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Effects of Cranberry Against Urinary Adverse Events Associated with Radiotherapy in Iraqi Patients with Bladder Carcinoma

A thesis

Submitted to the Department of Clinical Pharmacy and the Committee of Graduate Studies of the College of Pharmacy/ Al-Mustansiriayah University in Partial Fulfillment of the Requirements for the degree of Master of Science in Pharmacy (Clinical Pharmacy)

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بسم الله الرحمن الرحيم

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلاً﴾

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We certify that this thesis, **(Effects of Cranberry Against Urinary Adverse Events Associated with Radiotherapy in Iraqi Patients with Bladder Carcinoma)** was prepared under our supervision at the department of Clinical Pharmacy, College of Pharmacy/ Al-Mustansiriyah University as a partial fulfillment of the requirements for the degree of Master of Science in Pharmacy (Clinical Pharmacy).

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Dedication

I dedicate this work to…….

My family

My friends and colleagues who stand by my side to complete this work especially

Haider M. Badee, Noor Muneer, Hadiel Fadhil, Raghda Husham and Ibrahim Al-Nashmi

My Fiancée who supports me to the end

And to all patients suffering from cancer.

Mohammed Basim

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List of Abbreviations

Abbreviation	Meaning
AEs	Adverse events
ANOVA	Analysis of variance
AP-1	Activator protein-1
BC	Bladder cancer
CAFs	Cancer-associated fibroblasts
CIS	Carcinoma *in situ*
CRP	C-reactive protein
CT	Computed tomography
EBRT	External beam radiotherapy
EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EORTC	European organization for research and treatment of cancer
5-FU	5-flourouracil
GSH	Glutathione
GST	Glutathione s-transferase
GUCG	Genito-urinary cancer group
Gy	Gray unit of absorbed radiation
HDL-C	High density lipoprotein-cholesterol
Hs-CRP	High-sensitivity C-reactive protein test
HPF	High power field
HRP	Horseradish peroxidase
IARC	International association research on cancer
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IR	Ionized radiation
ISUP	International society of urological pathology

IVU	Intravenous urography
LDL-C	Low density lipoprotein-cholesterol
LUTS	Lower urinary tract symptoms
MDA	Malondialdehyde test
MIBC	Muscle-invasive bladder cancer
MMPs	Matrix-metalloproteinases test
MRI	Magnetic resonance imaging
M-VAC	Methotrexate-vinblastine-doxorubicin(Adriamycin®)-cisplatin
NBI	Narrow-band imaging
NF-κB	Nuclear factor kappa B
NMIBC	Non muscle-invasive bladder cancer
PACs	Proanthocyanidins
PAHs	Polycyclic aromatic hydrocarbons
PDD	Photodynamic diagnosis
PPIUS	Patient perception of intensity of urgency scale
QoL	Quality of life
ROS	Reactive oxygen species
RT	Radiotherapy
RTOG	Radiation therapy oncology group
RBCs	Red blood cells
STATs	Signal transducers and activators of transcription
SOD	Superoxide dismutase
SOD1	Copper/Zinc superoxide dismutase
TAC	Total anti-oxidant capacity
TAMs	Tumor associated macrophages
TCC	Transitional cell carcinoma
TGF-β	Transforming growth factor-beta
Tis	CIS or Carcinoma *in situ*
TNF-α	Tumor necrosis factor-alpha

TNM	Tumor, node, metastasis grading system
TURBT	Transurethral resection of bladder tumor
US	Ultrasound
UTIs	Urinary tract infections
UTUCs	Upper tract urothelial carcinomas
UUT	Upper urinary tract
VEGF	Vascular endothelial growth factor
WBCs	White blood cells
WLC	White-light cystoscopy
WHO	World health organization

Abstract

Background

The most common malignancy in the urinary tract is bladder cancer. It is classified into three categories differ in their prognosis and treatment plans; the non-muscle invasive bladder cancer; the muscle invasive bladder cancer and the metastatic lesions. Patients with MIBC can be treated with radiotherapy which is associated with acute and late adverse events. Acute adverse events can occur during the treatment course until several weeks after the end of the treatment and called radiation cystitis of acute lower urinary tract symptoms with or without urinary tract infections. Late adverse events are more dangerous and range from fibrosis and bladder contracture to life-threatening hemorrhagic cystitis that needs urgent urinary diversion. For the sake of increasing patients' quality of life, many preventive measures had been tried. American cranberry contains type-A proanthocyanidins and have a proven anti-inflammatory and anti-oxidant properties beside the anti-adhesive effect which is useful in preventing recurrent urinary tract infections.

Objective

This study was designed to evaluate the potential effects of cranberry- proanthocyanidins against urinary adverse events in patients with bladder carcinoma undergoing radiotherapy.

Patients and methods

This randomized placebo-controlled clinical study was carried out on 40 patients (30 males/10 females) with muscle-invasive bladder cancer (T2-T3 only) whom are candidates for multimodality bladder preserving treatment approach and fit for the curative dose (64 Gy) of radiotherapy.

These patients were with ages range of 60-70 years, and on diet restriction with any food or drink containing berries, red grapes, or red wine, and under a controlled hydration regimen (2-3 litters of water per day). These patients were diagnosed and treated in the Oncology Teaching Hospital/ Medical City Directorate under the supervision of specialist doctors after achieving ethical committee approval and taking patients oral consent, during the period from November 2014 to April 2016. These patients were randomly assigned into placebo group (500mg lactose cap. twice daily, N=20) and cranberry group (36mg proanthocyanidins tab. twice daily, N=20) both of them received the same radiation dose for 6-7 weeks. The studied parameters include subjective parameters (urinary frequency, nocturia, and urgency) assessed on weekly basis and objective parameters (pyuria, hematuria, serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), Cu/Zn superoxide dismutase [SOD1], and total anti-oxidant capacity [TAC]) assessed at the baseline and at the end of radiotherapy.

Results

Significant reduction of the subjective parameters was observed in the cranberry group at the end of the study when compared to the baseline level, while the placebo group showed a significant elevation at the end of the study compared to the baseline level. Also, the mean levels of the subjective parameters of the cranberry group were significantly lower than those of the placebo group at the end of the study. Pyuria and hematuria in the cranberry group at the end of radiotherapy were significantly lower than that in the placebo group. Cranberry group showed a significant reduction in inflammatory markers level post-radiation, whereas in the placebo group they were significantly elevated post-radiation compared to their baseline levels and they were

significantly lower in the cranberry group when compared to the placebo group at the end of radiotherapy. The SOD1 level was significantly elevated in the cranberry group at the end of the treatment compared to the baseline level, and its level in the placebo group was reduced significantly at the end of the treatment compared to the baseline level. The SOD1 level in the cranberry group was significantly higher than that of the placebo group at the end of the study. The TAC level in the cranberry group was maintained (no difference) during the study, while its level in the placebo group was reduced significantly post-radiation compared to the baseline level. Also, TAC level in the cranberry group was significantly higher than that of the placebo at the end of the study course, while its level was significantly lower than that of the placebo group at baseline.

Conclusion

This study introduces evidence that cranberry- proanthocyanidins reduced the incidence of radiation cystitis and depressed the inflammatory and oxidative stress responses from radiotherapy in patients with bladder cancer, suggesting its role in modulating the late adverse events of radiotherapy.

Chapter One
Introduction

1.1 Bladder Cancer

The higher contribution in the urinary tract malignancies is for bladder cancer (BC) [(1)]. Nowadays, BC is divided into three split categories that differ in prognosis, management, and therapeutic aims. These are: non-muscle invasive bladder cancer (NMIBC); muscle invasive bladder cancer (MIBC); and the third category represent those with metastatic lesions [(2)]. Almost all of bladder tumors are urothelial carcinomas (about 90%) and called transitional cell carcinoma (TCC), while more than 70% of those are confined to layers above the muscularis propria (NMIBC) [(3)]. Squamous cell carcinoma, adenocarcinoma, small cell carcinoma, sarcoma, carcinosarcoma / sarcomatoid tumors, paraganglioma, melanoma and lymphoma represent the rest 10% of BC [(4)]. Bladder cancer is the 9th in rank of the worldwide cancer in incidence. In males, it's the 7th most common cancer while in females it's the 17th most common cancer [(5)]. The average age of diagnosis is 65 years [(6)]. The burden of BC on the patients, families and healthcare systems is very high; it costs more than 100,000 USD per patient [(7)].

1.1.1 Epidemiology

Bladder cancer is a global health problem and its distribution is greatly variable geographically (figure 1.1). In Europe 2006, there were an estimated 104,400 incident cases of BC diagnosed (82,800 in men and 21,600 in women) that represent a 6.6% of the total cancers in men and 2.1% in women. The estimated ratio by gender (male/female) was 3.8:1. Bladder cancer represents a 4.1% of total deaths for cancer in men and 1.8% of total deaths in women [(8)]. In the European Union (27 countries), BC mortality rates were stable up to early 1990s, and declined, thereafter, by 16% in men and 12% in women, to reach values of 6 and 1.3/100,000 of the population, respectively, in the early years of the 2000s decade. This documented and quantified reduction in BC mortality seems related to a

decrease in tobacco smoking, while its relationship with other risk factors remains controversial [9].

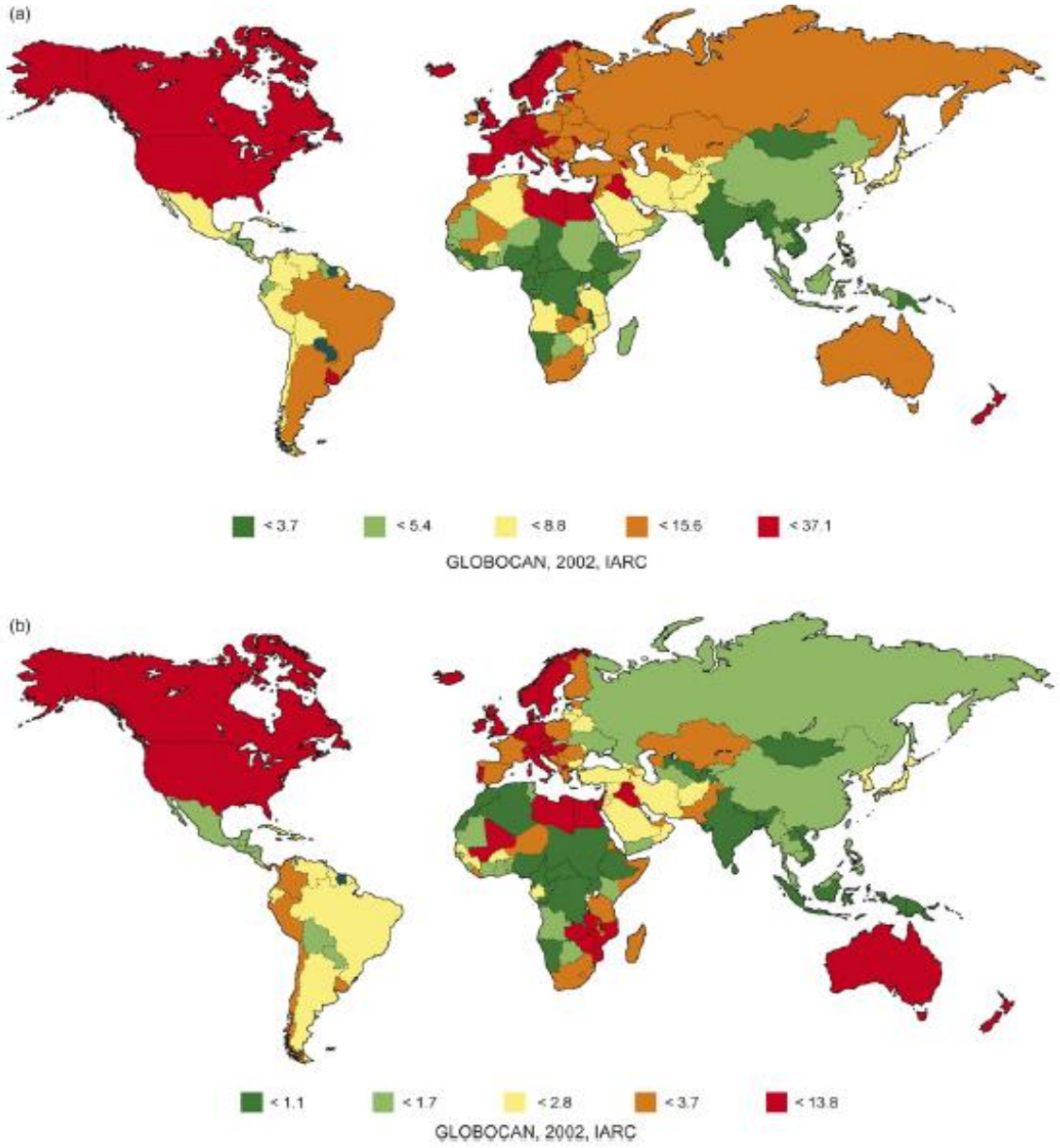


Figure 1.1: Incidence rates (per 100,000) for bladder cancer in (a) males and (b) females [9].

In the cooperation council states of the Arab Gulf, a ten years (1998-2007) cancer incidence report had reported that BC is considered the 4th and 5th most common cancer in Bahraini and Qatari males representing up to 7.9% and 6.9% of all cancers in men with average annual age standardized rates of 13.8/100,000 and 11.6/100,000 of the population, respectively [10].

In Iraq, the average annual age standardized rate is 19.9/100,000 of population for Iraqi males [8]. Figure 1.2 shows the age specific incidence rates for bladder cancer by gender in Iraq as published by the Iraqi Cancer Registry Center in 2009 [11].

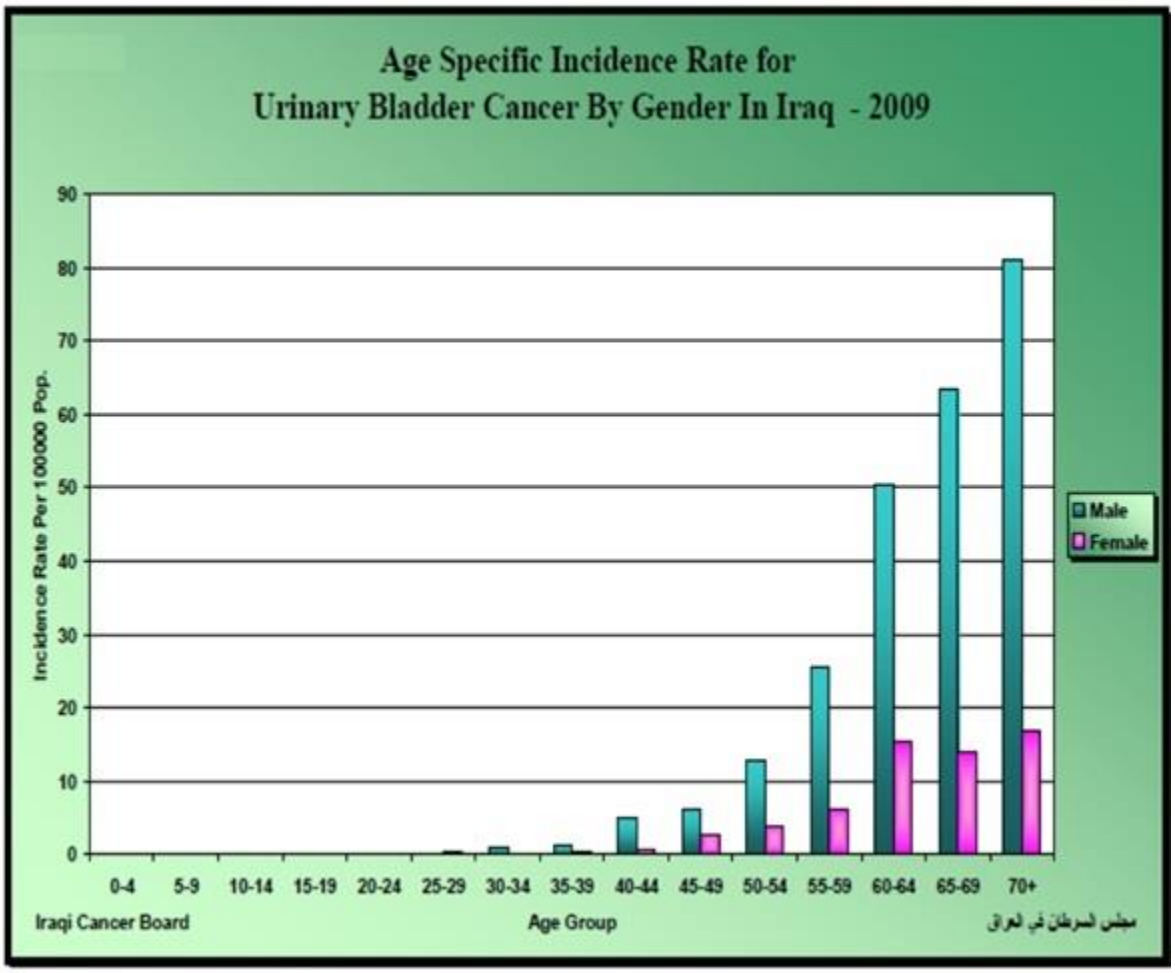


Figure 1.2: Incidence rates for urinary bladder cancer by gender with respect to the age in Iraq-2009 [11].

The environment and age are much related to BC. Several environmental carcinogens are directly correlated to this tumor. Furthermore, populations are increasing in number, and more people are living longer so more at potential risk [12]. The five-year relative survival rates are about 96% for carcinoma *in situ* (CIS) and its go down for around 6% for advanced stages with distant metastases (figure

1.3). About 75% of the newly diagnosed cases are NMIBC, whereas 25% of tumors have already spread to the muscle (MIBC) [13].

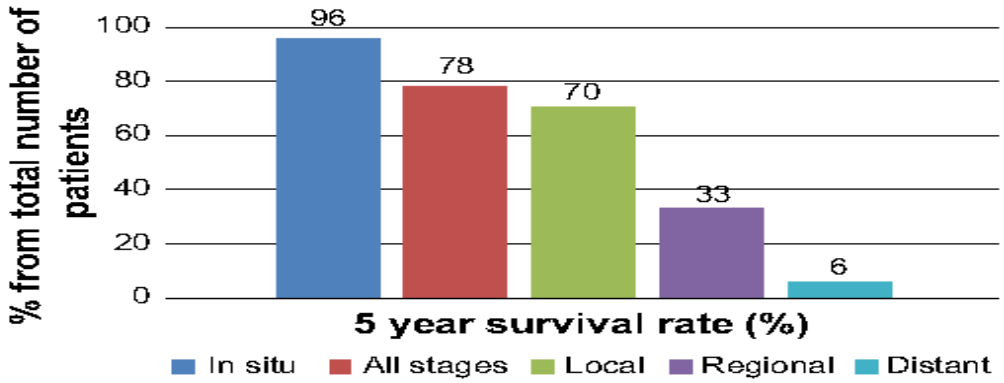


Figure 1.3: The five-year relative survival rates for bladder cancer with respect to the stage at time of diagnosis [13].

1.1.2 Etiologies and risk factors

Tobacco smoking is a well-recognized risk factor for BC, accounts for around 50-65% of male cases and 20-25% of female cases. It contains many carcinogenic compounds such as: arylamines, especially the potent carcinogen 4-aminobiphenyl (figure 1.4); Polycyclic Aromatic Hydrocarbons (PAHs); *N*-nitroso compounds; heterocyclic amines and various epoxides [12]. Few studies had investigated passive smoking and there were no good evidence between the passive smokers and BC [4].

Occupational exposure to carcinogenic compound accounts for 15-35% of cases in men and 1-6% cases of women. Exposure to aromatic amines used in the chemical, rubber, and dye industries and PAHs in the aluminum, coal, and roofing industries have been linked to the development of BC [14]. Painters, varnishers, and hair dressers also have increased risk for BC. Chronic urinary tract infections (UTIs) and the exposure to radiotherapy are associated with BC [15]. Cyclophosphamide may be associated with BC, although Hellmich *et al.* argued that idea saying that urinary tumors are not necessarily caused by cyclophosphamide alone; rather, bladder cancer appears to be related to the autoimmune disease itself [16].

Studies have failed to show any evidence of bladder carcinogenicity with saccharin and other sweeteners [17]. However, other studies have found that o-toluene sulfonamide (an impurity of saccharin) is responsible for BC cases associated with the use of saccharin [18]. Oil cooking fumes may be an important risk factor for BC. In the kitchens of Chinese homes, there were mutagenic 2-naphthylamine and 4-aminobiphenyl, which can cause bladder cancer [19]. Methenamine (with trade names of Cystex®, Hiprex®, and in Iraq as Uricol®) is an over the counter drug for the treatment of UTI. It is converted to formaldehyde in the acidic urine environment, which consequently kills bacteria [20]. Formaldehyde is a human carcinogen; the bladder will suffer the most exposure due to the storage of urine. It has been shown that formaldehyde exposure causes DNA damage in the urinary bladder transitional epithelium of rats [21].

