il-6.

Conclusion

In conclusion *E. coli* infection might play a role in the development of bladder cancer and this effect may be mediated by activation of NF-κB pathway resulting in inhibition of apoptosis and increased inflammation.

Table 1 NF-κBp65 tissue homogenate level (ng/ml), serum level of Bcl-2 (U/ml) and IL-6 (pg/ml)

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
Group I	Range	0.51- 0.67	0.51-0.75	0.60-0.87	293.30- 331.94	310.74-331.94	305.78- 348.24	12.65-15.20	13.55-16.95	14.5-17.00
	Mean ± S.D	0.57 ± 0.07	0.61 ± 0.08	0.70 ± 0.11	309.14 ± 14.55	320.61 ± 7.47	323.47 ± 14.33	14.09 ± 0.87	14.74 ± 1.23	15.63 ± 0.89
Group II	Range	0.52-1.13	0.91- 1.145	1.13-1.74	329.75-396.42	412.63-584.94	354.27- 681.57	17.77-22.44	20.91-25.11	24.76-29.69
	Mean ± S.D	$0.92 \pm 0.22^{a,d}$	$1.11 \pm 0.19^{a,d}$	$1.30 \pm 0.22^{a,c,d}$	$354.74 \pm 23.44^{a,d}$	$485.36 \pm 60.12^{a,c,d}$	485.58 ± 120.36^{a}	$19.76 \pm 1.64^{a,d}$	$23.13 \pm 1.46^{a,d}$	$26.41 \pm 1.89^{a,d}$
Group III	Range	0.57- 1.05	1.02-1.50	1.39- 2.09	315.83-351.77	341.94-374.09	362.57-407.20	17.22-21.78	20.08-24.22	22.50-25.59
	Mean ± S.D	$0.84 \pm 0.17^{a,d}$	$1.27 \pm 0.20^{a,d}$	$1.66 \pm 0.27^{a,b}$	$331.78 \pm 11.86^{a,d}$	$361.59 \pm 11.95^{a,b,d}$	$386.92 \pm 19.14^{a,d}$	$19.21 \pm 1.56^{a,d}$	$22.29 \pm 1.48^{a,d}$	$24.37 \pm 1.32^{a,d}$
Group IV	Range	0.94- 1.40	1.30-1.87	1.58-1.95	377.06-399.89	484.65-595.49	522.07- 726.57	22.00-27.46	26.61-34.78	35.87-43.51
	Mean ± S.D	$1.19 \pm 0.19^{a,b,c}$	$1.52 \pm 0.21^{a,b,c}$	$1.72 \pm 0.14^{a,b}$	$387.05 \pm 8.40^{a,b,c}$	$544.54 \pm 37.11^{a,b,c}$	$592.60 \pm 75.22^{a,c}$	$24.80 \pm 2.20^{a,b,c}$	$30.66 \pm 3.20^{a,b,c}$	$40.55 \pm 2.69^{a,b,c}$

Group I: Control group; Group II: *E. coli* group; Group III: Nitrosamine precursor group; Group IV: *E. coli* and nitrosamine precursor; a: Significantly different from control group (group I) at P<0.05; b: Significantly different from *E. coli* group (group II) at P<0.05; c: Significantly different from nitrosamine precursors group (group III) at P<0.05; d: Significantly different from *E. coli* + nitrosamine precursors group (group IV) at P<0.05.

Methods

Experimental animals and dosing

Ninety five male albino rats, weighing 50–60 gm were included in the study and divided into four groups; as follows: group I (**Gr I, 20 rats**): Normal control fed the standard diet . Group II (**Gr II, 25 rats**): *E. coli* infected; the rats infected by 0.1 ml saline containing suspension of *E. coli* in the bladder (approximately 2 X 10^6 organisms), according to [23]. Group III (**Gr III, 25 rats**): Received nitrosamine precursor; dibutyl amine (DBA) 1000 ppm and sodium nitrate 2000 ppm; in drinking water according to [21]. Group IV (**Gr IV, 25 rats**): Received nitrosamine precursor; dibutyl amine (DBA) 1000 ppm and sodium nitrate 2000 ppm; in drinking water and infected by *E. coli* in the bladder. At the end of the experiment, animals were decapitated and 5 ml of blood was collected. The present experiment was continued 36 weeks.

