Research Article

Effects of Cranberry-PACs against Urinary Problems associated with Radiotherapy in Iraqi Patients with Bladder Carcinoma

Mohammed Basim Mohammed[1]*, Bahir Abdul Razzaq[2], Manwar Abdul Elah Al-Naqqash[3], Suzan Yousif Jasim[4]
[1]Department of Clinical Pharmacy, College of Pharmacy, Al-Mustansiriyah University, M.Sc. program, Baghdad, Iraq.
[2]Assist. Prof., Department of Pharmacology and Toxicology, College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq.
[3]Lecturer, Oncology Teaching Hospital, Medical City Directorate, Baghdad, Iraq.
[4]Lecturer, Department of Clinical Laboratory Science, College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq.
***Corresponding author's E-mail:** mbm.clinical.86@gmail.com

Accepted on: 10-06-2016; **Finalized on:** 31-07-2016.

ABSTRACT

Bladder cancer (BC) is a very common tumor. It is categorized into non muscle-invasive, muscle-invasive, and metastatic bladder cancer according to many guidelines. Radiotherapy (RT) is a modality used in the management of bladder tumors, and as a consequence of this modality, a range of adverse events may affect the patients that might be devastating to the quality of life. Radiation cystitis is a term describes those adverse events ranging from increased incidence of lower urinary tract symptoms (LUTS) and UTIs, to the development of fibrosis and tumor recurrence. Proanthocyanidins (PACs) from American cranberry had been credited for their anti-inflammatory and anti-oxidant properties, so it was rational to test the radioprotective effects of PACs in patients with bladder cancer undergoing RT for 6 weeks duration. The patients were allocated into cranberry group and placebo group, randomly. The LUTS (urinary frequency, nocturia, and urgency) were taken on weekly bases from each patient. Data of urinalysis (pyuria and hematuria), inflammatory markers (TNF-α and IL-8 serum levels), and oxidative stress parameters (SOD1 and TAC serum levels) were assessed before and at the end of RT course. It was obvious that PACs significantly reduced the incidence of LUTS ($P<0.05$) and the serum levels of TNF-α, IL-8; significantly elevated SOD1 serum level ($P<0.05$), and maintained TAC serum level ($P>0.05$) in cranberry group patients at the end of RT, when compared to the placebo group patients who suffered from more significant pyuria and hematuria ($P<0.05$). This study suggests that PACs can reduce the incidence of acute adverse events (LUTS) and may play a positive role in reducing the late adverse events (fibrosis) of radiation cystitis.

Keywords: Bladder cancer, Radiation cystitis, Proanthocyanidins.

INTRODUCTION

Bladder cancer (BC) is one of the most common malignancy in the urinary tract.[1] It can be divided into three main categories; non muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), and metastatic tumor.[2]The most useful staging system for BC is the tumor, node, and metastasis system (TNM).

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).[3] According to the TNM, stages Tis, Ta, and T1 are NMIBC; while stages T2 and above are MIBC.[4]

The three categories of BC differ in their management approaches.[2] For MIBC, the recommended treatment is radical cystectomy, especially for resectable (T2-T4a) tumor stages.[5]

Bladder sparing approaches include transurethral resection of bladder tumor (TURBT) alone, TURBT followed by chemotherapy alone, radiotherapy (RT) 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. Patients within the categories of T2-T3a urothelial carcinomas may be considered for bladder-sparing approaches.[6]

When the bladder is exposed to radiation during RT for pelvic tumors, a series of histopathological changes are induced that in turn have clinical consequences. In addition to the increased micturition urgency, nocturia, frequency during day time, and dysuria, the appearance of hematuria of highly variable intensity represents one of the most complex complications.[7] These histopathological changes occur in two phases: acute and chronic. The acute phase is observed during the treatment until 6 months after treatment.[8] The chronic phase begins 6 months after RT. 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.[7] Radiation is rarely used in combination with radical cystectomy, so most of the radiation-induced urinary adverse events with respect to bladder cancer come from the bladder conservation literature using trimodal therapy. One of the most common acute radiation-induced adverse events is transient cystitis.[9]