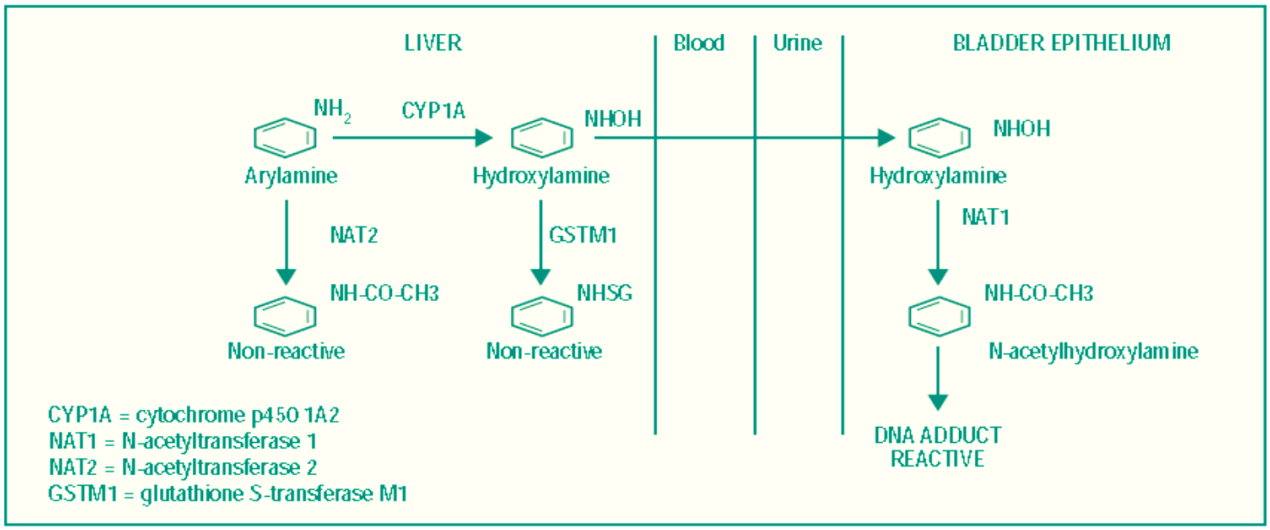


Figure 1.4: Arylamine metabolism pathway for bladder carcinogenesis. Arylamines may be N-acetylated by NAT2, which is highly expressed in the liver, rendering them nonreactive. Alternatively, they may be N-hydroxylated in the liver by CYP1A2, transported to the bladder, and taken up by the bladder epithelium. There they may undergo O-acetylation by NAT1, which is highly expressed in the bladder, to form a highly reactive species. Alleles that lead to decreased NAT2 activity and those that lead to increased NAT1 activity would be expected to increase cancer risk from arylamine exposure [22].

Chlornaphazine (a derivative of 2-naphthylamine, a nitrogen mustard that was developed in the 1950s), used for the treatment of polycythemia and Hodgkin's disease, was found to cause bladder cancer [23]. Chloronaphazine was

classified by the International Association Research on Cancer (IARC) in 2012 as carcinogenic to human [(24)]. Phenacetin, but not its metabolite acetaminophen, and phenacetin-containing analgesics were related to bladder cancer [(25)]. Also nitrosamines are cancerous agents that may lead to BC. Many nitrosamines are produced in the stomach when the food preservative nitrite reacts with amino acid [(26)].

The association between BC and coffee-drinkers had been mentioned for more than 30 years ago, but the recent epidemiological studies allowed the exclusion of this theory due to the strong confounding bias in that old studies as many coffee drinkers are smokers or they were smoke for many years [(27)]. Also, alcohol consumption was thought to be correlated with BC, but the epidemiological studies suggest no association between alcohol and BC. Although there was a moderate increase in the risk of getting BC in alcoholics reported by some investigations, it can be attributed again (as with coffee-drinkers) for the residual confounding by smoking [(28)].

Bilharziasis caused by *Schistosoma haematobium* infections are associated with increased oxidative DNA damages, accompanied by increased production of reactive oxygen species (ROS) and subsequently higher expression of repair genes. Schistosomiasis is likely a cause of bladder cancer [(29)].

Few promising preventive strategies emerge from the available data on bladder cancer risk. The most notable is abstinence from smoking. Any exposure to known hazardous materials in the workplace must be minimized [(30)]. When treating with alkylating agents, the co-administration of mesna (sodium 2-mercaptoethane sulphonate) is recommended to reduce bladder cancer risk; mesna lowers the risk of acute hemorrhagic cystitis, at least in animal studies, and exhibits anti-tumor efficacy [(31)]. Pre-clinical and limited clinical data demonstrate that bladder cancer is responsive to primary and secondary prevention efforts.

Furthermore, epidemiologic studies imply that natural products, such as vitamins and herbal compounds, may provide preventive benefits [32].

1.1.3 Staging and prognosis for bladder cancer

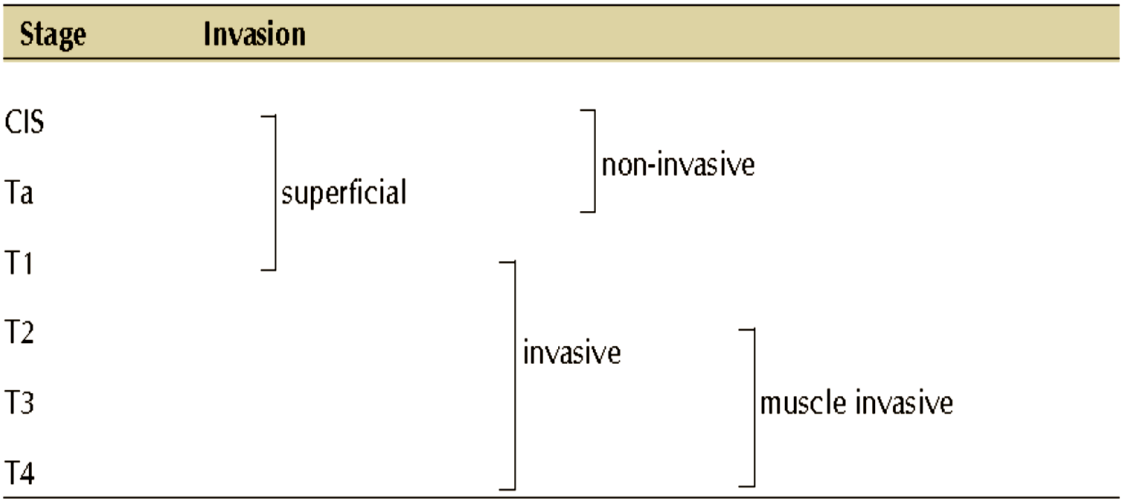
The most useful staging system for BC is the tumor, node, and metastasis (TNM) system. It gives a precise and simultaneous description of the primary stage (T stage), the status of lymph nodes (N stage) and metastatic sites (M stage) [33]. Table (1-1) illustrates the T staging system where T refers to the size of the tumor; N-stage or nodal system is defined as Nx (cannot be assessed), N0 (no nodal metastasis), N1 (single node < 2 cm involved), N2 (single node 2-5 cm in size or multiple nodes none > 5 cm are involved), and N3 (one or more nodes > 5 cm involved); M-stage or metastatic stage is defined as Mx (cannot be defined), M0 (no distant metastases), and M1 (distant metastases present) [33]. This system classifies non-muscle invasive tumors into: papillary tumors confined to the epithelial mucosa (stage Ta), tumors invading the subepithelial tissue (i.e., lamina propria; T1) and Carcinoma *in situ* (Tis or CIS) [34]. The MIBC in this system are classified according to the level of invasiveness to the two muscular layers and the outer layers as detailed in table (1-2) [35].

Table (1-1): TNM classification of urinary bladder cancer [33].

T - Primary Tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma *in situ*: “flat tumor”
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscle
T2a	Tumor invades superficial muscle (inner half)
T2b	Tumor invades deep muscle (outer half)
T3	Tumor invades perivesical tissue
T3a	Microscopically
T3b	Macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
T4a	Tumor invades prostate stroma, seminal vesicles, uterus, or vagina

T4b	Tumor invades pelvic wall or abdominal wall
N - Regional Lymph Nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph-node metastasis
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2	Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3	Metastasis in common iliac lymph node(s)
M - Distant Metastasis	
M0	No distant metastasis
M1	Distant metastasis

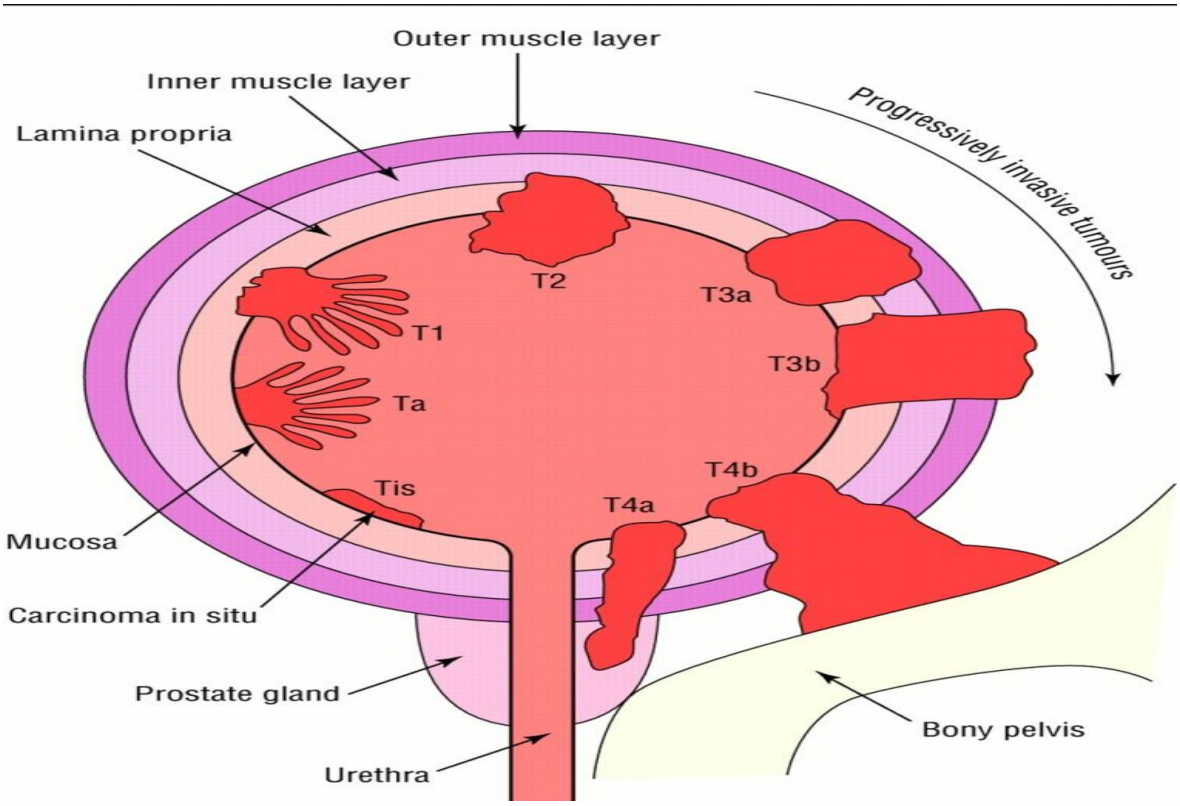
Table (1-2): Invasive and non-invasive bladder cancer as classified by TNM [(35)].



In 2004, the World Health Organization (WHO) and the International Society of Urological Pathology (ISUP) published a new histological classification of urothelial carcinomas which provides a different patient stratification between individual categories compared to the older 1973 WHO classification (table 1-3) [(36)]. In order to predict separately the short- and long-term risks of disease recurrence and progression in individual patients, the European Organization for Research and Treatment of Cancer (EORTC)/ Genito-Urinary Cancer Group (GUCG) has developed a scoring system and risk tables [(37)].

Table (1-3): WHO grading in 1973 and 2004 [(36)].

1973 WHO Grading
Urothelial Papilloma
Grade 1 Well differentiated
Grade 2 Moderately differentiated
Grade 3 Poorly differentiated
2004 WHO Grading System (papillary lesions)
Urothelial Papilloma (completely benign lesion)
Papillary urothelial neoplasm of low malignant potential (PUNLMP)
Low-grade papillary urothelial carcinoma
High-grade papillary urothelial carcinoma



Figures 1.5: staging of bladder cancer according to the TNM system [(38)].

The scoring system is based on the six most significant clinical and pathological factors which are shown in table (1-4). Table (1-5) shows the total scores stratified into four categories that reflect various probabilities of recurrence and progression at 1 and 5 years [(37)].

Table (1-4): Weightings used to calculate disease recurrence and progression scores [37]

Factor	Recurrence	Progression
Number of tumors		
Single	0	0
2-7	3	3
≥8	6	6
Tumor diameter		
< 3 cm	0	0
≥3	3	3
Prior recurrence rate		
Primary	0	0
≤ 1 recurrence/year	2	2
> 1 recurrence/year	4	2
Category		
Ta	0	0
T1	1	4
Concurrent CIS		
No	0	0
Yes	1	6
Grade		
G1	0	0
G2	1	0
G3	2	5
Total Score	**0-17**	**0-23**

Table (1-5): Probability of recurrence and disease progression according to total score [37]

Recurrence score	Probability of recurrence at 1 year		Probability of recurrence at 5 year	
	%	(95% CI)	%	(95% CI)
0	15	(10-19)	31	(24-37)
1-4	24	(21-26)	46	(42-49)
5-9	38	(35-41)	62	(58-65)
10-17	61	(55-67)	78	(73-84)

Progression score	Probability of progression at 1 year		Probability of progression at 5 year	
	%	(95% CI)	%	(95% CI)
0	0.2	(0-0.7)	0.8	(0-1.7)
2-6	1	(0.4-1.6)	6	(5-8)
7-13	5	(4-7)	17	(14-20)
14-23	17	(10-24)	45	(35-55)

Bladder cancer staging can be divided into clinical and pathological stages. Clinical stage obtained from the histologic findings at transurethral resection of bladder tumor (TURBT), the clinician's physical exam (including bimanual exam under anesthesia), and findings on radiologic imaging. Pathological staging (known as surgical staging) depends on the extent of disease following surgical resection of the bladder (partial versus radical cystectomy) and of the adjacent pelvic lymph nodes [(39)].

1.1.4 Clinical presentation and diagnosis

A thorough patient history is crucial to evaluate the contribution of each risk factor to the suspicion of bladder cancer. Hematuria, continuous or intermittent and either visible (gross) or microscopic, occurs in the majority of patients with bladder cancer. All patients with hematuria, particularly those without evidence of infections, stones, or other causative factors, should undergo cystoscopy and upper tract imaging [(40)]. Diagnosis of bladder cancer is considered in patients with irritative voiding symptoms (frequency, urgency, and dysuria) in the absence of infection, as this presentation may be associated with CIS [(39)].

The CIS or Tis can be defined as a flat, high-grade, non-invasive urothelial carcinoma that can be missed at cystoscopy or be considered as an inflammatory lesion if it is not biopsied. It's often multifocal and can occur outside the bladder, like in the upper urinary tract, prostatic ducts, and prostatic urethra [(41)]. The CIS can be further categorized into primary isolated CIS with no previous or concurrent papillary tumors and no previous CIS, secondary CIS detected during follow-up of

patients with a previous tumor that was not CIS, and concurrent CIS in the presence of any other urothelial tumor in the bladder [(42)].

Physical examination should include rectal and vaginal bimanual palpation for MIBC, while physical examination of patients with bladder cancer does not reveal NMIBC. A bimanual exam at the time of TURBT may help with clinical staging, especially for patients with MIBC [(43)]. Diagnosis of BC can be confirmed by the following procedures as documented in the most updated European and American guidelines for the management of BC.

a. Imaging:

- *Computed tomography, magnetic resonance imaging and intravenous urography:*

Computed Tomography (CT) scan detects papillary tumors in the urinary tract; however, Intravenous Urography (IVU) can be an alternative if CT is not available, particularly in muscle-invasive tumors of the bladder and in upper tract urothelial carcinomas (UTUCs). Computed tomography scan gives more information than IVU does (including status of lymph nodes and neighboring organs) [(44)]. Magnetic resonance imaging (MRI) has superior soft tissue contrast resolution compared with CT, but poorer spatial resolution [(45)].

- *Ultrasound (US):*

Characterization of renal masses, detection of hydronephrosis, and visualization of intraluminal masses in the bladder can be achieved by transabdominal US. Ultrasound has a comparable accuracy to that of IVU for the diagnosis of UTUCs. Therefore, it represents a useful tool for detection of obstruction in patients with haematuria. However, the diagnosis of CIS cannot be made with imaging methods (CT scan, IVU or US) [(40)].

b. Urinary Cytology:

Cytology is the most widely adopted non-invasive urine test (examination of voided urine samples of bladder washings for exfoliated cancer cells). It has a good specificity and sensitivity for the detection of high-grade tumors or CIS, but poor sensitivity for low-grade tumors, and has a delay in result availability. Early morning urine specimen is not recommended because cytolysis may be present [(46)].

c. Cystoscopy:

- *White-light cystoscopy (WLC):*

The standard WLC remains a fundamental investigative tool in the detection, resection and surveillance of BC for decades. Because small papillary tumors or CIS can be easily missed by the standard white-light cystoscopy, the development of newer technologies (narrow-band imaging cystoscopy and photodynamic diagnosis) was mandatory [(47)].

- *Photodynamic diagnosis (PDD):*

Photodynamic diagnosis (or fluorescence cystoscopy) enhances the detection of occult papillary lesions and CIS over WLC. It's performed using a violet light after intravesical instillation of 5-aminolevulinic acid dye or hexyl ester hexaminolevulinate into the bladder and absorbed by dysplastic tissue, to facilitate photosensitization (Figure 1.6). Abnormal tissue will emit a red color under blue reference light, while normal tissue appears blue [(48)].

- *Narrow-band imaging (NBI):*

Narrow-band imaging cystoscopy facilitates the contrast between normal urothelium and hyper-vascular cancer tissue without the use of dyes. Deeper penetration of the bladder mucosal surface is achieved by directing longer wavelengths of light. In contrast to PDD, which requires pre-operative instillation

of photosensitizing agents via a urethral catheter, NBI cystoscopy does not require extra invasive steps. It is convenient for outpatient setting [49].

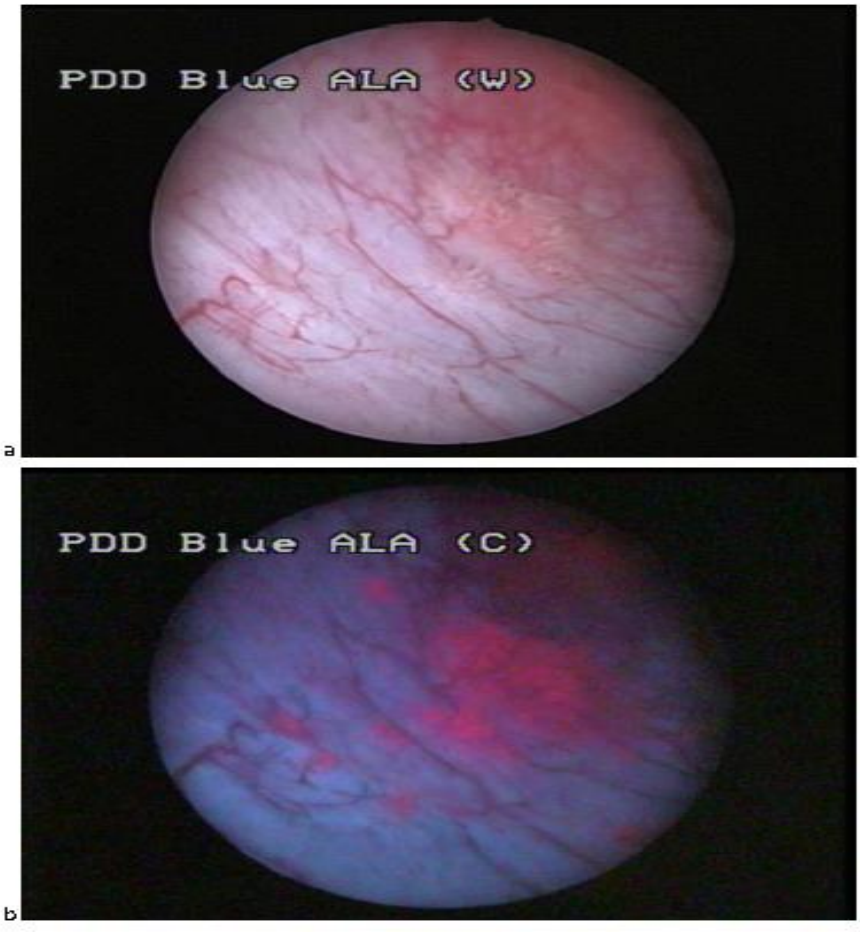


Figure 1.6: (a) White-light and (b) blue-light endoscopic image of flat lesions adjacent to a small papillary tumor [50].

d. Transurethral resection of bladder tumors (TURBT):

- *Conventional procedure :*

Transurethral resection of bladder tumor is indicated in BC to make the correct diagnosis, staging, and completely remove (in case of NMIBC) all visible lesions. It is the most important procedure in the diagnosis and treatment of BC. The strategy of resection depends on the size of the lesion. Separate resections of larger tumors give good histopathological information about the tumor and improve resection completeness [51]. In order to reduce the risk of understaging, a second TURBT is often required to determine the future treatment strategy [45].

- *Fulguration:*

In patients with a history of small lesions (like Ta tumors) fulguration of small papillary recurrences on an outpatient basis can reduce the therapeutic burden and can be a treatment option [(52)].

- *Bipolar electrocautery resection:*

It was claimed that bipolar electrocautery system has been introduced to reduce the risk of complications when compared to monopolar resection (e.g., bladder perforation due to obturator nerve stimulation) and produce better specimens for the pathologist [(53)].