Laboratory procedures

At three months interval (3, 6 and 9 months) animals were sacrificed and bladder tissue was separated and blood was collected into vacutainer clotted tubes. For histopathological studies bladder tissue pieces were fixed in 10% formalin, blocked in paraffin, sectioned, and stained with hematoxyline and eosin. Finally, the samples were examined by a pathologist.

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 [24] for NF-κB p65 determination by ELISA kit (Glory Science Co., Ltd, USA) following the manufacturer instructions.

Sera were obtained by centrifugation at 4000 rpm for 10 minutes. Sera were separated. Aliquoted sera were kept frozen at −80°C until used for 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 $P \leq 0.05$.

Abbreviations

E. coli: Escherichia coli; NF-κBp65: Nuclear factor kappa p65; Bcl-2: B-cell lymphoma 2; IL-6: Interleukin 6; UTIs: Urinary tract infections; IL-1β: Interleukin 1β; TNF: Tumor necrosis factor; EGF: Epidermal growth factor; I-κBα: Inhibitor of Kappa B alpha; TLR4: Toll-like receptors-4; LPS: Lipopolysaccharide; CD14: Cluster of differentiation 14; p38 MAPKs: p38 mitogen- activated protein kinase; Stat3: Signal transduction and transcription 3; DBA: Dibutyl amine; S.D: Standard deviation; ANOVA: Analysis of variance.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HE: Wrote the manuscript and participated in the study design. TS: Revised the manuscript and assisted with data analysis. AS: participated in the study design and supervised the ELISA work. NO: Performed the ELISA work and performed the data analysis. All authors read and approved the final manuscript.

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References

1. Beaglehole R, Irwin A, Prentice T: **Changing history.** *The World Health Report* 2004, **157:**122.
2. El Mawla NG, El Bolkainy MN, Khaled HM: **Bladder cancer in Africa: update.** *Semin Oncol* 2001, **28**(2):174–178.
3. Abd El Gawad IA, Moussa HS, Nasr MI, El Gemae EH, Masooud AM, Ibrahim IK, El Hifnawy NM: **Comparative Study of NMP-22 Telomerase and BTA in the detection of bladder cancer.** *J Egypt Natl Canc Inst* 2005, **17**(3): 193–202.
4. Janković S, Radosavljević V: **Risk factors of bladder cancer.** *Tumori* 2007, **93:**4–12.
5. Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein GP: **Superficial Bladder Cancer: An Update on Etiology Molecular Development Classification and Natural History.** *Rev Urol* 2008, **10**(1):31–43.
6. Lax AJ, Thomas W: **How bacteria could cause cancer: one step at a time.** *Trends Microbiol* 2002, **10**(6):293–299.
7. Karin M, Greten FR: **NF-κB: linking inflammation and immunity to cancer development and progression.** *Nature Rev* 2005, **5**(10):749–759.
8. Shishodia S, Chaturvedi MM, Aggarwal BB: **Role of Curcumin in Cancer Therapy.** *Curr Probl Cancer* 2007, **31**(4):243–305.
9. Pacifico F, Leonardi A: **NF-κB in solid tumors.** *Biochem Pharmacol* 2006, **72:**1142–1152.
10. Rose-John S, Schooltink H: **Cytokines are a therapeutic target for the prevention of inflammation-induced cancers.** *Recent Results Cancer Res* 2007, **174:**57–66.
11. Andrews B, Shariat SF, Kim JH, Wheeler TM, Slawin KM, Lerner SP: **Preoperative plasma levels of interleukin-6 and its soluble receptor predict disease recurrence and survival of patients with bladder cancer.** *J Urol* 2002, **167:**1475–1481.
12. Schetter AJ, Heegaard NH, Harris CC: **Inflammation and cancer: interweaving microRNA free radical cytokine and p53 pathways.** *Carcinogenesis* 2010, **31:**37–49.
13. Ashmawey AM, Mohamed WS, Abdel-Salam IM, El-Gendy SM, Ali AI, El-Aaser AA: **Role of urinary tract bacterial infection in the process of bladder carcinogenesis (molecular and biochemical studies).** *AJMS* 2011, **2:**31–40.
14. Kawai T, Akira S: **Signaling to NF-κB by Toll-like receptors.** *Trends Mol Med* 2007, **13:**460–469.
15. Feng D, Ling W, Duan R: **Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK p38MAPK and NF-κB in macrophages.** *Inflamm Res* 2010, **59:**115–121.