American cranberry (*Vaccinium macrocarpon*) contains many polyphenols, which have been associated *in vitro* with antibacterial, antiviral, antimutagenic,

anticarcinogenic, antiangiogenic, anti-inflammatory, and antioxidant properties.[10] *In vivo*, animal models reveal that cranberry extracts reduce C-reactive protein (CRP) and pro-inflammatory interleukins, increase nitric oxide synthesis, and suppress *Helicobacter pylori* infection.[11]

Proanthocynidins (PACs) extracts from American cranberry 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.[12]

Iron and copper existence in the free-state in biological systems catalyzes free radical reactions, like in the Fenton reaction when iron catalyzes the generation of hydroxyl radicals. PACs have the ability to bind such metals effectively, reduce their concentrations and thus the extent of oxidative activity.[13] The purpose of this study was to evaluate the effects of cranberry-PACs in reducing lower urinary tract symptoms, the inflammatory, and oxidative stress responses in patients with bladder carcinoma undergoing radiotherapy.

SUBJECTS AND METHODS

Forty five patients with MIBC of stages T2-T3 (eligible for RT with a total dose of 64 Gy) with age range of 60-70 years were randomly allocated into two groups: cranberry group and placebo group.

Twenty two patients (16 males/ 6 females) were taking two tablets per day of 36 mg pure PACs extracted from American cranberry according to the American extraction method (Urinal Akut®, by Walmark) during the course of RT (6 weeks) and 23 patients (17 males/ 6 females) were administered two placebo capsule per day of 500 mg pure lactose for the same duration as with cranberry group patients. All the patients were adjusted to drink 2 – 3 L water/day.

Certain exclusion criteria were adopted in order to exclude any expected interference with the aim of study, these were: cranberry allergy, UTIs and/or severe lower urinary tract symptoms (LUTS) at baseline and those having urethral catheterization during or around the course of RT, a history of pelvic RT, prostate and other pelvic malignancy or patients with MIBC stages T4a and T4b, patients taking or have been taken chemotherapy within the previous three months before the study, diabetes, neurogenic bladder, history of renal dysfunction, severe macrohematuria, irritable bowel syndrome, patients using medications like non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antibiotics, antispasmodics and other analgesics, and those on warfarin therapy.

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 incompliance with cranberry-PACs tablets, 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/Baghdad, under supervision of specialist doctors after achieving ethical committee approval and taking patients oral consent, during the period from November 2014 to April 2016.

Any patient planned to have radiotherapy course were given a simulation appointment, in which the radiation dosage/fraction set were 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)/Volumetric Modulated Arc Therapy (VMAT) with three-dimensional radiation therapy techniques (Elekta, Infinity™).

Each patient was requested to fill a daily diary card to record the number of urinations during day time (frequency) and number of urinations after sleeping (nocturia) which is assessed on weekly bases along with a weekly assessment for the sudden desire to pass urine (urgency) using the patient's perception of the intensity of urgency scale (PPIUS)[14] that gives urgency a scale from 0-5 in intensity.

Urine and blood samples were collected from each patient at baseline and after 6 weeks of RT to assess pyuria, hematuria, tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), Cu/Zn superoxide dismutase (SOD1) and total antioxidant capacity (TAC) before and after RT.

Demographic and baseline characteristics (age, sex, smoking and family history for bladder cancer, and T-stage within TNM system) were evenly distributed for both cranberry and placebo groups at the baseline level.

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, while blood samples were 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.

The TNF-α, IL-8, and SOD1 serum levels were measured by enzyme linked immunosorbent assay (ELISA) kits from CUSABIO, China[15-17], while serum TAC was assessed by the colorimetric assay kit from Biovision, USA.[18]

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM) or fractions. Student's t-tests (both the pooled and paired tests) were used to analyze most of the parameters.

Test of ANOVA was 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.