- *Bladder and prostatic urethral biopsies:*

The prostatic urethra and ducts in men may be involved with bladder tumors. The exact risk is not known, but it seems to be higher if the tumor is located on the trigone or bladder neck, in the presence of bladder CIS, and in multiple tumors [(54)]. Involvement of the prostatic urethra can be determined either at the time of primary TURBT or by frozen section during the cysto-prostatectomy procedure [(55)].

e. Urinary molecular marker tests:

Recently, there has been an intense search for noninvasive adjunctive urine-based markers in an attempt to improve or perhaps replace cytology and cystoscopy for diagnosis and the surveillance of patients with non-muscle invasive bladder cancers. None of these urinary markers have been accepted for diagnosis or follow-up in routine urology or in guidelines. Some of these urine tests are listed in table (1-6) [(40)].

Table (1-6): Urinary markers used for bladder cancer [40].

Markers (or test specifications)	Overall sensitivity (%)	Overall specificity (%)	Sensitivity for high-grade tumours (%)
UroVysion (FISH)	30-86	63-95	66-70
Microsatellite analysis	58-92	73-100	90-92
Immunocyt/uCyt +	52-100	63-79	62-92
Nuclear matrix Protein 22	47-100	55-98	75-92
BTA stat	29-83	56-86	62-91
BTA TRAK	53-91	28-83	74-77
Cytokeratins	12-88	73-95	33-100

BTA: bladder tumor antigen, FISH: fluorescence *in situ* hybridization

1.1.5 Treatment of muscle invasive bladder cancer (MIBC)

a. Resectable tumors:

Resectable bladder tumors are T2-T4a, N0-Nx, &M0 according to the TNM staging system when there is no involvement of the pelvic or abdominal wall (figure 1.7) [45].

- Radical cystectomy:

In western countries, radical cystectomy is the standard treatment for localized MIBC. Interest in patients' quality of life has increased the trend toward bladder preservation treatment modalities, such as radio- and/or chemotherapy [56]. Formerly, radical cystectomy was recommended for patients with MIBC T2-T4a, N0-Nx, and M0 [57]. Salvage cystectomy can be indicated for non-responders to conservative therapy, recurrence after bladder-sparing treatment, and non-urothelial carcinoma (these tumors respond poorly to chemo- and radiotherapy). Also, it is used as a purely palliative intervention, including in fistula formation, for pain or recurrent visible haematuria (macrohaematuria) [56].

Patient's age is a prognostic factor for radical cystectomy [58]. Advanced age was considered as a risk factor for complications due to radical cystectomy. Other

risk factors for morbidity include prior abdominal surgery, extravesical disease, and prior radiotherapy [(59)], while an increased body mass index is associated with a higher rate of wound ruptures and hernia [(60)].

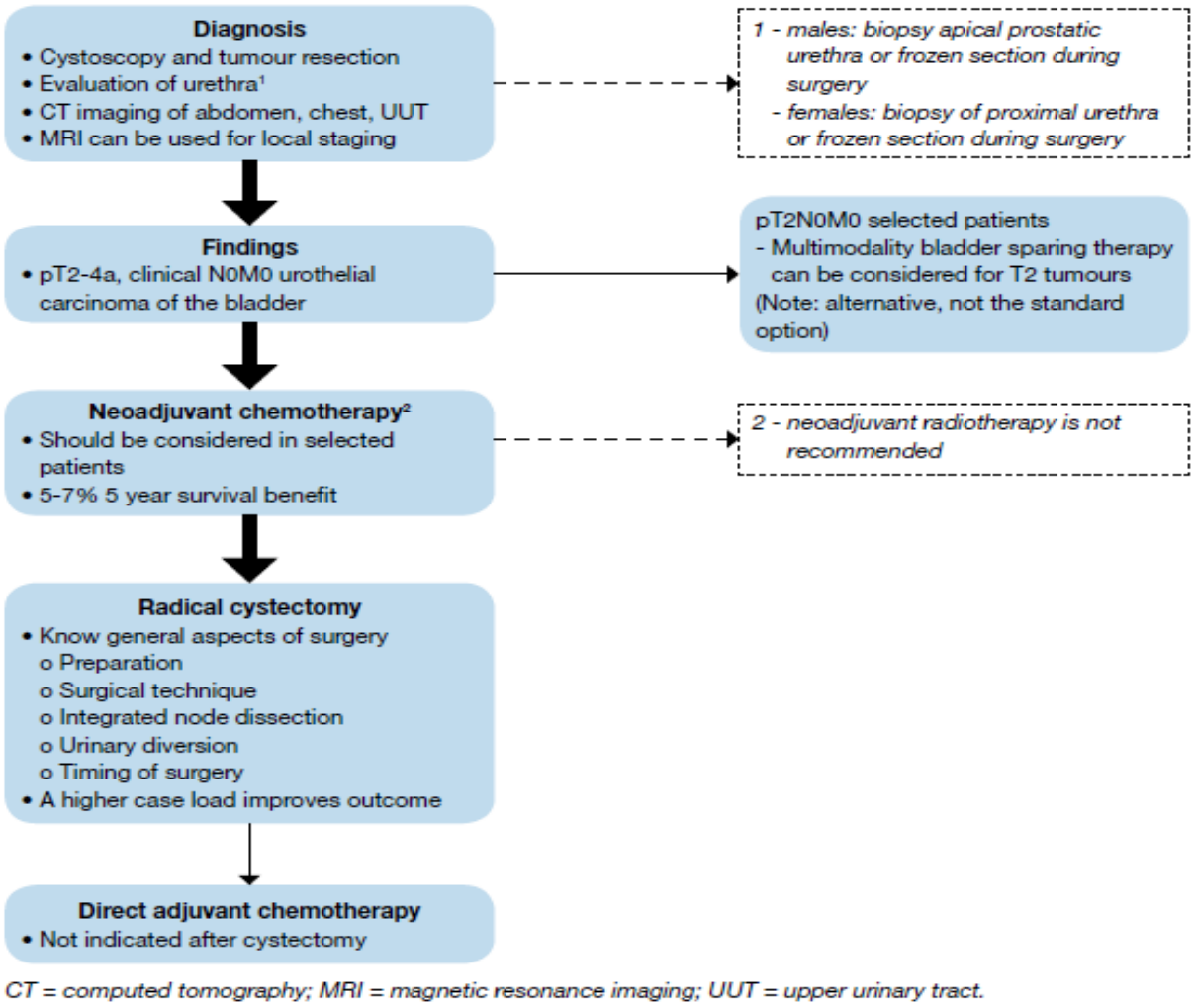


Figure 1.7: Flowchart for the Treatment of T2-T4a N0 M0 BC [(45)].

❖ <u>Pre- and post-operative chemotherapy (neoadjuvant and adjuvant chemotherapy):</u>

Radical cystectomy is the standard treatment for patients with muscle-invasive bladder cancer. However, this standard approach provides five-year survival in about 50% of muscle-invasive bladder cancer patients. The use of pre-operative chemotherapy (neoadjuvant chemotherapy) has been explored since the 1980s to improve these results, but neoadjuvant chemotherapy is still infrequently used [(61)]. Three independent meta-analyses showed a benefit in overall survival of

5% for cisplatin-based neoadjuvant chemotherapy, with an absolute disease-free survival rate of 9% at five years [62].

Even the most updated guidelines for muscle invasive bladder tumors give no recommendation for post-operative chemotherapy (adjuvant chemotherapy) as no improvement in the overall survival has been shown in this setting [2]. In addition, the exact timing for adjuvant chemotherapy (immediately after surgery versus treatment at the time of relapse) is still a controversy due to a lack of a sufficient clinical data [45, 62]. Adjuvant chemotherapy after radical cystectomy may be indicated for patients with T3/4 and/or lymph node positive (N+) disease without clinically detectable metastases (M0) [63]. At least three cycles of cisplatin-based regimen may be used for patients undergoing adjuvant chemotherapy. Cisplatin-based regimen may be gemicitabine-cisplatin or methotrexate-vinblastine-doxorubicin [Adriamycin®]-ciplastin (M-VAC) [64].

- Pre- and post-operative radiotherapy (neoadjuvant and adjuvant radiotherapy):

No data exist to support that pre-operative radiotherapy for resectable MIBC increases survival. Pre-operative radiotherapy for resectable MIBC, using a dose of 45-50 Gy in fractions of 1.8-2 Gy, results in down-staging after 4-6 weeks [65]. Limited high-quality evidence supports the use of pre-operative radiotherapy to decrease the local recurrence of MIBC after radical cystectomy [56].

Considering radiation post cystectomy, data are very scarce supporting this practice. Adjuvant radiotherapy is reasonable in patients with T3-T4 as the risk for local recurrence is high after surgery [2]. There is an old randomized study demonstrating improvement in five-year disease free survival and local control in patients received radiotherapy post-operative compared to surgery alone [66].

b. Non-resectable tumors:

Locally advanced tumors (T4b, those invading the pelvic or abdominal wall) may be associated with many debilitating symptoms, including bleeding, pain, dysuria and urinary obstruction. Patients with such presentation are candidates for palliative treatments, such as palliative radiotherapy [(67)]. In such cases, cystectomy with urinary diversion is the most invasive treatment and it carries the greatest morbidity and should be considered only if there are no other options. In these cases, palliative radical cystectomy with urinary diversion is usually performed for symptom relief [(68)].

Severe problems can affect the quality of life in patients with invasive, non-resectable, bladder cancer and in those who have not undergone cystectomy because of metastatic disease. These problems include pain, bleeding, voiding problems and obstruction of the upper urinary tract (UUT). Pain and bleeding can be relieved by radiotherapy or intravesical rinsing with silver nitrate, alum or formalin. Unilateral or bilateral nephrostomy tubes used for UUT obstruction [(69)].

c. Bladder-sparing approaches:

Patients within the categories of T2-T3a urothelial carcinomas may be considered for bladder-sparing approaches [(70)]. These approaches include TURBT alone, TURBT followed by chemotherapy alone, radiotherapy alone, or a combination of the three approaches (multimodality bladder-preserving approach or trimodal therapy). These approaches are reasonable for patients unfit for surgery and those seeking alternatives for radical cystectomy [(71)].

- ❖ Transurethral resection of bladder tumor alone (TURBT):

It is possible to use only TURBT as a therapeutic option if the tumor growth is limited to the superficial muscle layer of the bladder and if the re-staging biopsies are negative for residual tumor. It should only be considered alone as a

therapeutic option when the patient is unfit for cystectomy or a multimodality bladder-preserving approach, or refuses open surgery [56].

❖ External beam radiotherapy alone (EBRT):

The target dose for curative radiotherapy in case of bladder cancer is 60-66 Gy, with a subsequent boost using external radiotherapy (figure 1.8) or interstitial brachytherapy. The daily dose is usually 1.8-2 Gy and the course of radiotherapy should not extend beyond 6-7 weeks to minimize the repopulation of cancer cells [72]. The target field of radiation usually comprises the bladder only, with a safety margin of 1.5-2 cm to allow for unavoidable organ movements. Any beneficial effect with larger pelvic fields has not been demonstrated [73].

Figure 1.8: External beam radiotherapy apparatus [74].

Prognostic factors for radiotherapy outcomes include: tumor size; hydronephrosis; and completeness of the initial TURBT. The overall five-year

survival rates in patients with MIBC range between 30% and 60%, depending on whether they show a complete response following radiotherapy [75].

External beam radiotherapy alone should only be considered as a therapeutic option when the patient is unfit for cystectomy or a multimodality bladder-preserving approach (as mentioned in non-resectable tumors treatment). It also can be used to stop bleeding from the tumor when local control cannot be achieved by transurethral manipulation because of extensive local tumor growth [56].

- Chemotherapy:

Chemotherapy alone is considered insufficient without additional treatments for the bladder. Pathological complete responses of bladder primary tumors were reached in 12-50% of patients after methotrexate, vinblastine, doxorubicin plus cisplatin (M-VAC) and in 12-22% of patients after gemcitabine/cisplatin for 2-3 cycles in phase II and phase III trials [56].

- Multimodality bladder-preserving treatment:

Several groups have investigated organ-preservation strategies combine TURBT, chemotherapy and radiation. Radiation following TURBT is intended to achieve local tumor control, while systemic chemotherapy (most commonly in this practice as methotrexate, cisplatin and vinblastine) aims for the eradication of micrometastasis [45]. Most protocols use cisplatin and/or 5-FU, and gemcitabine with radiation, because of their established role as radio-sensitizers. Cisplatin-based chemotherapy in combination with radiotherapy, following TURBT, results in a cure rates of 60-80% [76].

1.2 Radiation cystitis

When the bladder is exposed to radiation during radiotherapy for pelvic tumors, a series of histopathological changes are induced that in turn have clinical consequences. In addition to irritative micturition syndrome characterized by

micturition urgency, frequency during day time and dysuria, the appearance of hematuria of highly variable intensity represents one of the most complex complications [(77)]. These histopathological changes occur in two phases: acute and chronic. The acute phase is observed during the treatment until 6 months after treatment [(78)]. Clinically, patients may experience micturition urgency, dysuria and/or frequency. Macrohematuria is observed in 7.7% of the cases, and although it is more frequent between 6 months and 5 years after treatment, this interval can be expanded from 6 weeks to 14 years [(79)]. The chronic phase begins 6 months after radiotherapy. The effect of radiation upon the bladder may lead to ischemia, which leads to changes at vascular and muscle level. Vascular endothelial damage causes hyperplasia, occlusion and perivascular fibrosis [(77)]. Some studies have shown the increased incidence of urinary tract infections (UTIs) and asymptomatic bacteriuria during the course of pelvic radiotherapy (RT) for the treatment of pelvic malignancies [(80-82)]. One study had shown the progression of UTIs even with the use of prophylactic trimethoprim during the course of RT [(82)].

Late urinary adverse effects (AEs) are graded by the radiation therapy oncology group (RTOG) system, which grades AEs on a scale of 0-5 (table 1-7) [(83)].

Table (1-7): RTOG system grades for late urinary adverse effects [(83)].

Grade	Description
0	No complications.
1	Microscopic hematuria. (Minor effects on quality of life)
2	Moderate urinary frequency, generalized telangiectasia, or intermittent macroscopic hematuria. (Minor effects on quality of life)
3	Severe frequency or dysuria, severe generalized telangiectasia (often with petechiae), frequent hematuria, or a reduction in bladder capacity to less than 150 mL. (Significant effects on quality of life)
4	Severe hemorrhagic cystitis, reduction in bladder capacity to less than 100 mL, and necrosis. (Significant effects on quality of life)
5	Any death resulting from late complications of radiation.

The incidence of RTOG grade 1 and 2 urinary AEs after external beam radiation therapy (EBRT) is reported to be 20-43% and 7-19%, respectively, with a follow-up of up to 10 years. Mild symptoms can resolve either spontaneously or with treatment within 42 months after EBRT. Grade 3 urinary AEs occur at a rate of 5-13%. Radiation cystitis with gross macroscopic hematuria is the most common grade 3 AE of EBRT [(83)].

Bladder conservation techniques using a combination of TURBT, chemotherapy and EBRT have yielded favorable results [(70)]. Thus, bladder conservation protocols are now a treatment option for selected patients with muscle invasive bladder cancer [(84)]. Radiation is rarely used in combination with radical cystectomy, so most of the radiation-induced urinary AEs with respect to bladder cancer come from the bladder conservation literature using trimodal therapy. Common acute radiation-induced AEs include transient cystitis and enteritis [(85)].

1.2.1 Radiotherapy-associated inflammation and oxidative stress

Radiation therapy with ionized radiation (IR) activate both pro- and anti-proliferative signal pathways altering the homeostatic balance between survival and cell death, regulated by several genes and factors involved in cell cycle progression, DNA repair, inflammation and cell death induction [(86)]. An increasing amount of data suggests that there is a direct relationship by which radiation stimulate the immune system, which in turn contributes to tumor cell death [(87)]. It has become evident that, in particular for solid tumors, the inhibition of neoplastic cell proliferative capacity following irradiation can occur through different modes of cell death that could also be induced by immunological factors (i.e. apoptosis, necrosis, senescence, etc.) [(86)].

Direct action of IR is the interaction of radiation beams or particles with critical target molecules in cells, such as DNA, to cause various types of damage in DNA structure, leading to lethal chromosome aberrations [(88)]. The indirect action of

IR takes place when the localized release of radiation energy generates free radicals, mainly by ionization of water, which constitutes about 80% of cell mass, and produces various reactive oxygen species (ROS). The ROS can then rapidly diffuse and react with other molecules to damage DNA, protein, and lipid targets (figure 1.9). This ROS-mediated effect of IR is suspected to have caused a majority of radiation-induced damage [89].

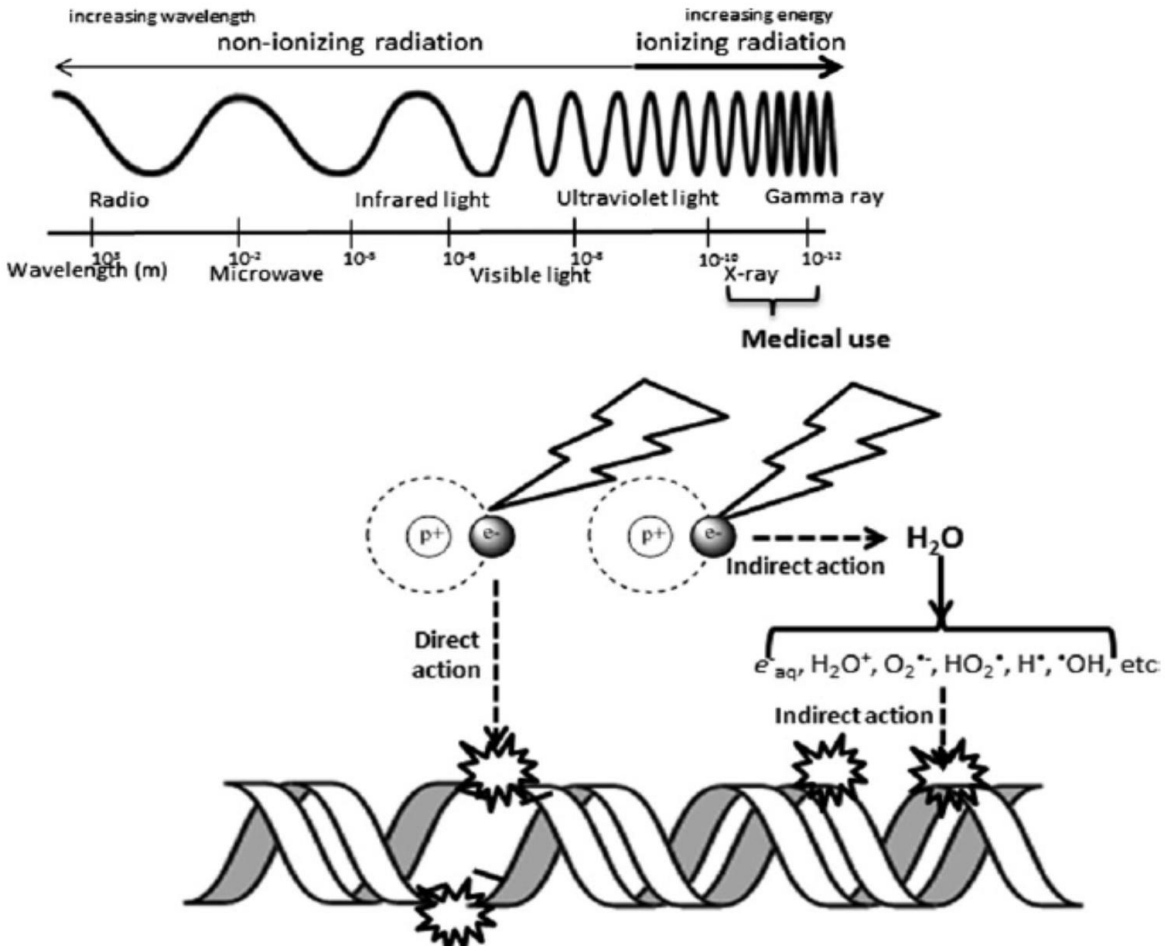


Figure 1.9: Electromagnetic spectrum of radiation and medical use of ionizing radiation (IR) showing the direct and indirect actions of IR [90].

Different types of cells in tumor tissues are subjected to complex regulatory mechanisms depending on their interactions with other cells and cellular products in the tumor microenvironment, such as interleukin-1β (IL-1β), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) [91]. Altered cytokine expression can alter many signaling pathways that focus on a few

important transcription factors, including nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), and signal transducers and activators of transcription (STATs). These transcription factors also upregulate the expression of several cytokines, such as IL-1β and TNF-α [(92)]. Such positive feedback loops amplify radiation- or oxidative-stress-induced inflammation, which may persist chronically [(91)].

The IR-induced mitochondrial dysfunction, especially decreased electron transport chain complex activity, produces a feed forward loop that contributes to persistent oxidative stress after irradiation [(93)]. Since the mitochondrion is the most important energy-generating organelle, mitochondrial dysfunction due to direct effects of IR or indirect effects mediated by ROS may result in alteration or adaptive responses of metabolic pathways involved in cancer development [(90)]. While it is possible to increase radiation doses to a level that ensures complete irradiation of cancer, the use of higher doses of radiation may cause unacceptable serious adverse effects to normal tissues. Thus, it is important to develop strategies that can sensitize tumor cells to radiation treatment and/or can protect normal tissue from radiation damage [(91)].

Biological organisms are able to maintain a delicate redox homeostasis (reduction/oxidation state) because they contain a complex intracellular ‘‘redox buffer’’ network, including both enzymatic and non-enzymatic antioxidants. The major enzyme defense system against ROS includes SOD, catalase, glutathione peroxidase, peroxiredoxin, and glutathione S-transferase (GST) [(91)]. In addition to these antioxidant enzymes, small thiol-containing peptides, such as glutathione (GSH), glutaredoxin, and thioredoxin systems, also help to scavenge ROS and maintain appropriate redox homeostasis [(94)]. An increase in production of reactive species and/or a decrease in antioxidants can lead to oxidative stress, which can damage DNA, inhibit cellular enzyme activities, and induce cell death through activation of kinases and caspase cascades [(95)].