16. Hsieh N, Chang AS, Teng C, Chen C, Yang C: **Aciculatin inhibits lipopolysaccharide-mediated inducible nitric oxide synthase and cyclooxygenase-2 expression via suppressing NF-κB and JNK/p38 MAPK activation pathways.** *J Biomed Sci* 2011, **18:**28.
17. Saban MR, Hellmich H, Nguyen N, Winston J, Hammond TG, Saban R: **Time course of LPS-induced gene expression in a mouse model of genitourinary inflammation.** *Physiol Genomics* 2001, **5:**147–160.
18. Schilling JD, Martin SM, Hunstad DA, Patel KP, Mulvey MA, Justice SS, Lorenz RG, Hultgren SJ: **CD14- and Toll-like receptor-dependent activation of bladder epithelial cells by lipopolysaccharide and type 1 piliated *Escherichia coli*.** *Infect Immun* 2003, **71:**1470–1480.
19. Faherty CS, Maurelli AT: **Staying alive: bacterial inhibition of apoptosis during infection.** *Trends Microbiol* 2008, **16:**173–180.
20. Palsson-McDermott EW, O'Neill LAJ: **Signal transduction by the lipopolysaccharide receptor Toll-like receptor-4.** *Immunology* 2004, **133**(2):153–162.
21. El Gendy S, Hessien M, Abdel Salam I, Morad M, EL-Magraby K, Ibrahim HA, Kalifa MH, El-Aaser AA: **Evaluation of the possible antioxidant effects of Soybean and Nigella Sativa during experimental hepatocarcinogenesis by nitrosamine Precursors.** *Turk J Biochem* 2007, **32**(1):5–11.
22. Neuhaus J, Schlichting N, Oberbach A, Stolzenburg JU: **Lipopolysaccharide-mediated regulation of interleukin-6 in cultured human detrusor smooth muscle cells.** *Urologe A* 2007, **46:**1193–1197.
23. Higgy NA, Verma AK, Erturk E, Bryan GT: **Augmentation of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) bladder carcinogenesis in Fischer 344 female rats by urinary tract infection.** *Proc Amer Assoc Cancer Res* 1885, **26:**118–124.
24. Tripathi DN, Jena GB: **Effect of melatonin on the expression of Nrf2 and NF-κB during cyclophosphamide-induced urinary bladder injury in rat.** *J Pineal Res* 2010, **48:**324–331.

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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): 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): Group I was normal control group, group II received nitrosamine precursor; 1000 ppm DBA and 2000 ppm NaNO3; in

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drinking 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 scarified; 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

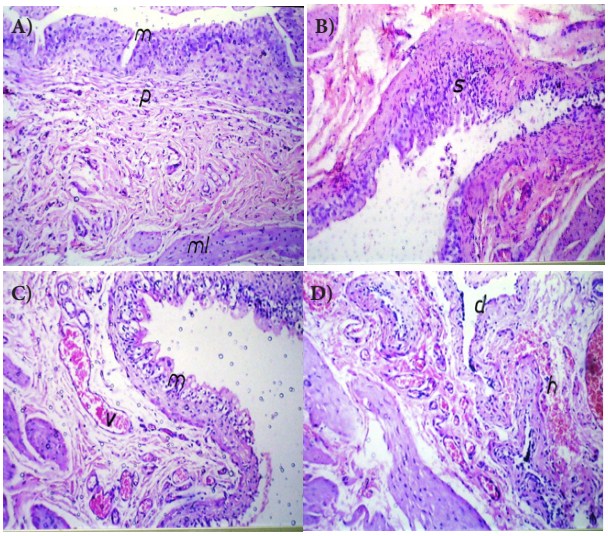

Figure 1. Histopathological Changes in Different Treated Groups; Control Group (Group I), *E. coli* and Nitrosamine Precursors Group (Group II), and *E. coli*, Nitrosamine Precursors and Curcum Group (III). 1A) Urinary Bladder of Rat in Control Group (Group I). Showing Normal Histological Structure of 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.