RESULTS

Effects of cranberry-PACs on lower urinary tract symptoms (LUTS)

There was no significant difference (P>0.05) between the two groups at baseline regarding frequency and nocturia, while the baseline level of urinary urgency in the cranberry group was significantly higher (P<0.05) than that of the placebo group.

At the end of the study, the three parameters were significantly reduced (P<0.05) from baseline in the cranberry group. In the placebo group, there were significant elevations (P<0.05) at the end of RT in those parameters from baseline.

When comparing the two groups at the end of the study, the LUTS in the placebo were significantly higher (P<0.05) than those in the cranberry group (Table 1).

The weekly mean changes of LUTS throughout treatment with both groups are clarified in Fig 1.

Effects of cranberry-PACs on urinalysis parameters (pyuria and hematuria)

At baseline, there was no significant difference (P>0.05) between the two groups regarding the two parameters of urinalysis (pyuria and hematuria). After RT, these two parameters were significantly elevated (P<0.05) in the placebo group.

In the cranberry group, the elevation in these parameters was consider significantly lower (P<0.05) than those of the placebo group (Table 2, Fig. 2).

Table 1: Mean Changes of LUTS for both Treatment Groups

LUTS Parameters	Cranberry Group (n=20)			Placebo Group (n=20)		
	Pre-treat	Post-treat	Post/Pre	Pre-treat	Post-treat	Post/Pre
Frequency	8.556±0.28^{a}	7.080±0.14^{b}*	0.82	9.305±0.35^{a}	14.485±0.17^{b}	1.55
Nocturia	1.421±0.067^{a}	1.176±0.024^{b}*	0.82	1.522±0.057^{a}	2.398±0.080^{b}	1.57
Urgency	2.200±0.19^{a}*	1.155±0.14^{b}*	0.52	1.200±0.16^{a}	3.500±0.14^{b}	2.91

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)

Table 2: Mean Changes of Pyuria (WBCs/HPF) and Hematuria (RBCs/HPF) for both Treatment Groups

Urinalysis Parameters (Cells/HPF)	Cranberry Group (n=20)			Placebo Group (n=20)		
	Pre-treat	Post-treat	Post/Pre	Pre-treat	Post-treat	Post/Pre
Pyuria	4.00±0.56^{a}	11.15±1.1^{b}*	2.78	4.70±0.56^{a}	57.30±4.7^{b}	12.19
Hematuria	1.85±0.39^{a}	8.15±0.80^{b}*	4.40	2.00±0.47^{a}	33.75±2.60^{b}	16.87

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)

Table 3: Mean Serum Levels of TNF-α and IL-8 (pg/mL) for both Treatment Groups

Inflammatory Markers	Cranberry Group (n=20)			Placebo Group (n=20)		
	Pre-treat	Post-treat	Post/Pre	Pre-treat	Post-treat	Post/Pre
TNF-α (pg/ml)	105.38±9.23^{a}	44.30±4.20^{b}*	0.42	114.30±15.40^{a}	408.70±36.60^{b}	3.57
IL-8 (pg/ml)	66.51±5.67^{a}	34.77±1.76^{b}*	0.52	63.40±6.00^{a}	386.50±22.30^{b}	6.09

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)

Table 4: Mean Serum Levels of SOD1 (ng/mL) and TAC (nmol/µL) for both Treatment Groups

Oxidative Stress Markers	Cranberry Group (n=20)			Placebo Group (n=20)		
	Pre-treat	Post-treat	Post/Pre	Pre-treat	Post-treat	Post/Pre
SOD1 (ng/ml)	0.789±0.078[a]	1.055±0.077[b]*	1.33	0.878±0.062[a]	0.616±0.066[b]	0.70
TAC (nmol/nl)	7.095±0.082[a]*	7.102±0.077[a]*	1.00	7.834±0.101[a]	7.062±0.104[b]	0.90

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)