Ionized radiation exposure commonly induces stromal cells, especially cancer-associated fibroblasts (CAFs), into a senescence-like phenotype in an altered tumor microenvironment. The so-called senescence-activated secretory pathways in senescent stromal fibroblasts generate an inflammatory environment through the secretion of pro-inflammatory cytokines and proteases [(96)]. The IR generates highly reactive free radicals, and it has been well documented that stromal components, such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and endothelial cells, enhance oxidative stress, which promotes tumor progression (figure 1.10). The CAFs can also exert their cancer-promoting roles through release of growth factors, such as TGF-β and epidermal growth factor, as well as chemokines [(97)]. Many types of cancer-infiltrating immune cells, such as macrophages, dendritic cells, and T cells, are important stromal components of a tumor as well as being prominent bystander targets of radiotherapy [(98)]. Thus, activated immune cells are not limited to induction of antitumor immunity; they are also involved in creating an immunosuppressive and pro-oxidant network that promotes tumor progression and facilitates immune evasion [(91)].

Interleukin-8 (IL-8) is a chemo-attractant chemokine. It's usually associated with inflammation that predisposes cells to produce different chemokines for malignant transformation or progression [(99)]. The secretion of IL-8 is increased by oxidative stress from either intracellular or extracellular sources. It can stimulate the recruitment of inflammatory cells, which further elevates oxidant stress mediators, thereby making IL-8 a key parameter in localized inflammation [(91)]. In addition to establishing the importance of IL-8 in developing chemo-resistance [(100)], it has been found that the RelB-mediated NF-κB alternative pathway plays a crucial role in IL-8 upregulation, which enhances the radio-resistance of prostate cancer cells [(101)]. Thus, it will be interesting to investigate the relationship of radio-

sensitizing effects of IL-8 signaling blockage with either inhibitors of IL-8 receptors or monoclonal antibodies against IL-8 [91].

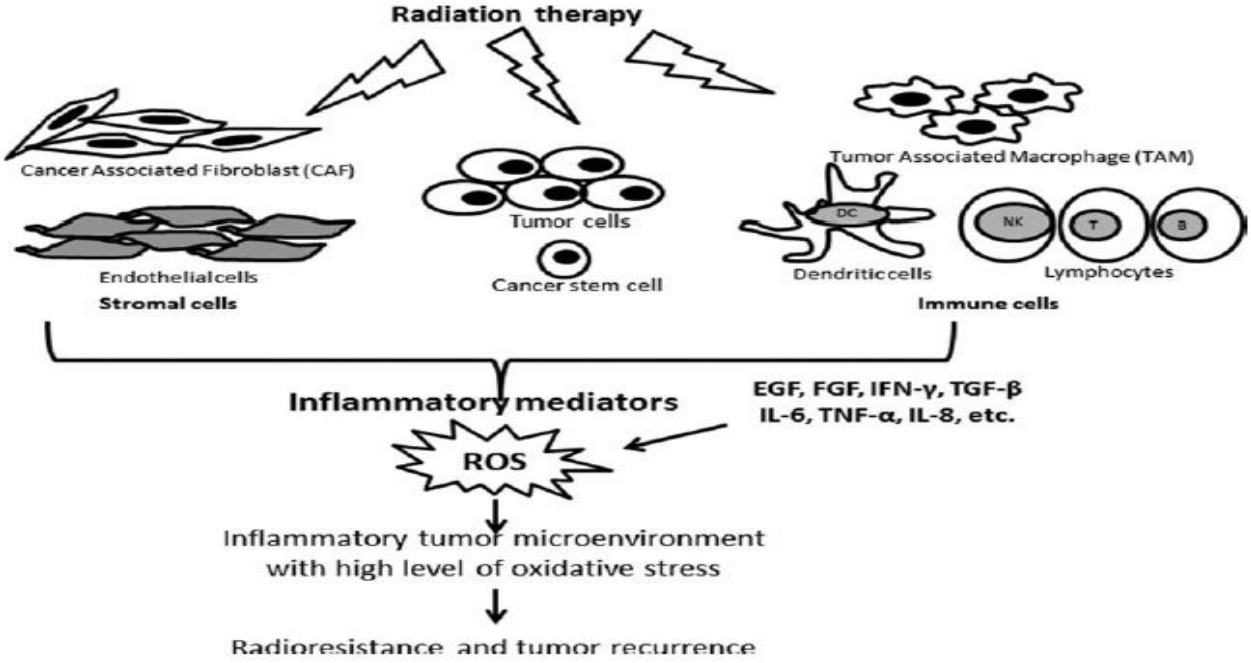


Figure 1.10: Inflammatory mediators induced by IR include epidermal growth factor (EGF), fibroblast growth factor (FGF), interferon-γ (IFN-γ), transforming growth factor-beta (TGF-β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and IL-8. Both radiation treatment and IR-induced inflammatory mediators increase ROS levels within a tumor microenvironment, which contributes to radio-resistance and recurrence of cancer [91].

Tumor necrosis factor-α (TNF-α) is one of the central factors involved in stress responses, including response to radiation exposure, because of its ability to induce rapid hemorrhagic necrosis via selective destruction of tumor blood vessels and generate specific T-cell antitumor immunity [102]. Antagonists of TNF-α action have been developed for the treatment of rheumatoid arthritis and other inflammatory diseases [103].

When present chronically in the tumor microenvironment, TNF-α is a major mediator of cancer-related inflammation. Increased mitochondrial ROS production induced by TNF-α leads to activation of nuclear genes, especially NF-κB. In human and mouse ovarian cancer, TNF-α maintains TNF receptor1-dependent IL-17 production by CD4 + cells, which leads to myeloid cell recruitment into the tumor microenvironment and enhances tumor growth [104]. Tumor necrosis factor-α

can be produced when NF-κB is activated and TNF-α is also an important stimulus of NF-κB signaling and additional cytokine production [91].

In contrast to normal cells, tumor cells are usually under a higher oxidative stress and secrete more pro-inflammatory mediators. So, increased oxidative stress to the extent that still within the adaptive redox buffering capability of normal cells may overwhelm that of cancer cells, thereby selectively disrupting the redox state in tumor cells and activating the apoptotic or necrotic pathway, which leads to selective killing of tumor cells [91]. The balance between pro-inflammatory and anti-inflammatory cytokines (figure 1.11) is critical in determining a positive or a negative outcome, adverse reaction and resistance to radiation treatment [105]. Thus, modulation of IR-induced oxidative stress and inflammatory cytokine signaling may provide a better basis for enhancing radiation-mediated killing in cancer treatment with minimal normal tissue damage [106].

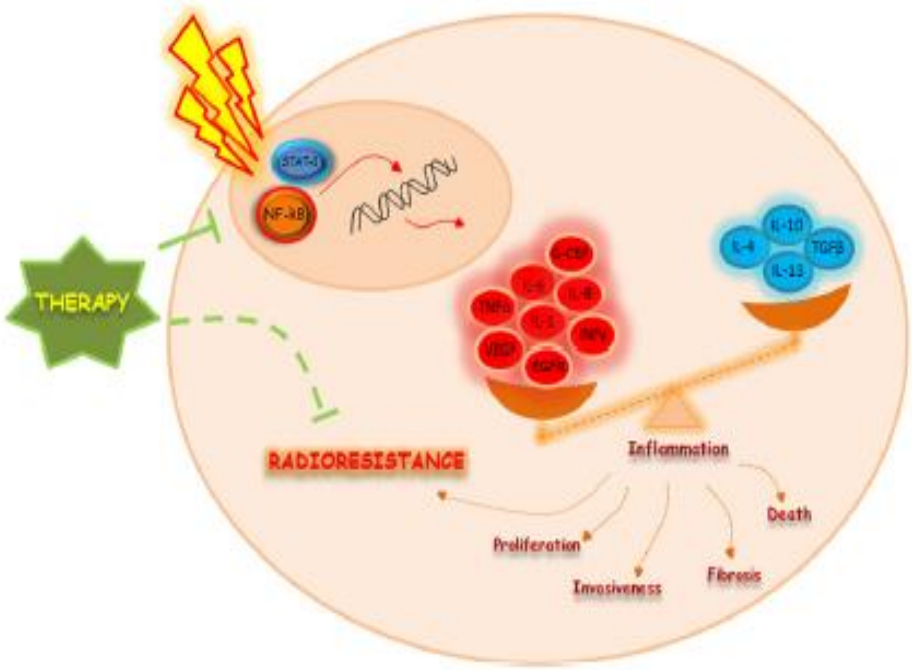


Figure 1.11: How IR could stimulate key transcription factors modulating inflammatory gene expression profile and cytokines involved in invasiveness and radiation related fibrosis. Targeting Nf-kB and STAT-3 IR activated could offer the opportunity to improve radiation therapy by enhancing radiosensitivity [107].

1.2.2 Radiation-induced fibrosis and radio-protection

To obtain optimal results from radiotherapy, the normal tissues should be protected against radiation injury. Hence, the role of radio-protective compounds is of great importance in clinical radiotherapy [(108)].

Radio-protectors are synthetic compounds or natural products that are immediately administered before irradiation to reduce injuries caused by ionizing radiation. Initial attempts focused on synthetic thiol compounds, like amifostine (the only radio-protector approved by FDA for mitigating side effects of radiotherapy). This drug offers good protection but is relatively toxic (nausea, vomiting and hypotension being some of the most common adverse effects) [(88)].

Radiation-induced fibrosis is a new theory that accounts for the damage to normal tissues, including bone, after radiotherapy [(109)]. The establishment of radiation-induced fibrosis involves free radical production immediately after the irradiation, resulting in complex multistep activation processes that persist for months and years [(88)]. Such tissue damage results in replacement of the native tissue with fibrocytes, which are unable to synthesize collagen, and also impaired vascularization of the tissue as the basement membranes of vessels are likewise affected by the radiation effects [(110)]. The destruction of endothelial cells, coupled with vascular thrombosis, lead to necrosis of micro-vessels, local ischaemia, and tissue loss. Loss of the natural endothelial cell barrier allows seepage of various cytokines that cause fibroblasts to become myofibroblasts [(111)]. Pelvic radiation affects both the gastrointestinal and genitourinary tracts and presentation of the above mentioned pathophysiologic processes include colonic strictures, fistulas and radiation colitis as well radiation cystitis, prostatic necrosis, and urethral strictures. The latter are the result of periurethral fibrosis, atrophy, and subsequent tissue contraction [(112)].

1.3 Cranberry

Cranberry, this term is derived from the contraction of ‘‘crane berry’’ (a name taken from the nickname of the bilberry flower, which, when it withers, is similar in appearance to the head and neck of the sand crane, a bird that often feeds on the berries of this plant) [(113)]. Cranberry belongs to the *Ericaceae* family of the subgenus *Oxycoccus*, the species called *Vaccinium macrocarpon* and naturally grows in acidic swamps full of peat moss in humid forests [(114)]. It is widespread throughout Northern Europe, Northern Asia and Northern North America. Cranberry is an evergreen ground cover native plant of North America. They are shrubs which grow about four meters having dark pink colored flowers and bears reddish black berries. Most of the cranberries today are cultivated in bogs and floated in water (figure 1.12) by Water Bog method or by water harvesting [(115)].

Cranberries contain water (88%), organic acids (including salicylate), fructose, vitamin C (high levels as much as 200 mg/kg of fresh berries), flavonoids, proanthocyanidins (PACs), anthocyanidins, catechins, and triterpinoids. Anthocyanidins and proanthocyanidins are tannins (stable polyphenols) only available in *Vaccinium* berries and act as a natural plant defense system against microbes [(116)].

Figure 1.12: Cranberry fruit in humid forest in North America [(117)].

The building blocks of PACs can be condensed either via a single C-C bond between C4 of the upper unit and C8 or C6 of the lower unit (B-type PACs) or with an additional ether-type bond between C2 of the upper unit and the hydroxyl group at C7 of the lower unit (A-type PACs) (Fig. 1.13). The PACs with at least 1 A-type linkage account for 51–91% of total PACs in cranberry [118]. The distinction between A- and B-type PAC structures is of importance because the difference can influence their biological properties. The A-type PACs exhibit significantly greater inhibition of *in vitro* adhesion of P-fimbriated *Escherichia coli* bacteria to uroepithelial cells than the B-type PACs, the initial step of UTI [119]. Many plant foods (such as apple, grape, and chocolate) contain high amounts of PACs, but only a few (plums, peanuts, avocados, cinnamon, and lingonberry) contain A-type PACs, and none (except for lingonberry) at the amount found in cranberries [120].

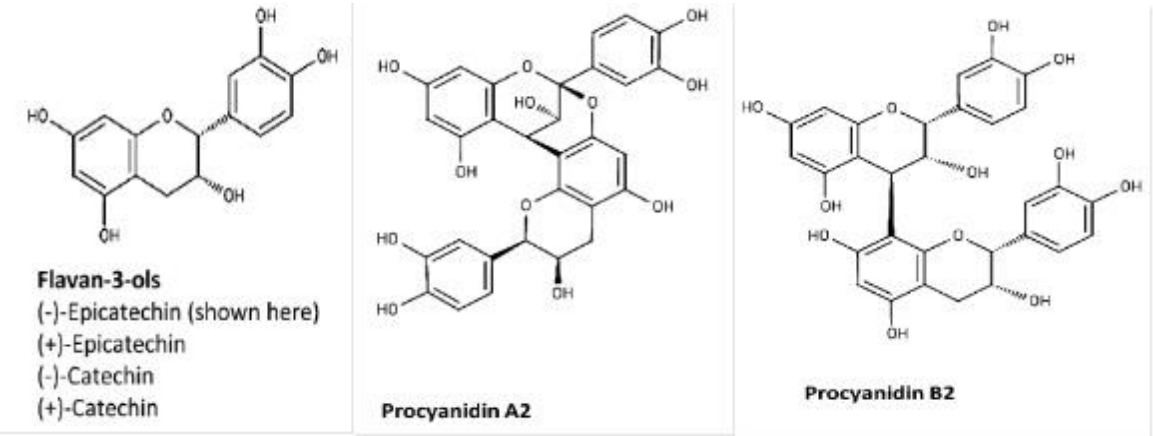


Figure 1.13: Proanthocynidins (PACs) chemical structures in cranberry [119].

1.3.1 Cranberry-PACs activity in the urinary tract

The American cranberry (*Vaccinium macrocarpon*) has been credited with preventing recurrent UTIs. Proanthocyanidins blocks the adherence of bacteria to the urothelium, preventing mucosal infection, by binding to different types of fimbriae of uropathogenic bacteria, particularly E. coli [121]. One of the important virulence properties of E. coli is its adherence to the host tissue. This phenomenon is related to a main protein structure called the adhesin protein, and its name is

based on its shape: pili or fimbriae [122]. The adhesion of bacteria is accomplished by the binding of lectins exposed on the cell surfaces of these fimbriae to complementary carbohydrates on the host tissues. Pili or fimbriae are small filaments that enable bacteria to adhere to the host tissue (urothelium); these proteins can be either mannose-resistant or mannose-sensitive [123]. Mannose-sensitive pili, called type 1 pili, permit bacterial adhesion to the urothelium; these fimbriae are inhibited by fructose (present in grapes, oranges, and cranberries). The more virulent strains of E. coli, isolated from patients with pyelonephritis and recurrent UTIs, have other types of fimbriae called p-fimbriae (pyelonephritis fimbriae) which bind to glycolsphingolipids of the lipid double membrane of renal cells, which precedes renal parenchymal invasion [124]. These P-fimbriae are inhibited by PAC [122]. Another suggested mechanism of cranberry activity is the *in vitro* reduction in the expression of p-fimbriae in *E. coli* or by inducing conformational changes in the surface macromolecules of p-fimbriated by specifically reducing fimbrial length and density [125].

Many studies have focused on uropathogenic E. coli type 1and p-fimbriated E. coli. Still there are many *in vitro* studies showing an inhibition of adherence for *Proteus species, Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus, Salmonella typhimurium and Klebsiella pneumonia* [126]. Even with multi-drug resistant strains of E.coli, cranberry had shown inhibition of adherence of these types to uroepithelial cells due to the presence of proanthocyanidins [127].

Another mechanism suggests that the juice may affect the concentration of Tamm-Horsfall glycoproteins in urine which interferes with the *E. coli* adherence to the human kidney [115]. The healthy bladder also produces two substances that make physical barrier to prevent the direct exposure of urine to the urothelium and inhibit bacterial adherence. These are Tamm-Horsfall glycoprotein (Raffi *et al*, 2005, reported that the severity of bacteriuria & inflammatory changes in the

urinary tract were much greater in Tamm-Horsfall glycoprotein-deficient mice) and Glycosaminglycan, both are secreted from the urothelial cells [(6)].

1.3.2 Cranberry-PACs anti-oxidant and anti-inflammatory actions

Proanthocynidins exert their anti-oxidant effect through free radical scavenging property and metal chelating activity. The scavenging capacity of PACs depends on the high ability to donate hydrogen, and it is related to the great number of hydroxyl groups on the flavonoid nucleus. The electronic configuration of PACs allows for easy release of electrons from electron donating -OH groups (attached to the aromatic rings in PAC) to free radical species; however, the hydrogen atom is more readily to be abstracted [(128)]. The PACs have the ability to bind iron and copper effectively, reduce their concentrations, and the extent of oxidative activity. The anti-inflammatory effects of PACs may come from restraining the inflammatory responses of activated neutrophils, they could prevent the oxidative discharge at the site of their adhesion [(129)].

1.3.3 Cranberry-PACs effects from clinical trials

Vaccinium macrocarpon is a rich source of polyphenols, which have been associated *in vitro* with antibacterial, antiviral, antimutagenic, anticarcinogenic, antitumorigenic, antiangiogenic, anti-inflammatory, and antioxidant properties [(130)]. *In vivo*, animal models reveal that cranberry extracts reduce C-reactive protein (CRP) and proinflammatory interleukins and increase nitric oxide synthesis; decrease angiotensin-converting enzyme, angiotensin II, and angiotensin II type 1 receptor; suppress *Helicobacter pylori* infection; and improve pancreatic beta-cells glucose responsiveness and functional beta-cells mass [(119)].

The mechanism of action may results from clinical studies showing that cranberry products can lower LDL cholesterol (LDL-C) and total cholesterol [(131)], increase HDL cholesterol (HDL-C), while lowering the oxidative modification of LDL-C [(132)], improve endothelial function [(133)], lower glycemic responses, elevate

plasma antioxidant capacity, modulate ulcerogenic gastric *Helicobacter pylori* colonization, decrease cariogenic *Streptococcus mutans* and total bacterial counts in saliva, reduce biomarkers of metabolic syndrome, and protect against urinary tract infections (UTIs) [(119)].

The A-type cranberry-PACs prevented biofilm formation and reduced adherence of *Candida albicans* to oral epithelial cells and attenuated the inflammatory response induced by this pathogen, represents potential novel therapeutic agents for the prevention/treatment of oral candidiasis [(134)]. The A-type cranberry PACs neutralized all the virulence properties of *Porphyromonas gingivalis* in a dose-dependent fashion and also inhibited the secretion of IL-8 but did not affect the secretion of IL-6 by epithelial cells stimulated with *P. gingivalis*. This anti-inflammatory effect was associated with reduced activation of the NF-κB pathway [(135)].

Consumption of the cranberry beverage modified the *ex vivo* proliferation of T cells. As these cells are located in the epithelium and serve as a first line of defense, improving their function may be related to reducing the number of symptoms associated with cold and flu [(136)].

1.4 Aim of the study

The aim of this study is to evaluate the effects of cranberry-PACs in reducing adverse events of lower urinary tract in patients with bladder carcinoma undergoing radiotherapy via its anti-inflammatory and anti-oxidant effects.

Chapter Two
Patients and Methods

2.1 Materials

2.1.1 Kits

Kits used in this study are mentioned in table (2-1) with their manufactures and origins.

Table (2-1): Kits used in the study.

Kit	Manufacture	Origin
Copper/Zinc superoxide dismutase (SOD1)	CUSABIO	China
Interleukin-8 (IL-8)	CUSABIO	China
Tumor necrosis factor-alpha (TNF-α)	CUSABIO	China
Total anti-oxidant capacity (TAC)	Biovision	United States

2.1.2 Chemicals

Specific chemicals with highest purity are used in this study and presented in table (2-2) with their manufacturers and origins.

Table (2-2): Chemicals and drugs used in the study.

Chemical	Manufacturer	Origin
Blood Agar	Microgen	India
Lactose	Luna Company	Egypt
MacConkey Agar	Microgen	India
Urinal Akut® (each tablet contains 36mg of pure proanthocyanidins extracted according to the American method)	Walmark	Czech Republic

2.1.3 Instruments

Instruments used in this study are summarized in table (2-3) with their manufactures and origins.

Table (2-3): Instruments used in the study.

Instrument	Manufacturer	Origin
3D helical CT-scan.	Siemens	Germany
Cold Centrifuge -Universal 16A	Hettich	Germany
Comprehensive image-guided radiation therapy system (Linac) with Volumetric Modulated Arc Therapy (VMAT)	Elekta (Infinity™)	Sweden
Deep freezer	Froilabo	France
ELISA plate reader and washer	BioTek	USA
Incubator	SHEL LAB	USA
Light microscope	Olympus	Japan
Micropipette	SLAMED	Germany
Sensitive Balance	A and D company Ltd.	Japan

2.2 Patients

2.2.1 Patients selection

This randomized placebo-controlled clinical study was carried out on 45 patients (33 males/12 females) with MIBC (T2-T3 only) proven by radiologic

tests, cystoscopy and histopathological staging whom are candidates for multimodality bladder preserving treatment approach and fit for curative doses (64 Gy) of radiotherapy. These patients were with ages range of 60-70 years (mean: 65.84 years), and on diet restriction with any food or drink containing berries, red grapes, or red wine, and under a controlled hydration regimen (2-3 litters of water per day). Certain exclusion criteria were followed to avoid interference with the study design and include:

1. Patients with UTIs and/or severe urinary symptoms at baseline and those having urethral catheterization during or around the course of radiotherapy.
2. Patients with history of pelvic radiotherapy.
3. Patients with prostate and other pelvic malignancy or patients with MIBC stages T4a and T4b.
4. Patients taking or have been taken chemotherapy within the previous three months before the study.
5. Patients with diabetes, neurogenic bladder, history of renal dysfunction, severe macrohematuria or patients with irritable bowel syndrome.
6. Patients using medications like warfarin, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antibiotics, antispasmodics, and other analgesics.

Only 40 patients (30 males/10 females) completed the study, the other 5 were excluded from the study (2 females from the cranberry group due to poor compliance and 3 males from the placebo group due to the development of UTI approved by urine culture at the 3rd week of RT). These patients were diagnosed and treated in the Oncology Teaching Hospital/ Medical City Directorate under supervision of specialist doctors after achieving ethical committee approval and taking patients oral consent, during the period from November 2014 to April 2016.

2.2.2. Study design

Patients of this study were randomly allocated into two groups as follow (figure 2.1):

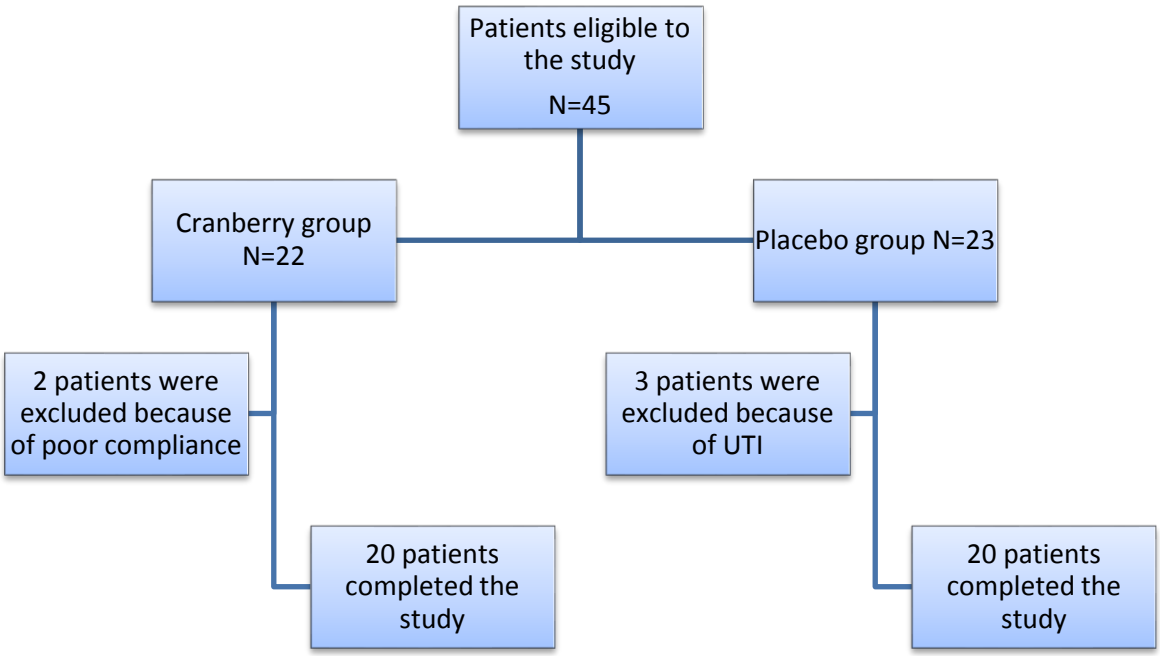


Figure 2.1: Patients flow diagram.

- **Cranberry group:** include 20 patients receiving cranberry tablets twice per day during their radiotherapy regimen which prolonged for 6-7 weeks (under Urinal Akut® trademark which contain 36 mg of PACs from American cranberry per tablet according to American extraction method). Those patients were receiving 64 Gy of radiotherapy fractionated as 32 fractions (2 Gy per fraction), five fractions per week.
- **Placebo group:** include 20 patients receiving placebo capsules (500 mg of lactose/cap) twice per day during their radiotherapy regimen which also prolonged for 6-7 weeks. Those patients were receiving 64 Gy of radiotherapy fractionated as 32 fractions (2 Gy per fraction), five fractions per week.

Any patient planned to have radiotherapy course was given a simulation appointment, in which the radiation dosage/ fraction set was assigned using 3-dimention helical CT-scan. Radiotherapy was exposed in a supine position after emptying the bladder and performed using comprehensive image-guided radiation therapy system (Linear accelerator or Linac) with Volumetric Modulated Arc Therapy (VMAT) and by three-dimensional radiation therapy techniques (Elekta, Infinity^{TM}).

At baseline, before starting radiotherapy, all the patients filled a specially designed questionnaire, recording their medical history and pretreatment characteristics. During treatment, all patients underwent weekly examination to assess the subjective lower urinary tract symptoms (mean weekly urinary frequency, nocturia, and urgency) which were recorded in a daily diary card (urinary urgency was given a scale of 0-5 according to the patient perception of intensity of urgency scale questionnaire [PPIUS] [137]). Two urine cultures and urinalysis tests were performed for each patient, one at the baseline and the second at the end of treatment, to assess their UTIs, pyuria, and hematuria. A further urine culture was performed if the intensity of lower urinary tract symptoms (LUTS) were present to be sure if the patient cannot complete the study protocol. Also, patients were instructed to report any drug-related adverse effects.

Inflammatory markers (IL-8 and TNF-α) and Oxidative stress markers (SOD1 and TAC) were determined at baseline and at the end of radiation treatment, for both patient groups.

The demographic and baseline characteristics were evenly distributed for both groups and summarized in table (2-4).

Table (2-4): Baseline characteristics for patient groups.

Demographic & baseline characteristics	**Cranberry group N=20**	**Placebo group N=20**	**Total N=40**
Age (60-70 yrs)	65.68±3.414	65.81±3.647	65.84±3.805
Sex (male/ female)	16(80)/4(20)	14(70)/6(30)	30(75)/10(25)
Family history for BC	Negative	Negative	Negative
Smoking history			
Negative smoking history	3(15)	6(30)	9(22.5)
Past/current smokers	17(85)	14(70)	31(77.5)
T-Stage			
T2	10(50)	12(60)	22(55)
T3	10(50)	8(40)	18(45)

Data expressed as numbers (%) and as mean ± standard error of mean (SEM).

2.3 Methods

2.3.1 Samples collection and preparation

Using a sterile cup of urine samples, each patient was requested to give 5ml of midstream urine at baseline and at the end of the treatment for both patient groups. Urine for urinalysis was centrifuged at 3000 rpm in order to get the supernatant ready for microscopical assay and the rest of urine was cultured to check for any bacterial growth.

Venous blood (5 ml) was obtained from the forearm of each patient by vein puncture at baseline and at the end of treatment and for both patient groups. Each blood sample was placed in EDTA-free tube to be centrifuged for 10 minutes at 3000 rpm. Serum was then divided into several eppendorf tubes and kept frozen at -40°C until the time of assay.

2.3.2 Laboratory analysis

i. Urine tests:

General urine examination was carried out for the detection of pyuria (expressed as WBCs/high power field), hematuria (expressed as RBCs/high power field), and bacteriuria (expressed as no. cells/high power field). The collected samples were streaked on MacConkey agar and blood agar plate. Plates were incubated at 37ºC for 24 hrs. Colonies on each plate were counted to determine the number of microorganisms per milliliter in the original specimen. Urinary tract infection was assumed when the findings of bacteriuria exceeded 100,000 U/mL, accompanied by LUTS.

ii. Serum tests:

a) Determination of serum IL-8, TNF-α & SOD1

Measurement of these markers was achieved according to their manufacturer kits manuals and as following [138-140]:

Principle

A particular antibody for IL-8, TNF-α & SOD1 is pre-coated onto a microplate that contains 96 wells. The standards and samples specimens are pipetted into the wells and any IL-8, TNF-α & SOD1 present are bound by the immobilized antibody. Then, a biotin-conjugated antibodies specific for IL-8, TNF-α & SOD1 are added to the wells. After washing, avidins conjugated Horseradish Peroxidase (HRP) are added to the wells. Another wash to remove any

unbound avidin-enzyme reagent before adding a substrate solution to the wells and color develops in proportion to the amount of IL-8, TNF-α & SOD1 bound in the initial step. The color development is stopped and the intensity of the color is measured (figure 2.2). Results were expressed as ng/mL of serum for SOD1 and as pg/mL of serum for both IL-8 and TNF-α.

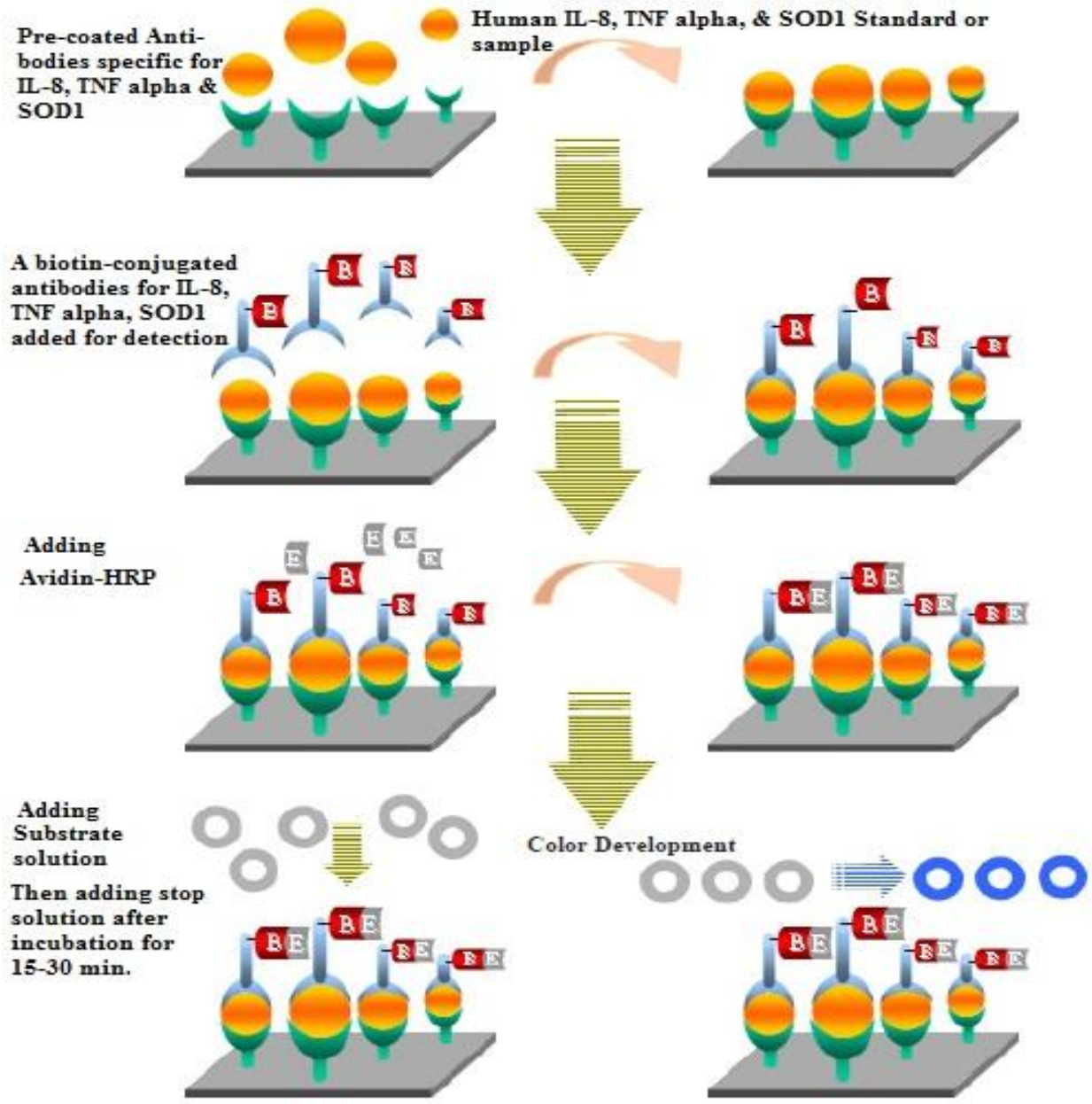


Figure 2.2: Quantitative sandwich enzyme immunoassay technique. IL-8: interleukin 8, TNF alpha: tumor necrosis factor alpha, SOD1: superoxide dismutase (Cu-Zn) & HRP: horseradish peroxidase *(138-140).*

Procedure of the three assays:

1. Biotin-antibody and HRP-avidin vials were centrifuged before opening.
2. Biotin-antibody and HRP-avidin were diluted 100 times using Biotin-antibody and HRP-avidin Diluents, respectively.
3. Wash Buffer solutions were prepared by adding 20 mL of washing buffer to 480 mL of deionized water.
4. Standard vials for each kit were centrifuged at 6000 rpm for 30 seconds then reconstituted with 1.0 mL of Sample diluent to produce stock solutions of 2000 pg/mL IL-8, 500 pg/mL TNFα, and 1000 pg/mL SOD1. Those stock solutions were used to prepare a 2-fold dilution series.
5. Just in SOD1 kit, serum samples have been diluted 2000 folds before starting the assay.
6. The Assay Layout Sheets were used to determine the number of wells to be used and the remaining wells were kept into the pouches, sealed and stored at 4°C.
7. 100µl of standards and samples have been added per well and the wells were covered with an adhesive strip, and then incubated for 2 hours at 37°C.
8. The liquid in each well removed by the ELISA plate washer using the aspiration program (not the washing program).
9. 100µl of Biotin-antibody solution were added to each well and covered with a new adhesive strip then incubated for 1 hour at 37°C.
10. Each well were aspirated then washed for 3 times by the ELISA plate washer.
11. 100µl of HRP-avidin solution were added to each well and covered with a new adhesive strip, then incubated for 1 hour at 37°C.

12. Another Aspiration/washing processes were performed for 5 times.
13. 90µl of TMB Substrate solution were added to each well (colors had been developed in the wells) then incubated for 15-30 minutes at 37°C immediately avoiding direct light.
14. 50µl of Stop Solution were added to each well.
15. Optical densities were determined within 5 minutes of the last step by the ELISA plate reader set at 450 nm. Anther reading taken immediately after the 1st one but at 540 nm, the last reading is subtracted from the 1st one to correct any optical imperfections in the plate.

Calculations:

Standard curves were created through reducing the data by computer software (provided by the manufacturer called Expert Curve 1.4) that generates a four parameter logistic (4-PL) curve-fit. The software was able to give the concentrations of each parameter with specific formulas for each standard curve. In the case of SOD1, the concentrations read from the standard curve were multiplied by the dilution factor (2000).

b) Determination of serum total anti-oxidant capacity level

Measurements of this marker were achieved according to its manufacturer kit manual and as following [141]:

Principle

This assay depends on the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that gives a color with a maximum absorbance at 570 nm. The absorbance values of antioxidants are compared with a known uric acid standard curve. Absorbance values are proportional to the

sample's total reductive capacity (figure 2.3). Results were expressed as "nmol/µL Copper Reducing Equivalents".

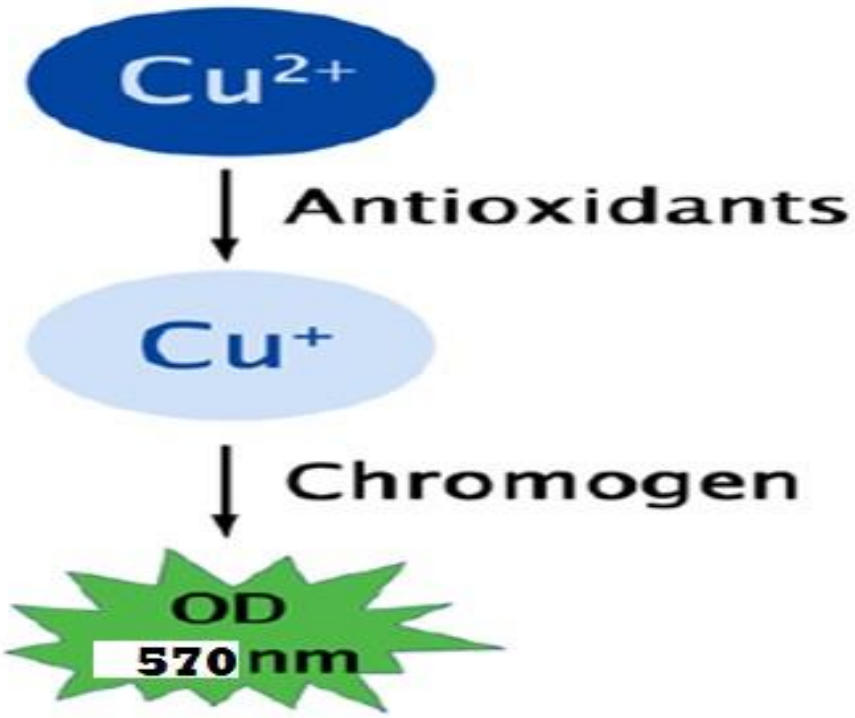


Figure 2.3: Chemical principle of Total anti-oxidant capacity assay [141].

Procedure of the assay:

1. The lyophilized standard was dissolved in 20 µl of pure dimethylsufoxide solution, then 980 µl of distilled water were added and mixed well, generating a 1 nmol stock solution.
2. To create a standard solution: 0, 4, 8, 12, 16, 20 µl of the stock solution to individual wells. The total volume for each well had been adjusted to 100 µl with deionized water to give 0, 4, 8, 12, 16, 20 nmol of Stock solution.
3. Working solution was prepared by diluting one part of Cu^{2+} reagent with 49 parts of Assay diluent.
4. 0.1 µl of serum samples were added to the wells.
5. 100 µl Cu^{2+} of working solution were added to all standard and sample wells.

6. The plate had been covered and incubated at room temperature for 1.5 hours.
7. The absorbance was read at 570 nm using the plate reader.

Calculations of TAC results:

1. Standard curve was drawn on a semi-logarithmic paper, the absorbance plotted as a function of stock solution concentrations (0, 4, 12, 16, 20 nmol)
2. From the standard curve **(**y=0.0353x-0.001). This equation used to determine samples amount in nmol read from the standard curve.
3. TAC of sample= Sa/Sv in nmol/µL of Cupper reducing equivalents, where: Sa=y, and Sv= the undiluted sample volume added to the wells.

2.4 Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). Student's t-tests (both the pooled and paired) were used to analyze most of the parameters. Analysis of variance test (ANOVA) used to analyze LUTS of weekly means. *P*-values < 0.05 were considered significant. Microsoft® Excel software program (2010) and Minitab® statistical software were used for statistical analysis.

Chapter Three

Results

3.1. Effects of cranberry-PACs on LUTS during RT

A. Effects on urinary frequency

Table (3-1), figures (3-1) and (3-2) showing the following findings:

A significant elevation in the mean urinary frequency of the placebo group was observed at the end of treatment when compared to that at baseline *(P<0.05)*.In the cranberry group, the mean urinary frequency at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline.

When compared to the mean urinary frequency of the placebo group at baseline, the mean urinary frequency of the cranberry group was not significantly differ (*P>0.05)*. On the other hand, the mean urinary frequency of the cranberry group was significantly lower *(P<0.05)* than that of the placebo group at the end of the treatment.

There was a significant elevation (gradual increment) in the weekly mean urinary frequency of the placebo group throughout the treatment course *(P<0.05)*, while the weekly mean urinary frequency of the cranberry group showed a significant reduction (gradual decrement) throughout the treatment course *(P<0.05)*.