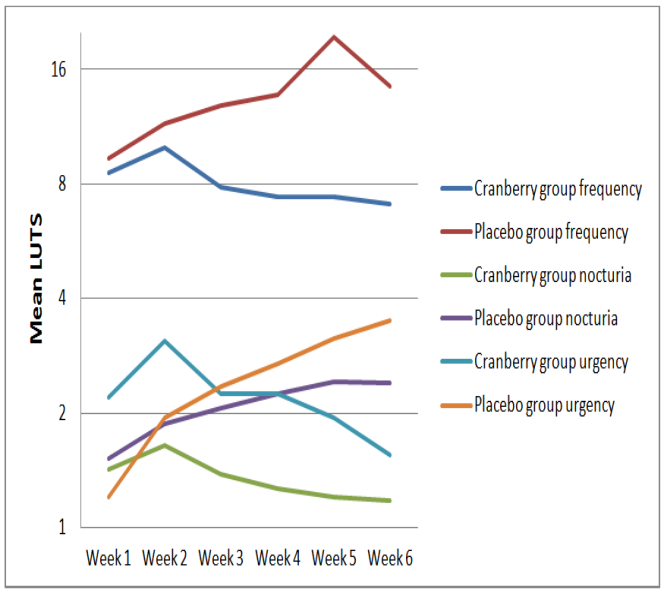

Figure 1: Weekly Mean Changes of LUTS during the Treatment for both Groups (p<0.05).

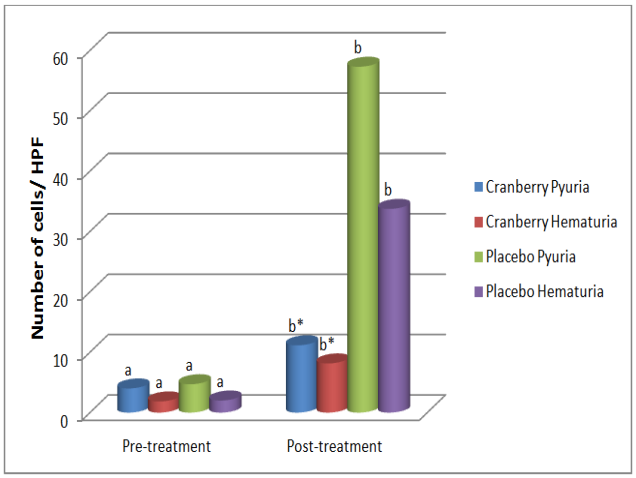

Figure 2: Mean changes of pyuria (WBCs/HPF) and hematuria (RBCs/HPF) for both treatment groups. Non-identical (a, b) superscripts indicate a significant difference within the group *(P<0.05)*. Superscript (*) indicates significant difference compared with placebo group (P<0.05).

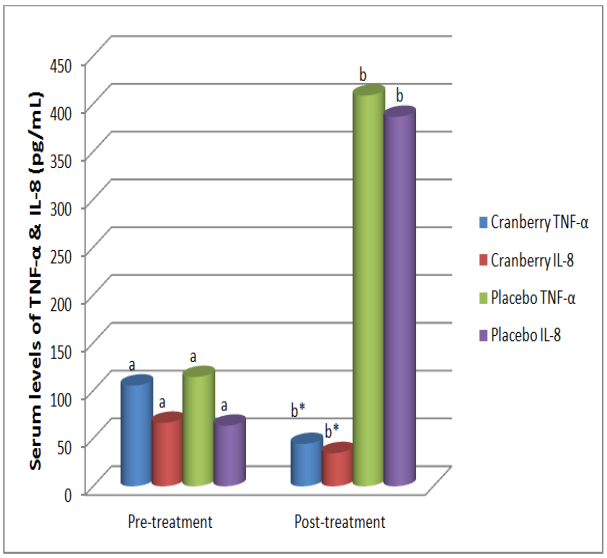

Figure 3: Mean serum levels of TNF-α and IL-8 (pg/mL) for both treatment groups. Non-identical (a, b) superscripts indicate a significant difference within the group (P<0.05). Superscript (*) indicates significant difference compared with placebo group (P<0.05).