Table (3-1): Mean urinary frequency changes during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	9.305 ± 0.35^{a}	14.485 ± 0.17^{b}
Cranberry	20	8.556 ± 0.28^{a}	$7.080 \pm 0.14^{b*}$

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

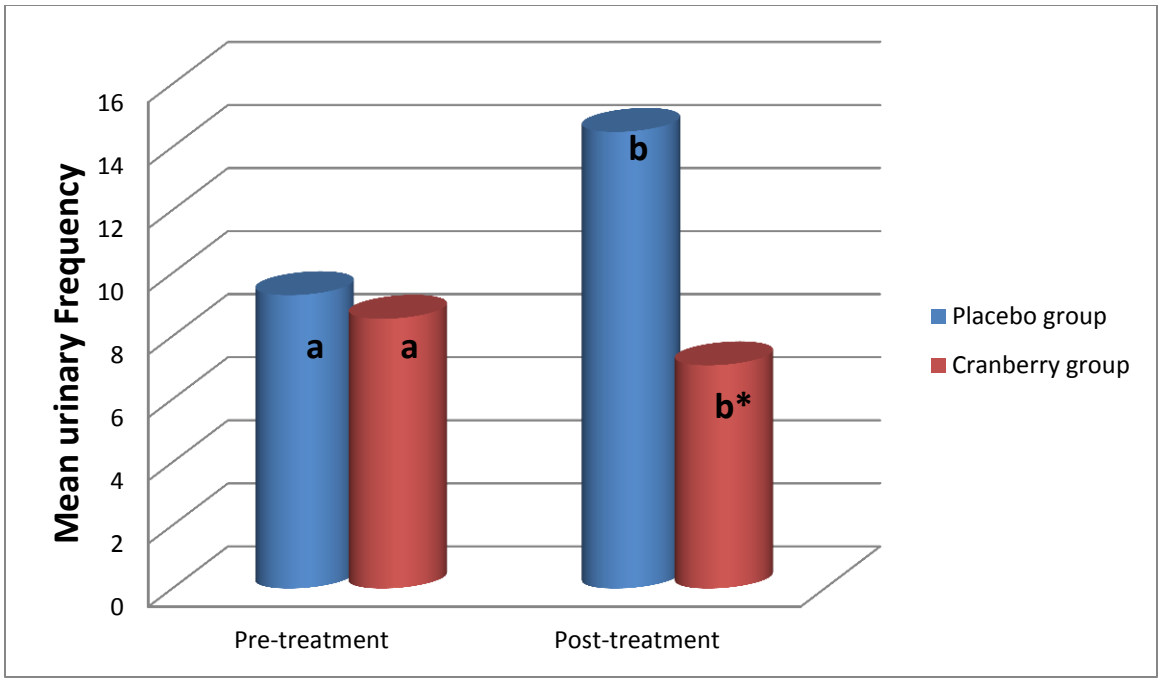


Figure 3.1: Mean urinary frequency changes during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

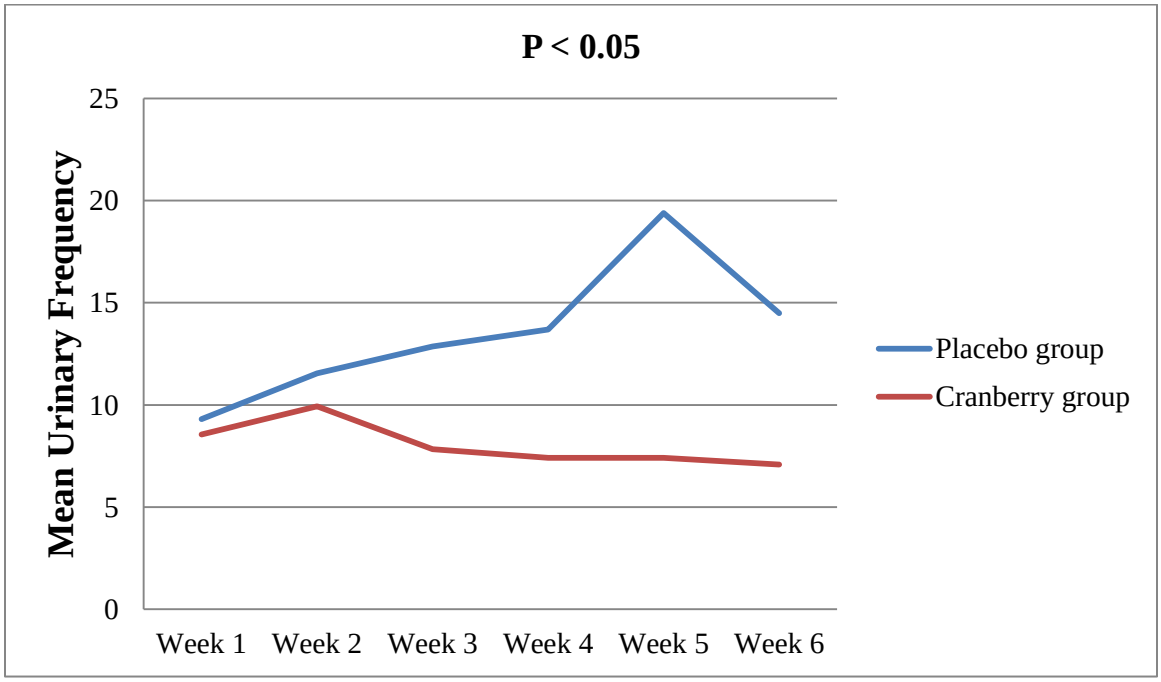


Figure 3.2: Changes of the weekly mean urinary frequency during the study for both groups.

B. Effects on nocturia

Table (3-2), figure (3-3) and (3-4) clarifying the following findings:

A significant elevation in the mean nocturia of the placebo group was observed at the end of the treatment when compared to that at baseline *(P<0.05)*. The mean nocturia of the cranberry group at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline.

When compared to the mean nocturia of the placebo group at baseline, the mean nocturia of the cranberry group was not significantly differ *(P>0.05)*. The mean nocturia of the cranberry group was significantly lower *(P<0.05)* than that of the placebo group at the end of the treatment.

There was a significant elevation (gradual increment) in the weekly mean nocturia of the placebo group throughout the treatment course *(P<0.05)*, while the weekly mean nocturia of the cranberry group showed a significant reduction (gradual decrement) throughout the treatment course *(P<0.05)*.

Table (3-2): Mean nocturia changes during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	1.522 ± 0.057^{a}	2.398 ± 0.080^{b}
Cranberry	20	1.421 ± 0.067^{a}	$1.176 \pm 0.024^{b*}$

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

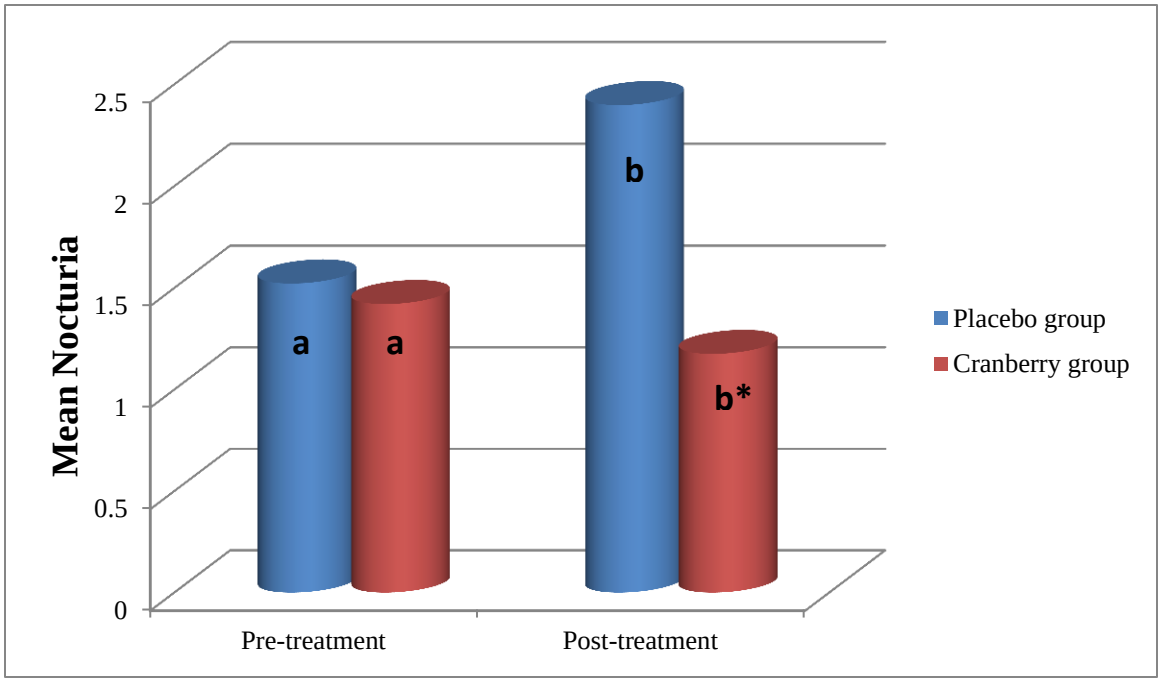


Figure (3-3): Mean nocturia changes during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

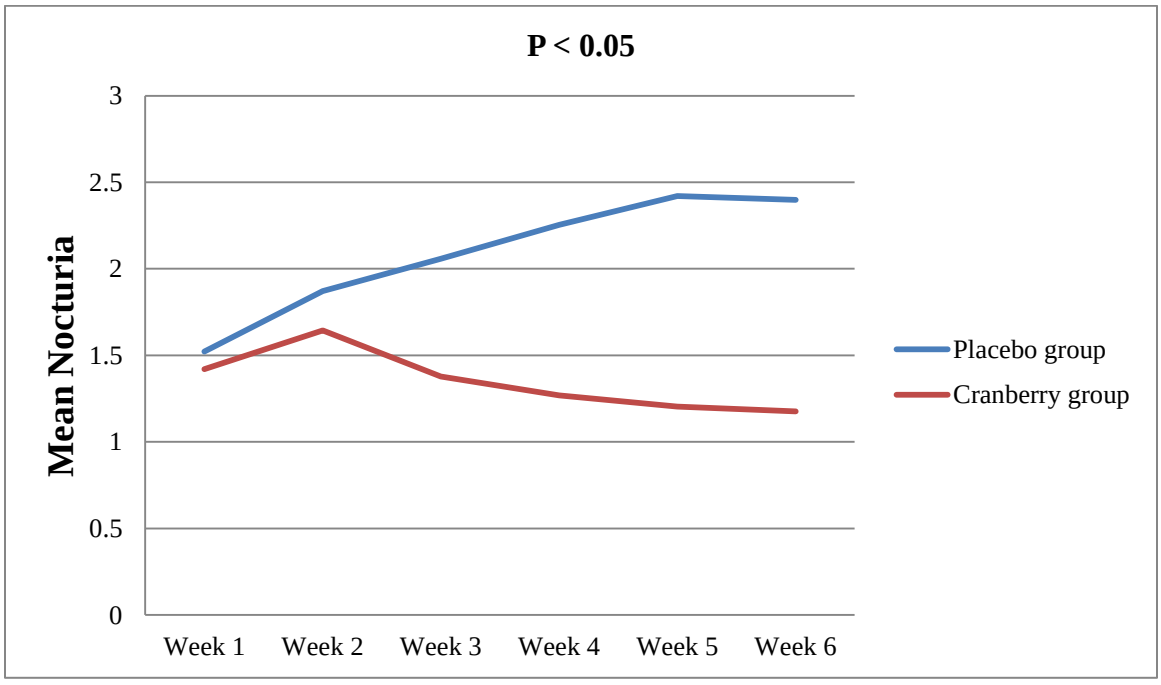


Figure (3-4): Changes of the weekly mean nocturia during the study for both groups.

C. Effects on urinary urgency

Table (3-3), figures (3-5) and (3-6) show the following findings:

A significant elevation in the mean urinary urgency of the placebo group was observed at the end of treatment when compared to baseline *(P<0.05)*. The mean urinary urgency of the cranberry group at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline.

The mean urinary urgency at baseline of the cranberry group was significantly higher *(P<0.05)* than that of the placebo group. Meanwhile, the mean urinary urgency of the cranberry group was significantly lower *(P<0.05)* than that of the placebo group at the end of the treatment.

There was a significant elevation (gradual increment) in the weekly mean urinary urgency of the placebo group throughout the treatment course *(P<0.05)*, while the weekly mean urinary urgency of the cranberry group showed a significant reduction (gradual decrement) throughout the treatment course *(P<0.05)*.

Table (3-3): Mean urinary urgency changes during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	1.200 ± 0.16^{a}	3.500 ± 0.14^{b}
Cranberry	20	$2.200 \pm 0.19^{a*}$	$1.155 \pm 0.14^{b*}$

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

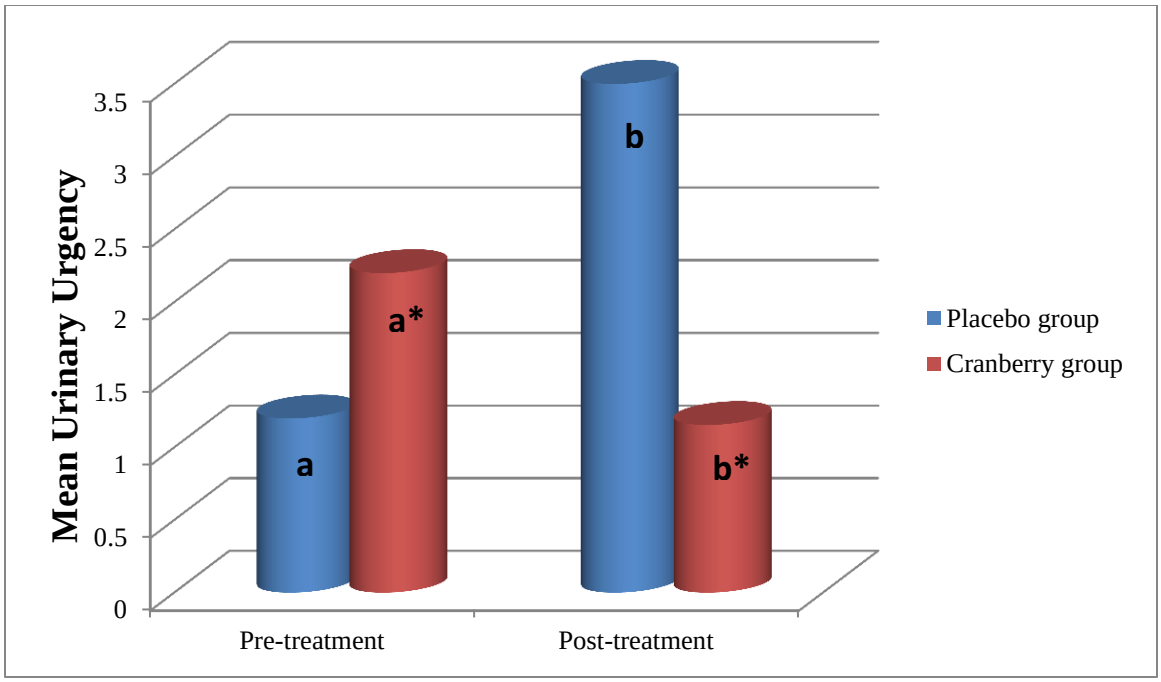


Figure (3-5): Mean urinary urgency changes during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

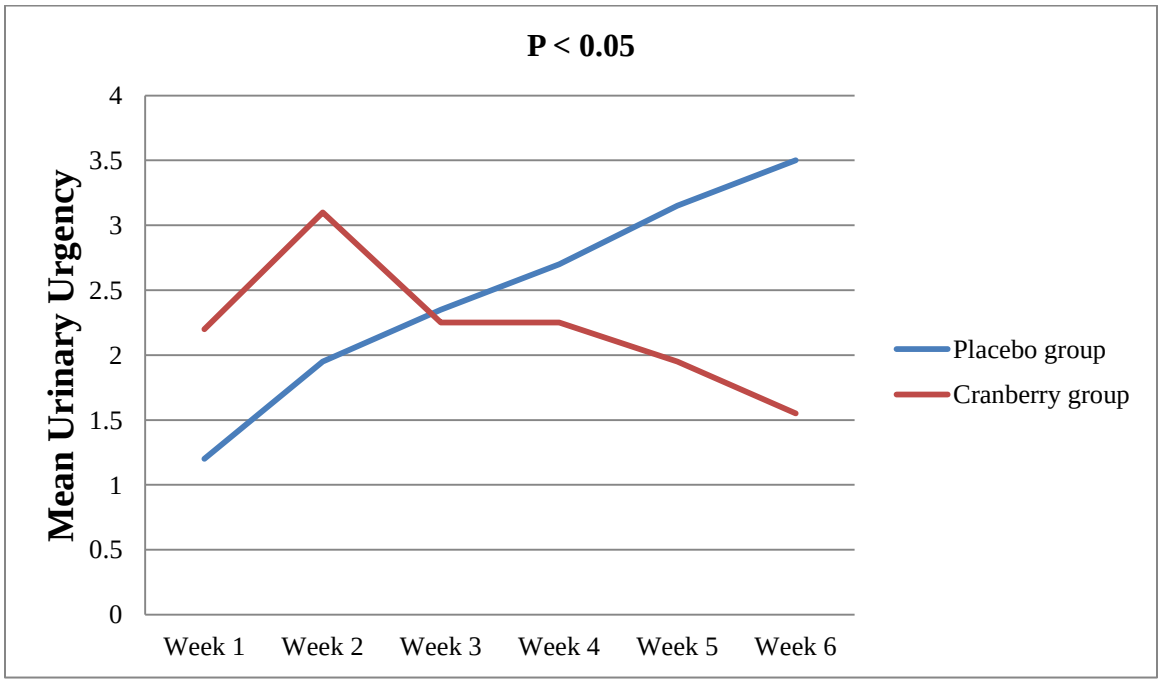


Figure (3-6): Changes of the weekly mean urinary urgency during the study for both groups.

3.2 Effects of cranberry-PACs on urinalysis during RT

A. Effects on pyuria

Table (3-4) and figure (3-7) illustrating the following results:

The mean level of pyuria at the end of the treatment was significantly elevated *(P<0.05)* from the baseline level in the placebo group. The mean level of pyuria in the cranberry group was elevated significantly *(P<0.05)* at the end of the treatment when compared to that of baseline, but it's still significantly lower *(P<0.05)* than that of the placebo group post treatment. Regarding the mean level of pyuria of the two groups at baseline, there was no significant difference *(P>0.05)* between them.

Three patients (6.6 %) from the placebo group were withdrawn from the study at the 3rd week due to the development of severe LUTS associated with bacteriuria in urinalysis and approved by a heavy growth of *E. coli* in the culture and sensitivity test.

Table (3-4): Mean pyuria changes (WBCs/HPF) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	4.70 ±0.56[a]	57.30 ±4.7[b]
Cranberry	20	4.00 ±0.56[a]	11.15 ±1.1[b*]

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

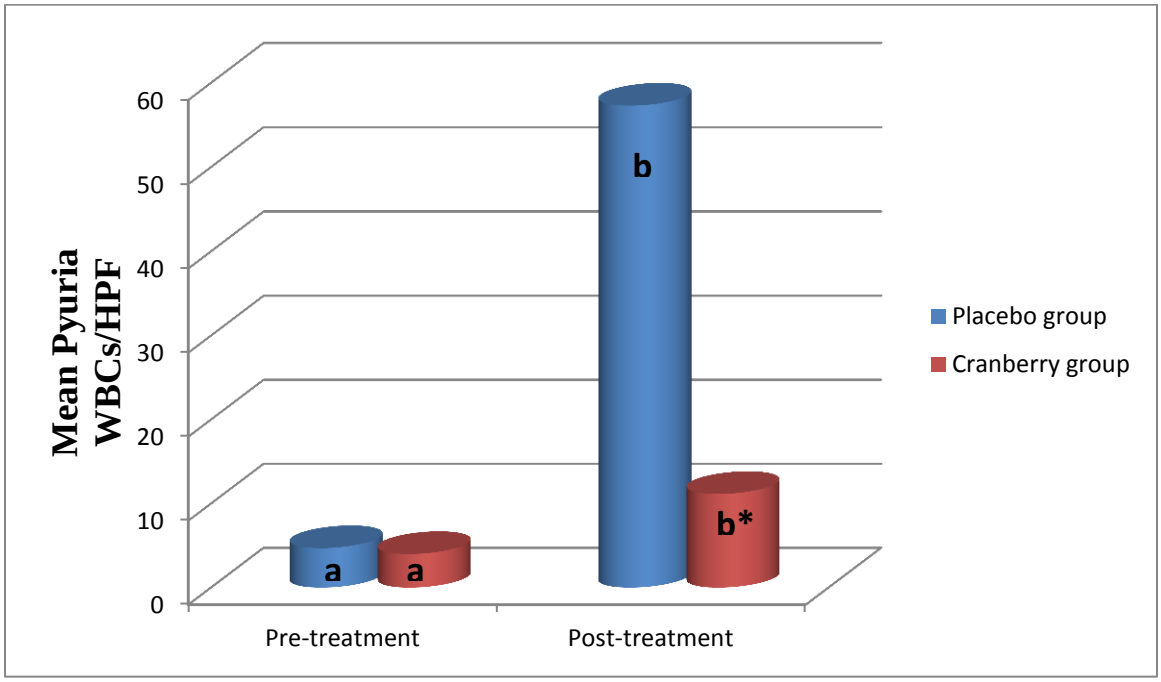


Figure (3-7): Mean pyuria changes (WBCs/HPF) during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

B. Effects on hematuria

Table (3-5) and figure (3-8) show the following results:

The mean level of hematuria at the end of the treatment was significantly elevated *(P<0.05)* from the baseline level in the placebo group. Also, the mean level of hematuria in the cranberry group was elevated significantly *(P<0.05)* at the end of the treatment when compared to that of baseline, but it's still significantly lower *(P<0.05)* than that of the placebo group post treatment. Regarding the mean level of hematuria of the two groups at baseline, there was no significant difference *(P<0.05)* between them.