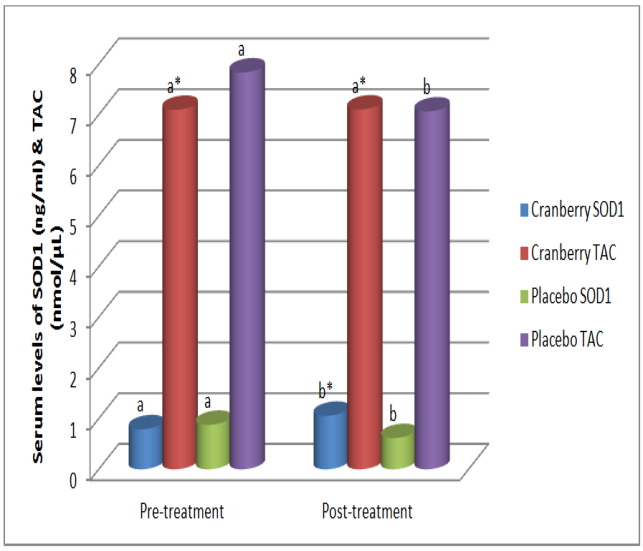

Figure 4: Mean serum levels of SOD1 (ng/mL) and TAC (nmol/µL) for both treatment groups. Non-identical (a, b) superscripts indicate a significant difference within the group (P<0.05). Superscript (*) indicates significant difference compared with placebo group (P<0.05).

Effects of cranberry-PACs on inflammatory markers (TNF-α and IL-8)

A significant elevation in the mean TNF-α and IL-8 serum levels 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 and IL-8 serum levels of the cranberry group at the end of the treatment were reduced significantly (P<0.05) from that at baseline. When compared to the mean TNF-α and IL-8 serum levels of the placebo group at baseline, the mean TNF-α and IL-8 serum levels of the cranberry group were not significantly differ (P>0.05). Meanwhile, the mean TNF-α and IL-8 serum levels of the cranberry group were significantly lower (P<0.05) than that of the placebo group at the end of the RT (Table 3, Fig. 3).

Effects of cranberry-PACs on oxidative stress markers (SOD1 and TAC)

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 (P<0.05) in the mean SOD1 serum level of the cranberry group was observed at the end of treatment when compared to that at baseline. 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 RT (Table 4, Fig. 4).

In the placebo group, a significant reduction (P<0.05) was observed in the mean serum level of TAC at the end of RT when compared to that at baseline. In the cranberry group, 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 baseline level. 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 4, Fig. 4).

DISCUSSION

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 RT 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.[19]

Because these symptoms are linked to the stage of the tumor[20,21], 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[19,20,&22]. 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.[23]

The difference of the present study from the previous trials 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 RT. 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 RT (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.[19] 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.[23] 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.[22,23]

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.[24-26]

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.[23] 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 patients with no comorbid medical conditions.[27] 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.[19] 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.[27] 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.[19] 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.[27] Urinary urgency is one of the LUTS that can be highly increased with RT.[19]. 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.[22,23] Vidlar A[28] reported that cranberry-PACs showed a clinically relevant, dose-dependent, and significant reduction in LUTS in men over 45 years. Ledda A[29] indicated the effectiveness and safety of a well-standardized cranberry extract in the prevention of recurrent UTI.

Infective (bacterial) cystitis is a common type of lower UTIs. Non-infective (sterile) cystitis can be the result of RT for pelvic tumors. This type of cystitis is more severe and cause more intensive pain, irritative voiding symptoms, and hematuria.[30] 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.[31] Pyuria is a common observation seen with radiation cystitis.[32] 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.[33]

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.[34]

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).[35] 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 the 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 in the cranberry group. This fact had been elegantly reviewed by many other researchers.[11,35-37]

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.[22,23]

Radiotherapy has a significant effect in modulating the immune system through the activation of cytokine cascades.[38] 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.[39] 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.[40]

Radiation energy leads to the formation of ROS that cause the majority of radiation-induced damage to the tumor and normal tissues. Many cells in the tumor microenvironment are affected by these ROS and release many cytokines and inflammatory mediators as a response. These mediators can modulate the signaling pathways of the transcription factors, like NF-κB, which in turn up-regulate the expression of many cytokines and inflammatory mediators. This feedback can amplify radiation-induced inflammation that may persist chronically, leading to a radio-resistant tumor tissue and/or fibrosis of normal neighboring tissues.[41]