Table (3-5): Mean hematuria changes (RBCs/HPF) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	2.00 ±0.47[a]	33.75 ±2.60[b]
Cranberry	20	1.85 ±0.39[a]	8.15 ±0.80[b*]

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

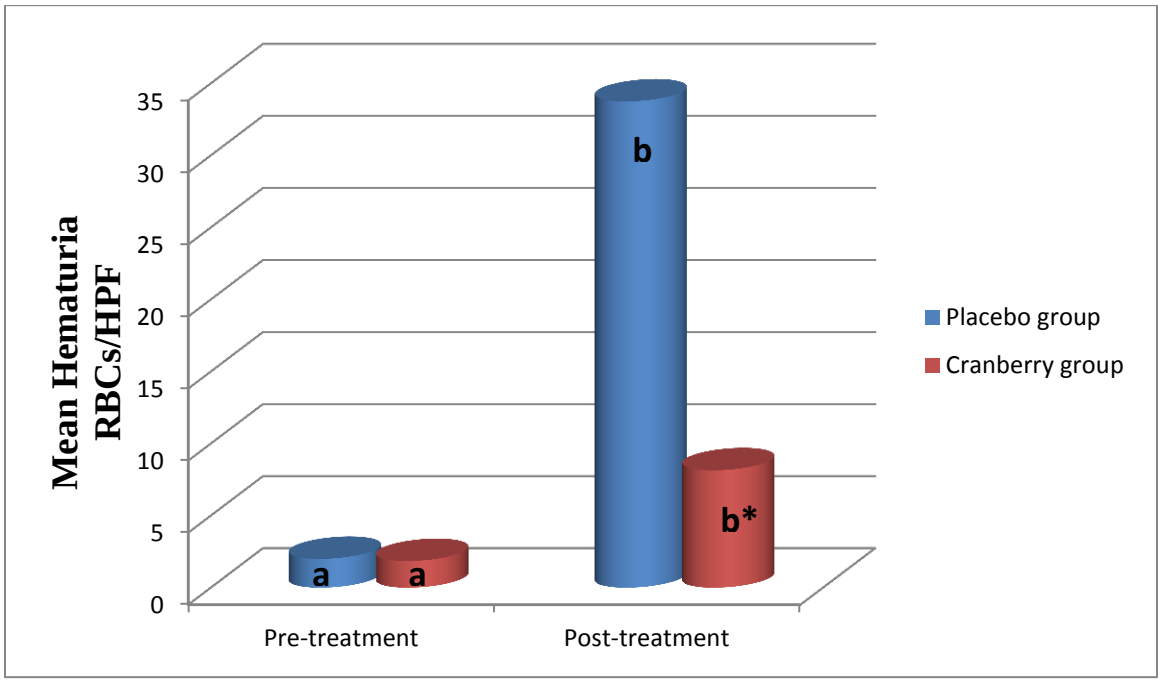


Figure (3-8): Mean hematuria changes (RBCs/HPF) during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

3.3 Effects of cranberry-PACs on inflammatory markers during RT

A. Effects on serum level of TNF-α

Table (3-6) and figure (3-9) showing the following findings:

A significant elevation in the mean TNF-α serum level of the placebo group was observed at the end of treatment when compared to the baseline level *(P<0.05),*whereas the mean TNF-α serum level of the cranberry group at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline.

When compared to the mean TNF-α serum level of the placebo group at baseline, the mean TNF-α serum level of the cranberry group was not significantly differ *(P>0.05)*. Meanwhile, the mean TNF-α serum level of the cranberry group was significantly lower *(P<0.05)* than that of the placebo group at the end of the treatment.

Table (3-6): Mean serum level of TNF-α (pg/mL) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	114.30 ±15.40[a]	408.70 ±36.60[b]
Cranberry	20	105.38 ±9.23[a]	44.30 ±4.20[b*]

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

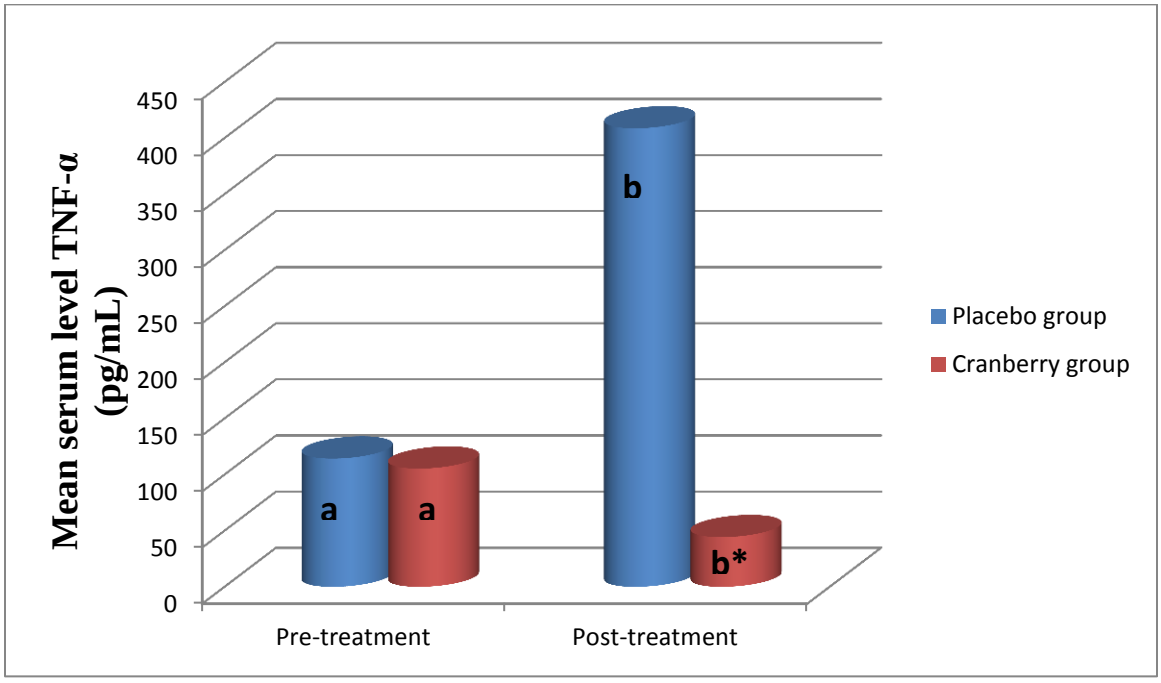


Figure (3-9): Mean serum level of TNF-α (pg/mL) during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

B. Effects on serum level of IL-8

Table (3-7) and figure (3-10) showing the following findings:

A significant elevation in the mean IL-8 serum level of the placebo group was observed at the end of treatment when compared to that at baseline *(P<0.05).* The mean IL-8 serum level of the cranberry group at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline.

When compared to the mean IL-8 serum level of the placebo group at baseline, the mean IL-8 serum level of the cranberry group was not significantly differ *(P>0.05)*. However, the mean IL-8 serum level of the cranberry group was significantly lower *(P<0.05)* than that of the placebo group at the end of the treatment.

Table (3-7): Mean serum level of IL-8 (pg/mL) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	63.40 ±6.00[a]	386.50 ±22.30[b]
Cranberry	20	66.51 ±5.67[a]	34.77 ±1.76[b*]

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

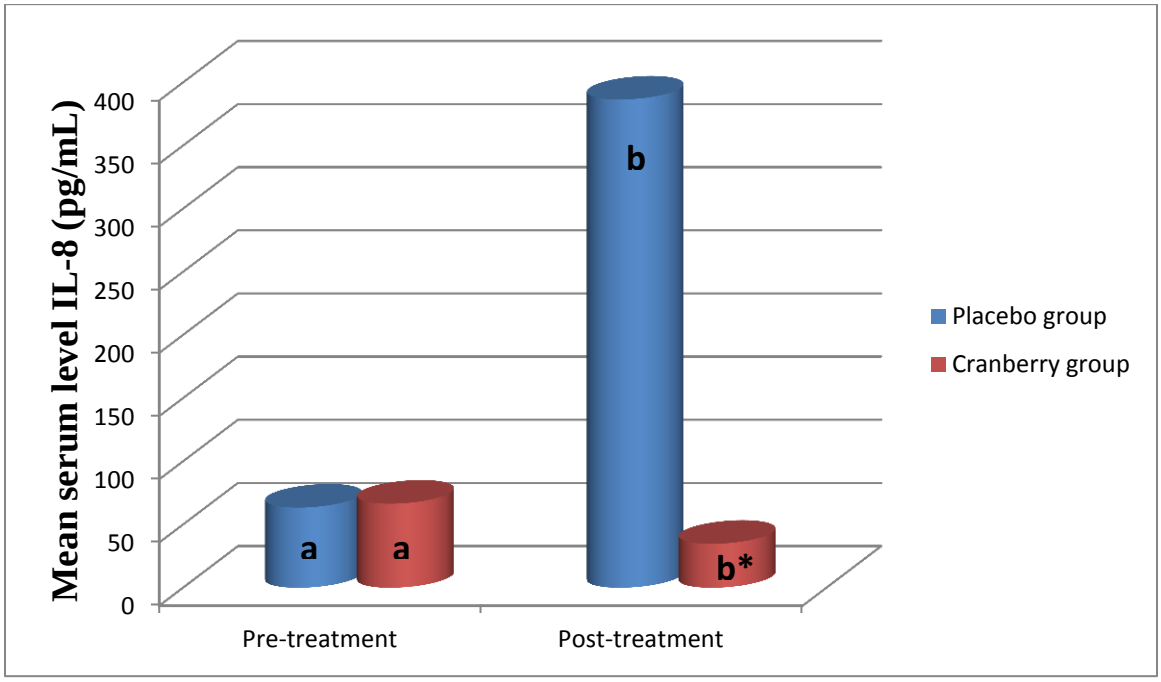


Figure (3-10): Mean serum level of IL-8 (pg/mL) during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

3.4 Effects of cranberry-PACs on the oxidative status during RT

A. Effects on serum levels of SOD1

Table (3-8) and figure (3-11) show the following findings:

The mean SOD1 serum level of the placebo group at the end of the treatment was reduced significantly *(P<0.05)* from that at baseline, while significant elevation in the mean SOD1 serum level of the cranberry group was observed at the end of treatment when compared to that at baseline *(P<0.05).*

When compared to the mean SOD1 serum level of the placebo group at baseline, the mean SOD1 serum level of the cranberry group was not significantly differ *(P>0.05)*. Meanwhile, the mean SOD1 serum level of the cranberry group was significantly higher *(P<0.05)* than that of the placebo group at the end of the treatment.

Table (3-8): Mean serum levels of SOD1 (ng/mL) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	0.878 ±0.062[a]	0.616 ±0.066[b]
Cranberry	20	0.789 ±0.078[a]	1.055 ±0.077[b*]

Data are expressed as mean ± SEM

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

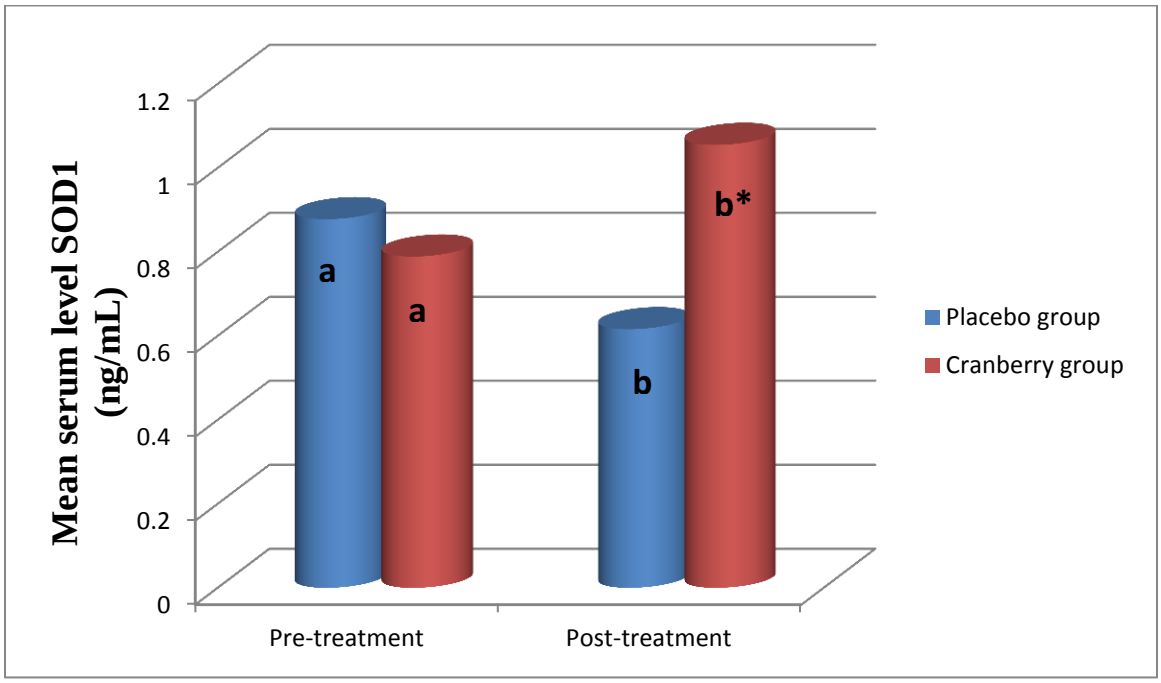


Figure (3-11): Mean serum level of SOD1 (ng/mL) during the treatment.

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

B. Effects on serum level of TAC

Table (3-9) and figure (3-12) showing the following observations:

In the placebo group, a significant reduction *(P<0.05)* was observed in the mean serum level of TAC at the end of treatment when compared to that of the baseline. Meanwhile, there was no significant elevation *(P>0.05)* in the mean serum level of TAC at the end of the treatment when compared to that of the baseline level in the cranberry group.

When comparing the two groups, the baseline mean serum level of TAC for the cranberry group was significantly lower *(P<0.05)* than that of the placebo group. On the other hand, the mean serum level of TAC for the cranberry group at the end of the treatment was significantly higher *(P<0.05)* than that of the placebo group.

Table (3-9): Mean serum level of TAC (nmol/µL) during the treatment.

Group	N	Pre-treatment	Post-treatment
Placebo	20	7.834 ±0.101[a]	7.062 ±0.104[b]
Cranberry	20	7.095 ±0.082[a*]	7.102±0.077[a*]

Data are expressed as mean ± SEM

Identical (a, a) superscripts indicate no significant difference within the group *(P>0.05)*

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

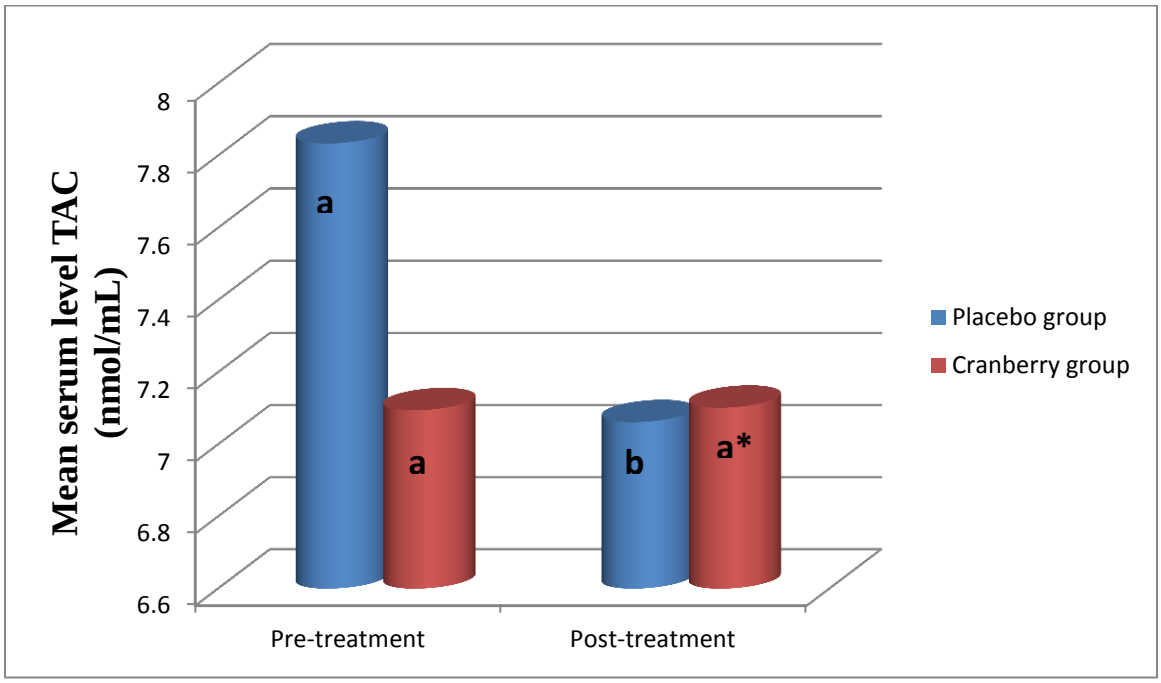


Figure (3-12): Mean serum level of TAC (nmol/µL) during the treatment.

Identical (a, a) superscripts indicate no significant difference within the group *(P>0.05)*

Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*

Superscript (*) indicates significant difference compared with placebo group *(P<0.05)*

Chapter Four
Discussion

4.1 Effects of cranberry-PACs on LUTS during RT

The LUTS in patients with BC may present before treatment with curative doses of radiotherapy and can be exacerbated by the treatment. In addition, acute LUTS may develop during radiotherapy course of treatment (bladder toxicity). These symptoms may range from increased frequency, nocturia, urgency, to the development of UTIs; collectively can be defined as radiation cystitis [(142)].

Because these symptoms are linked to the stage of the tumor [(143, 144)], this study excluded any patient with a stage that involve any area in the bladder neck or the prostate (T4 and above stages) that might hinder bladder urinary flow in order to rule out the variation between the patients in their baseline urinary symptoms (frequency, nocturia, and urgency) and trying to concentrate on the effects of RT in increasing the incidence of these LUTS. This approach was consistent with older studies excluded patients with LUTS at the baseline that might severely affect the recruitment criteria for those studies [(142, 143, & 145)]. Also, any patient having these criteria but presented with baseline UTI was excluded.

In order to limit the variation between the patients of both groups, all the patients were advised to drink 2-3 liters of water per day to avoid any difference between the patients regarding the development of urinary symptoms. Previous study tried different hydration regimen with inconclusive result about the most effective regimen in reducing urinary symptoms [(121)].

The difference of the present study from the previous trails was derived from these selection criteria for the patients, as it focused on MIBC patients and specifically grades of T2 and T3 with their subgroups when the tumor is available in the muscular layer of the bladder and away from the involvement of bladder neck, prostate, and the adjacent organs. These highly restricted criteria were tailored in order to rule out any contribution to the increased incidence of LUTS, radiation cystitis, and UTIs due to reasons other than curative radiotherapy. In such

a way, one can judge the effects of PACs in reducing these adverse events more reliably and logically.

Severe acute bladder toxicity is not so common with the use of new techniques of radiotherapy (IMRT and 3D image-guided RT), but it is still an important concern because it can lead to insufficient radiation dose delivery to the tumor [142]. For this reason, this study was interested to maximize the benefit from RT concomitantly with reducing the acute and late radiation adverse events to increase patients' compliance with the treatment protocols and finally increasing their quality of life (QoL).

More specifically, acute LUTS usually present within the 2nd and 3rd week of RT, these also include increased hematuria, pyuria and UTIs [121]. Radiation cystitis is caused by damaging the umbrella cells that make up the apical part of the bladder urothelium through the pronounced production of ROS [145, 121]. This loss of urothelial integrity permits a direct contact between bladder irritants (uric acid, urea, creatinine, chloride, sodium, and potassium) and submucosa, leading to inflammation that increase mucosal damage and may lead to fibrosis of the submucosa as a late event [146-148].

The findings of this clinical study showed that taking standardized 36mg of PACs tablets twice daily from baseline to the end of RT course had decreased the incidence of LUTS in patients with BC treated with RT compared with placebo capsules. The decision to administer 36mg of PACs twice per day was agreed with the recommendations of Hamilton K. *et al.* [121] who reports a decrease in radiation cystitis and LUTS in males with prostate cancer treated with pelvic RT using 72mg capsules of standardized cranberry once daily during and for two weeks after completion of RT.

According to the American Urological Association (AUA), up to seven micturations episodes per day are considered normal for individuals with no comorbid medical conditions [(149)].

This study showed that the mean urinary frequency of the placebo and cranberry group was slightly elevated at baseline as the patients with BC may present with LUTS, including frequency, even before starting RT and this was matching with a previous study that indicates the same finding during RT course at baseline [(142)]. Another observation was a significant elevation in the weekly mean urinary frequency (gradual increment) in the placebo group throughout the study, while in the cranberry group there was a significant reduction in the weekly mean urinary frequency (gradual decrement) throughout the study.

Nocturia is the complaint of interrupting the sleep more than once due to the need to void [(149)]. The current study found that the mean nocturia of the placebo and cranberry group was slightly elevated at baseline as the patients with BC may present with LUTS, including nocturia, even before starting RT and this was matching with a previous study that indicates the same finding during RT course at baseline [(142)]. Also, it showed that there was a significant elevation in the weekly mean nocturia (gradual increment) in the placebo group throughout the study, whereas in the cranberry group there was a significant reduction in the weekly mean nocturia (gradual decrement) throughout the study.

Urinary urgency defined as the complaint of a sudden, compelling desire to pass urine which is difficult to defer [(149)]. Urinary urgency is one of the LUTS that can be highly increased with RT [(142)]. In the present study, the mean urinary urgency of the placebo group was considered mild at baseline, while that of the cranberry group was considered moderate and the difference between the two groups at baseline was statistically significant. It also found that there was a significant elevation in the weekly mean urinary urgency (gradual increment) in

the placebo group throughout the study (considered moderate to severe at the end of RT according to the PPIUS), while in the cranberry group there was a significant reduction in the weekly mean urinary urgency (gradual decrement) throughout the study (considered mild at the end of RT according to the PPIUS).

The significant differences in LUTS between the two groups were attributed to the potent anti-oxidant and anti-inflammatory properties of PACs extracted from the American cranberry that confers a good protection for the urothelium against de-epithelialization effects of RT. These observations are supported by many studies that also found the importance of PACs in reducing the incidence of radiation cystitis and associated LUTS [145, 121]. Vidlar A *et al.* [150] reported that cranberry-PACs showed a clinically relevant, dose-dependent, and significant reduction in LUTS in men over 45 years. Ledda A *et al.* [151] indicated the effectiveness and safety of a well-standardized cranberry extract in the prevention of recurrent UTI.

4.2 Effects of cranberry-PACs on urinalysis during RT

Infective (bacterial) cystitis is a common type of lower UTIs. Non-infective (sterile) cystitis can be the result of radiotherapy for pelvic tumors. This type of cystitis is more severe and cause more intensive pain, irritative voiding symptoms, and hematuria [152].

Pyuria or sterile pyuria is the presence of white blood cells in a urinalysis in the absence of bacteriuria. The finding of more than 5-8 WBCs/HPF is considered to be the cut-off for defining pyuria. The presence of leukocytes in the urine suggests an infectious or inflammatory process involving the genitourinary tract, either directly or indirectly [153]. Pyuria is a common observation seen with radiation cystitis [154].

The Canadian and American guidelines define hematuria as the presence of more than 2-3 RBCs/HPF in a properly collected specimen of urine when there is

no benign etiology such as menstruation, recent exercise, recent sexual activity or recent instrumentation of the urinary tract [(155)].

The pathogenesis of increased pyuria and hematuria from exposure to RT is attributed to the acute inflammation as a response to radiation injury of the bladder mucosa. It is characterized by vasodilation, increased vascular permeability and leukocytes (WBCs) migration to the urothelium, release of inflammatory mediators, cytokines, histamines, complement factors, clotting factors, nitric oxide, and proteases. These mediators cause bladder irritation which is responsible for increased frequency, urgency and other LUTS [(156)].

Radiation damages blood vessels and is always accompanied with increased hematuria. It also damages the basement membranes of blood vessels leading to occlusion, thrombosis and neovascularization (an important factor for radiation cystitis and subsequent hemorrhagic cystitis) [(157)].

To study the effects of RT on bladder mucosa, urinalysis tests were performed for each patient enrolled in this study before and after completing the course of RT in order to track changes in pyuria and hematuria (as the objective parameters of radiation cystitis) and to assess the protective effects of PACs-extracted from American cranberry in reducing these parameters when compared to the placebo group.

Three patients of the placebo group were withdrawn from the study at the 3rd week of RT course due to the development of severe LUTS accompanied by UTI approved with urinalysis and urine culture tests. This finding is supported by the fact that the anti-adhesive effects of PACs extracted from American cranberry prevent the attachment of bacteria to the urothelium, an important step in the development of UTI, thereby decreasing the incidence of UTIs and LUTS. This fact had been elegantly reviewed by many other researchers [(119, 157-159)].

In this clinical study, the urinalysis findings showed a significant elevation of the detected means of pyuria and hematuria of both groups at the end of RT course when compared to their baseline level. Although, the means of pyuria and hematuria of the cranberry group at the end of the study were significantly lower than that of the placebo group.

The difference between the two groups is attributed to the efficient anti-oxidant and anti-inflammatory properties of the cranberry-PACs that offer a good protection to the bladder mucosa against the damaging effects (ROS and acute inflammation production) of RT. These observations are supported by many studies that also found the importance of PACs in reducing the incidence and severity of radiation cystitis and its associated symptoms [145, 121].

4.3 Effects of cranberry-PACs on inflammatory markers during RT

Radiotherapy has a significant effect in modulating the immune system through the activation of cytokine cascades [160]. After exposure to IR, *in vivo* and *in vitro* cells and tissues increase the expression of many cytokines and growth factors, including TNF-α and IL-8 [161]. These cytokines produced in a time-dependent manner, peaking usually at 4–24 hours after irradiation, with subsequent decrease to baseline levels within 24 hours to a few days [162].

Due to the strong anti-inflammatory properties of PACs that may be useful in protecting normal tissues from the late adverse event of radiation (fibrosis) and increasing the radio-sensitivity of the tumor cells, this study was interested to evaluate the effects of PACs-extracted from American cranberry on the inflammatory changes induced by RT through the assessment of TNF-α and IL-8 serum levels before and after the course of RT.

One of the central factors involved in stress responses, including response to radiation exposure, is TNF-α [163]. It is an essential mediator of cancer-related

inflammation and it performs paradoxical roles in cancer promotion and progression pathways resulting in the activation of NF-κB and AP-1 transcription factor complexes [(103)]. For these reasons, this study evaluated the changes in serum levels of TNF-α before and after RT course of treatment.

The study found that the mean serum level of TNF-α in the cranberry group was significantly reduced post RT when compared with the pre-RT, while in the placebo group it's mean serum level at the end of the treatment was significantly elevated when compared to the baseline level. As a comparison between the groups, it is obvious that there is a significant difference between the mean serum levels of TNF-α at the end of the treatment, indicating that the PACs-extracted from American cranberry effectively reduced the level of TNF-α in the cranberry group due to its anti-inflammatory properties.

The secretion of IL-8 is elevated through oxidative stress from intracellular and extracellular sources. Interleukin-8 attracts inflammatory cells, which further elevates oxidative stress mediators, thereby making IL-8 a key parameter in localized inflammation [(164)]. It is always accompanied by inflammation that predisposes cells to produce different chemokines for malignant transformation or progression [(99)]. This study measured the changes in IL-8 levels before and after the course of RT for both groups.

The present study found that the mean serum level of IL-8 at the end of the treatment with RT in the cranberry group was significantly reduced compared with baseline level, while for the placebo group the opposite was observed. Also, the serum level of IL-8 for the cranberry group was significantly lower than that of the placebo group at the end of RT.

These findings show the strong anti-inflammatory properties of the PACs-extracted from American cranberry in reducing IL-8 levels. There are many previous studies support these findings regarding the anti-inflammatory effects of

the PACs. La VD *et al.* [135] reported that the PACs of American cranberry inhibited the phosphorylation state and expression of fibroblast's activator protein-1(AP-1), which prominently involved in the transcriptional regulation of many pro-inflammatory mediators, such as IL-6 and IL-8. Bodet *et al.* [165] also reported that the PACs of American cranberry inhibited IL-6, TNF-α, and IL-8 production by gingival fibroblasts stimulated with lipopolysaccharides (LPS) from five different periodontopathogens, whereas Feldman and Grenier *et al.* [134] showed that the PACs of American cranberry inhibited *Porphyromonas gingivalis* growth and biofilm formation and also reduced LPS-induced secretion of IL-1β, TNF-α, IL-6 and IL-8. Matsushima *et al.* [166] reported that the extract of cranberry suppresses IL-8 secretion from stomach cells when stimulated by *Helicobacter pylori* in every clinically separated strain but inhibits growth in part of the strains.

MacDougall *et al.* [167] reported that Cranberry extract reduces TNF-α-induced expression of cyclooxygenase-2 and inducible nitric oxide synthase in vascular smooth muscle cells. Another study had shown that PACs anti-inflammatory action comes from the interaction of leukocytes migration, so depressing the levels of cytokines which include TNF-α and IL-8 [168].

There are many other studies supporting the effect of cranberry-PACs in modulating immune cells signaling pathways. Déziel BA *et al.* [169] reported that cranberry-PACs decreases matrix metalloproteinase (MMP) activity by the stimulation and/or inhibition of specific temporal MMP regulators, and by affecting either the phosphorylation status and/or expression of NF-κB and AP-1 pathway proteins. Martina *et al.* [170] demonstrated that cranberry polyphenols may help protect liver cells against oxidative insult by modulating GSH concentration, ROS and MDA generation, antioxidant enzyme activity and cell signaling pathways. Denis MC *et al.* [171] reported that cranberry polyphenols fractions limited NF-κB activation.

4.4 Effects of cranberry-PACs on the oxidative status during RT

Water radiolysis is the indirect action of radiation that result in the production of free radicals, such as hydrated electrons (e^-_{aq}), ionized water ($H2O^+$), hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen radical ($H^{\cdot}$), and hydroxyl radical ($^{\cdot}OH$), which can diffuse far enough to reach and damage the DNA, protein, and lipid targets [(172)]. These ROS break chemical bonds, produce chemical changes, and start the chain of events that results in the final expression of biological damage. The intracellular ROS levels are suddenly increased after exposure to IR and that increased levels of ROS are sustained for several hours after initial IR exposure [(91)]. Radiotherapy induces cell death through the generation of oxidative stress, and cellular antioxidant status also affects normal tissue injury and tumor sensitivity to radiation treatment [(173)].

The NF-κB signaling pathway is essential in supporting cancer-related inflammation and malignant progression as well as sustaining the immunosuppressive phenotype of tumor-associated macrophages (TAMs) [(174)]. Inhibition of NF-κB has been proposed as a mean to treat cancer or to overcome chemo-resistance and radio-resistance in cancer therapy [(175)]. When compared to normal cells, cancerous cells are usually suffering from oxidative stress and secrete more pro-inflammatory mediators. Incremental elevations in oxidative stress to a level that is still within the adaptive redox buffering capacity of normal cells may overwhelm the less adaptive redox buffering capacity of tumor cells, thereby selectively disrupting the redox state in tumor cells and activating the apoptotic or necrotic pathway, which leads to selective killing of tumor cells [(91)].

This study put a hypothesis that PACs can modulate IR tissue responses in a way that suggests increase tumor cells sensitivity while protecting neighboring normal cells from the bystander effects of radiation which is mediated through the

excessive release of pro-inflammatory cytokines. This hypothesis is supported by a previous study reported that oxidative stress came from an imbalance between pro-oxidants and antioxidants that prefers the former is believed to play a critical role in prostate carcinogenesis and prostate cancer progression [(176)]. Another study reported that selective inhibition of NF-κB pathway can, to a remarkable degree, sensitize prostate cancer cells to IR induced killing [(173)].

Copper/Zinc superoxide dismutase (SOD-1) is a key enzyme in the dismutation of superoxide radicals resulting from cellular oxidative metabolism, converting them into hydrogen peroxide and as a result, serves a key antioxidant role [(177)]. Three types of SOD isozymes have been identified in human cells and Cu/Zn-superoxide dismutase (SOD1) contribute to approximately 70–80% of cellular SOD activity [(178)]. Previous study showed a significant reduction in the SOD1 levels post radiation, in addition to reduced TAC of the irradiated tissues [(179)]. This study was interested to track the changes in SOD1 and TAC serum levels during RT in order to assess the potential effect of PACs in maintaining the anti-oxidant capacity of those patients to overcome radiation-induced damage in an attempt to reduce future late adverse events of IR.

In this clinical study, SOD1 was significantly elevated in the cranberry group at the end of the treatment when compared to the baseline level. This finding matching a previous study that reported increased level and activity of SOD1 after ingestion of cranberry extract [(180)]. On the other hand, SOD1 level was significantly reduced in the placebo group at the end of the treatment when compared to the baseline level. This finding was consistent with a study reporting that SOD1 level is reduced by the enormous production of ROS during IR [(178)]. Elberry AA *et al.* [(181)] reported that American cranberry preserves SOD activities and protects against doxorubicin-induced cardio-toxicity in rats through the anti-oxidant activity of cranberry.

There was no significant difference in the TAC serum level of cranberry group before and after treatment, while its serum level in the placebo group was significantly reduced when compared to the baseline level. This is suggesting that the anti-oxidant effects of the cranberry-PACs maintained the TAC of the patients intact during the treatment through its free radical scavenging potency [(128)]. These findings are consistent with previous studies reporting that the scavenging capacity of catechin and epicatechin molecules of the PACs depends on the number of ortho-dihydroxyl and ortho-hydroxyketol groups and C2-C3 double bounds due to their hydrogen donating ability [(182, 183)]. The dimeric PACs are more effective than vitamin C in trapping ROS [(184)]. They have the ability to inhibit ROS generation as well as the release of lysosomal enzymes. Facino *et al.* [(185)] have indicated that PACs strongly complex iron and copper cations in the ratio of Fe^{2+}/procyanidin (2:1) and Cu^{2+}/procyanidin (4:1) respectively.

During the study, all the patients of the two groups were requested to record any side effects associated with their medication (cranberry tablets and placebo capsules) but no specific side effects were found.

One important limitation of this study was the difficulty in obtaining large size of sample (number of patients) due to the strict inclusion criteria in addition to the rarity of MIBC patients when compared to NMIBC. Other limitations include the short period of the study (no follow-up post treatment), unavailability of enteric-coated PACs tablets, and single cranberry arm dose.

In summary, patients with MIBC treated with radiotherapy can get benefit from cranberry-PACs in reducing the incidence of radiation cystitis that develop during the course of RT. Cranberry-PACs may assist in the prevention of the late adverse events like fibrosis of bladder tissue, bladder contracture and life-threatening hemorrhagic cystitis through modulation of the immune cells signaling

pathways in a way that favors increased tumor cells radio-sensitivity and protecting the normal neighboring tissues from the bystander effects of radiation.

4.5 Conclusion

From this study, one can conclude that cranberry-PACs can reduce bladder discomfort and incidence of urinary symptoms associated with RT in patients with bladder carcinoma by ameliorating the mucosal damage through the anti-inflammatory and anti-oxidant properties of PACs.

4.6 Recommendations

For future work, the following recommendations can be suggested:

1- Use larger sample size, longer-term study to assess PACs effects on the late adverse events of RT.

2- Test other dosage forms, formulations, and different doses of cranberry-PACs; also try to study other active components of cranberry.

3- Test the effects of cranberry on other types of pelvic tumors and on palliative and/or curative courses of RT.

4- Assess other parameters like hs-CRP, MMPs, isoprostane, VEGF, and MDA; also try to find more specific parameters to test the effects of PACs in the modulation of NF-κB.

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Appendix

Patient's daily diary card

Patient's name: date:

Please put √ in the blanks below each time you go for micturition at day time (Frequency):

Please put √ in the blanks below each time you go for micturition after sleeping at night (Nocturia):

Please indicate the degree of associated urgency for each day by selecting the appropriate number from the table below:

Patient Perception of Intensity of Urgency Scale (PPIUS):

0	No urgency	I felt no need to empty my bladder, but did so for other reasons.
1	Mild urgency	I could postpone voiding as long as necessary, without fear of wetting myself.
2	Moderate urgency	I could postpone voiding for a short while, without fear of wetting myself.
3	Severe urgency	I could not postpone voiding, but had to rush to the toilet in order not to wet myself.
4	Incontinence	I leaked before arriving to the toilet.
5	Urge incontinence	I cannot postpone any voiding always wetting my self

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

الجامعة المستنصرية

كلية الصيدلة

تأثيرات التوت البري على المشاكل البولية المرتبطة بالعلاج الاشعاعي لدى المرضى العراقيين المصابين بسرطان المثانة

رسالة مقدمة إلى فرع الصيدلة السريرية والى لجنة الدراسات العليا في كلية الصيدلة/الجامعة المستنصرية كجزء من متطلبات الحصول على شهادة الماجستير في علوم الصيدلة (الصيدلة السريرية)

من قبل

محمد باسم محمد

(بكالوريوس صيدلة 2009)

بإشراف

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١٤٣٧هـ ٢٠١٦ م

الخلاصة

الخلفية:

يعتبر سرطان المثانة الأكثر شيوعا من بين الامراض السرطانية التي تصيب المسالك البولية. يصنف سرطان المثانة الى ثلاثة اصناف تختلف في طرائق علاجها وانذاراتها, وهذه الأصناف كالتالي: غير المجتاح لعضل المثانة, المجتاح لعضل المثانة, وسرطان المثانة المنتشر خارجها. المرضى المصابين بنوع المجتاح للعضل يمكن علاجهم بالعلاج الاشعاعي والذي ينضوي على تأثيرات جانبية حادة سريعة الحدوث ومتأخرة الحدوث. التأثيرات الحادة سريعة الحدوث من الممكن ظهورها خلال فترة العلاج بالاشعاع وحتى عدة اسابيع بعد انتهاء مدة العلاج ويطلق عليها تسمية تهيج المثانة الاشعاعي وتصاحبها اعراض المسالك البولية السفلية الحادة مع امكانية حدوث التهاب المثانة.أما الاعراض المتاخرة الحدوث فتكون اكثر خطورة وتتراوح في حدتها بين تليف وتقلص المثانة الى تهيج المثانة النازف المهدد لحياة المريض والذي يحتم ازالة المثانة وتحوير المسلك البولي. من اجل تحسين نوعية حياة المريض المصاب بهذا المرض, العديد من الخطوات الوقائية تم تجربتها لتقليل هذه الاعراض. التوت البري الذي يحتوي على مستخلص خاص يتميز بقدرته على تقليل تهيج و اكسدة الانسجة الى جانب قدرته على منع التصاق البكتريا بجدار المثانة الداخلي المفيد بتقليل الاصابة بالتهاب المسالك البولية.

الهدف:

تم تصميم هذه الدراسة لتقييم تأثيرات التوت البري في تقليل اعراض المسالك البولية السفلية وتقليل تهيج واكسدة الانسجة الناتج من العلاج الاشعاعي لمرضى سرطان المثانة.

طرق العمل

اجريت هذه الدراسة السريرية العشوائية والمقومة بدواء غفل على اربعين مريضا (30 رجلا و10 نساء) مصابا بسرطان المثانة المجتاح للعضل (تحديدا مرحلة T2و T3) والمرشحين للعلاج الاشعاعي بجرعة 64Gy. تراوحت اعمارهم بين ال60 وال70 سنة وكانوا مقيدين بنظام غذائي يمنعهم من تناول الاطعمة والأشربة المحتوية على مستخلصات التوت البري بجميع انواعه والمشروبات الروحية الحمراء والعنب الاحمر وكانوا ملتزمين بشرب 2-3 لتر من الماء يوميا. تم تشخيص هؤلاء المرضى وعلاجهم في مستشفى الاورام التعليمي / دائرة مدينة الطب وتحت اشراف اطباء اخصائيين وبعد استحصال موافقة اللجنة الاخلاقية وموافقة المرضى الشفهية للدخول في اطار الدراسة للفترة مابين تشرين الثاني 2014 الى نيسان 2016 . تم توزيع المرضى عشوائيا الى مجموعة علاج غفل (عددهم 20 وأخذوا كبسول يحتوي على 500 ملغم سكر اللاكتوز مرتين يوميا) ومجموعة التوت البري (عددهم 20 واخذوا حبوب محتوية على مستخلص التوت البري بتركيز 36ملغم مرتين يوميا). استلم جميع المرضى في المجموعتين نفس الجرعة من العلاج الاشعاعي ولفترة تراوحت بين 6 و7 اسابيع. تمت دراسة المؤشرات التالية: التكرار البولي, التردد البولي الليلي, والالحاح البولي بصورة اسبوعية. باقي المؤشرات تمت دراستها ومتابعتها قبل البدء بالعلاج وبعد الانتهاء منه وهي كالتالي: البول

القيحي, البول الدموي, معامل النخر السرطاني نوع الفا (TNF-α), انترليوكين-8(IL-8), أنزيم SOD1, والسعة الكلية المقاومة للأكسدة (TAC).

النتائج

كان هنالك نقصانا ذو مغزى معنوي في مجموعة التوت البري في نهاية العلاج الاشعاعي فيما يخص الاعراض البولية للمسالك السفلية عند مقارنتها بمستوى هذه الاعراض قبل الدراسة, بينما ازدادت هذه الاعراض في مجموعة دواء غفل بعد انتهاء العلاج الاشعاعي مقارنة بمستواها قبل الاشعاع, وقد اعتبر الفرق بين المجموعتين فيما يخص هذه الاعراض بعد انتهاء العلاج الاشعاعي ذو مغزى معنوي. البول القيحي والدموي في المجموعتين ازداد في نهاية الدراسة زيادة ذات مغزى معنوي ولكن اعتبرت الزيادة في مجموعة دواء غفل اكثر بكثير وذات مغزى معنوي مقارنة بمجموعة التوت البري. أظهرت مجموعة التوت البري نقصانا ذو مغزى معنوي في معامل النخر السرطاني(نوع الفا) والانترليوكين-8 بعد انتهاء العلاج الاشعاعي مقارنة بمستويات ماقبل العلاج, بينما ازدادت مستويات هذه المؤشرات زيادة ذات مغزى معنوي في مجموعة دواء غفل بعد انتهاء العلاج الاشعاعي مقارنة بمستوياتها قبل البدء بالعلاج واعتبر الفرق بين المجموعتين في نهاية العلاج الاشعاعي ذو مغزى معنوي. كان مستوى أنزيمSOD1 في مجموعة التوت البري بعد انتهاء العلاج الاشعاعي اكثر من مستواه قبل البدء بالعلاج واعتبر الفرق ذو مغزى معنوي, اما فيما يخص مجموعة دواء غفل فقد حدث العكس تماما وكان الفرق بين المجموعتين ايضا ذو مغزى معنوي بعد انتهاء العلاج. اعتبرت مستويات سعة مقاومة الاكسدة الكلية في مجموعة التوت البري مستقرة قبل وبعد الانتهاء من العلاج الاشعاعي اذ اعتبر الفرق بين المستويين بدون مغزى معنوي, أما في مجموعة دواء غفل فقد تراجع هذا المؤشر كثيرا في نهاية العلاج الاشعاعي وقد اعتبر هذا التراجع ذو مغزى معنوي حين مقارنته بمستواه قبل البدء بالعلاج.

الاستنتاج

قدمت هذه الدراسة دليلا بان مستخلصات التوت البري قللت احتمالية حدوث التهاب المثانة الاشعاعي وثبطت تهيج واكسدة الانسجة المتاثرة بالعلاج الاشعاعي مفترضة بذلك دور هذه المواد في تقليل الاعراض الجانبية المتأخرة للعلاج الاشعاعي لدى المرضى المصابين بسرطان المثانة.