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 study 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.[42] 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.[43] 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.[44] It is always accompanied by inflammation that predisposes cells to produce different chemokines for malignant transformation or progression.[45] 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[46] 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[47] 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[48] 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[49] 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[50] 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.[51]

There are many other studies supporting the effect of cranberry-PACs in modulating immune cells signaling

pathways. Déziel BA[52] 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[53] 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[54] reported that cranberry polyphenols fractions limited NF-κB activation.

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 ($HO_2^\bullet$), hydrogen radical ($H^\bullet$), and hydroxyl radical ($^\bullet OH$), which can diffuse far enough to reach and damage the DNA, protein, and lipid targets.[55] 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.[41] 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.[56]

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).[57] Inhibition of NF-κB has been proposed as a mean to treat cancer or to overcome chemo-resistance and radio-resistance in cancer therapy.[58] 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.[41]

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.[59] Another study reported that selective inhibition of NF-κB pathway can, to a remarkable degree, sensitize prostate cancer cells to IR induced killing.[56]

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.[60] 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.[61] Previous study showed a significant reduction in the SOD1 levels post radiation, in addition to reduced TAC of the irradiated tissues.[62] 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.[63] 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.[61] Elberry AA[64] 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.[12]

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.[65,66] The dimeric PACs are more effective than vitamin C in trapping ROS.[67]

They have the ability to inhibit ROS generation as well as the release of lysosomal enzymes. Facino[68] 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.

From above, 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.

CONCLUSION

This study can conclude that cranberry-PACs may 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.

REFERENCES

1. Peggy S, Jessica B, Mary R, Jianyu R, Urine cytology and adjunct markers for detection and surveillance of bladder cancer, American Journal of Translational Research, 2(4), 2010, 412-440.
2. National comprehensive cancer network (NCCN) guidelines for bladder cancer, USA: National comprehensive cancer network, Inc., 2015.
3. Colombel M, Soloway M, Hideyuki A, Andreas B, Joan P, Roger B, Epidemiology, Staging, Grading, and Risk Stratification of Bladder Cancer, European Urology Supplements, 7, 2008, 618-626.
4. Scottish Intercollegiate Guidelines Network, Management of transitional cell carcinoma of the bladder: a national clinical guideline, Scotland (SIGN), 2005.
5. Hautmann RE, World Health Organization (WHO) Consensus Conference in Bladder Cancer, In: Abol-Enein H, Hafez K, Haro I, Mansson W, Mills RD, Montie JD, Sagalowsky AI, Stein JP, Stenzl A, Studer UE, Volkmer BG. (eds.), Urinary Diversion, Urology, 69(1), 2007, 17-49.
6. Gakis G, ICUD-EUA International Consultation on Bladder Cancer 2012: Radical Cystectomy and Bladder Preservation for Muscle-Invasive Urothelial Carcinoma of the Bladder, European Urology, 63(1), 2013, 45-57.
7. R Martínez-Rodríguez, Practical treatment approach in radiation-induced cystitis, ACTAS UROLOGICAS ESPANOLAS, 34(7), 2010, 603–609.
8. Pavlidakey P, MacLennan G, Radiation Cystitis, Journal of Urology, 182, 2009, 1172–3.
9. Smith ZL, Christodouleas JP, Keefe SM, Bladder preservation in the treatment of muscle-invasive bladder cancer (MIBC): a review of the literature and a practical approach to therapy, BJU international, 112(1), 2013, 13-25.
10. Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A. Dietary (poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases, Antioxidant & Redox Signaling, 18(14), 2013, 1818–92.
11. Jeffrey B, Cranberries and Their Bioactive Constituents in Human Health, Advances in Nutrition, 4(6), 2013, 618–632.
12. Govindaraj J, Free radical scavenging property of proanthocynidin: an *in vitro* evaluation, Biomedicine, 29, 2009, 263-267.
13. Govindaraj J, Therapeutic effects of proanthocynidins on the pathogenesis of periodontitis-an overview, Indian Journal of Experimental Biology, 49, 2011, 83-93.
14. Sherilyn M, Thomas S, Misun L, Zalmai H, Isaac O, Wen-Hung C, Dennis A, Content validity and test-retest reliability of patient perception of intensity of urgency scale (PPIUS) for overactive bladder, BMC Urology, 12, 2012, 26.
15. Human Interleukin 8 (IL-8) ELISA Kit [package insert], China, Hubei: Cusabio Life Science, 2015.
16. Human tumor necrosis factor-α (TNF-α) ELISA Kit [package insert], China, Hubei: Cusabio Life Science, 2015.
17. Human superoxide dismutase [Cu/Zn] (SOD1) ELISA Kit [package insert], China, Hubei: Cusabio Life Science, 2015.
18. Total Antioxidant Capacity (TAC) Colorimetric Assay Kit [package insert], Milpitas, CA: Biovision, 2015.
19. Cowan C, Hutchison C, Cole T, Barry SJE, Paul J, Reed NS, Russell JM, A randomized double-blind placebo-controlled trial to determine the effect of cranberry juice on decreasing the incidence of urinary symptoms and urinary tract infections in patients undergoing radiotherapy for cancer of the bladder or cervix, Clinical Oncology, 24, 2012, e31-e38.
20. Campbell G, Pickles T, D'yachkova Y, A randomised trial of cranberry versus apple juice in the management of urinary symptoms during external beam radiation therapy for prostate cancer, Clin Oncol, 15, 2003, 322–328.
21. Majewski W, Tarnawski R, Acute and late toxicity in radical radiotherapy for bladder cancer, Clinical Oncology, 21(8), 2009, 598-609.
22. Bonetta A, Di Pierro F, Enteric-coated, highly standardized cranberry extract reduces risk of UTIs and urinary symptoms during radiotherapy for prostate carcinoma, Cancer Manag Res, 4, 2012, 281–286.
23. Hamilton K, Bennett NC, Purdie G, Herst PM, Standardized cranberry capsules for radiation cystitis in prostate cancer patients in New Zealand: a randomized double blinded, placebo controlled pilot study, Supportive care in cancer, 23(1), 2015, 95-102.
24. Engles CD, Hauser PJ, Abdullah SN, Culkin DJ, Hurst RE, Intravesical chondroitin sulfate inhibits recruitment of inflammatory cells in an acute acid damage-leaky bladder‖ model of cystitis, Urology, 79(2), 2012, 483.e13-7.
25. Giberti C, Gallo F, Cortese PS, Shenone M, Combined intravesical sodium hyaluronate/chondroitin sulfate therapy for interstitial cystitis/bladder pain syndrome: a prospective study, Ther Adv Urol, 5, 2013, 175–179.
26. Porru D, Leva F, Parmigiani A, Barletta D, Choussos D, Gardella B, Impact of intravesical hyaluronic acid and chondroitin sulfate on bladder pain syndrome/interstitial cystitis, Int Urogynecol J, 23, 2012, 1193–1199.
27. American Urological Association. Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: aua/sufu guideline. San Diego, American Urological Association Education and Research, Inc., 2014.
28. Ales V, Vladimir S, Jitka V, Emilie F, Marc R,Vilím S, Cranberry fruit powder (Flowens™) improves lower urinary tract symptoms in men: a double-blind, randomized, placebo-controlled study, World Journal of Urology, 34(3), 2016, 419-424.
29. Ledda A, Bottari A, Luzzi R, Belcaro G, Hu S, Dugall M, Cranberry supplementation in the prevention of non-severe lower urinary tract infections: a pilot study, Eur Rev Med Pharmacol Sci, 19(1), 2015, 77-80.
30. Supriya M, Renu M, Pramod K, Goura K, Radiation Induced Cystitis and Proctitis - Prediction, Assessment and Management, Asian Pac J Cancer Prev, 16(14), 2015, 5589-5594.
31. Robert S, Sterile Pyuria: A Differential Diagnosis, Comp Ther, 26(3), 2000, 150–152.
32. Gilbert J, Peter N, Sterile Pyuria, The New England journal of medicine, 372, 2015, 1048-54.
33. Sing RI, Singal RK, What is significant hematuria for the primary care phycisian? Can J Urol, 19(1), 2012, 36-41.

34. Sonal G, Abhishek S, Richard L, Ashutosh K, Alexis E, Role of inflammation in bladder function and interstitial cystitis, Ther Adv Urol, 3(1), 2011, 19-33.
35. Mundy AR, Andrich DE, Posterior urethral complications of the treatment of prostate cancer, BJU Int, 110, 2012, 304-25.
36. Marcelo H, Homero B, Antonio C, Miguel S, Cranberries and lower urinary tract infection prevention, CLINICS, 67(6), 2012, 661-667.
37. Jepson RG, Craig JC, Cranberries for preventing urinary tract infections, Cochrane Database Syst Rev, 23(1), 2008.
38. Schaue D, Kachikwu EL, McBride WH, Cytokines in radiobiological responses:a review, Radiat Res, 178, 2012, 505–23.
39. Dancea HC, Shareef MM, Ahmed MM, Role of Radiation-induced TGF-beta Signaling in Cancer Therapy, Mol Cell Pharmacol, 1, 2009, 44–56.
40. Hong JH, Chiang CS, Campbell IL, Sun JR, Withers HR, McBride WH, Induction of acute phase gene expression by brain irradiation, Int J Radiat Oncol Biol Phys, 33, 1995, 619–26.
41. Miao L, Holley A, Zhao Y, St Clair, William H, Redox-Mediated and Ionizing-Radiation- Induced Inflammatory Mediators in Prostate Cancer Development and Treatment, Antioxidant & Redox Signaling, 20(9), 2014, 1481-1500.
42. Lejeune FJ, Clinical use of TNF revisited: improving penetration of anti-cancer agents by increasing vascular permeability, J Clin Invest, 110, 2002, 433–435.
43. Balkwill F, Tumour necrosis factor and cancer, Nature Reviews Cancer, 9, 2009, 361–371.
44. Vlahopoulos S, Boldogh I, Casola A, Brasier AR, Nuclear factor-kappa B-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation, Blood, 94, 1999, 1878–1889.
45. Singh RK, Sudhakar A, Lokeshwar BL, Role of chemokines and chemokine receptors in prostate cancer development and progression, Journal Cancer Science and Therapy, 2(4), 2010, 89–94.
46. La VD, Howell AB, Grenier D, Anti-Porphyromonas gingivalis and Anti-Inflammatory Activities of A-Type Cranberry Proanthocyanidins, Antimicrobial Agents and Chemotherapy, 54(5), 2010, 1778-84.
47. Bodet C, Grenier D, Chandad F, Ofek I, Steinberg D, Weiss E, Potential Oral Health Benefits of Cranberry, Critical Reviews in Food Science and Nutrition, 48, 2008, 1–9.
48. Mark Feldman, Cranberry proanthocyanidins inhibit the adherence properties of Candida albicans and cytokine secretion by oral epithelial cells, BMC Complementary and Alternative Medicine, 12, 2012, 6.
49. Matsushimaa M, Takayoshi S, Aya M, Tetsuya M, Atsushi T, Cranberry extract suppresses interleukin-8 secretion from stomach cells stimulated by Helicobacter pylori in every clinically separated strain but inhibits growth in part of the strains, Journal of Functional Food, 5(2), 2013, 729-735.
50. MacDougall C, Amoroso J, Neto C, Gottschall-Pass K, Hurta R, Cranberry (Vaccinium macrocarpon) extract reduces tumour necrosis factor alpha-induced expression of cyclooxygenase-2 and inducible nitric oxide synthase in vascular smooth muscle cells, FASEB Journal, 21(5), 2007, A249.
51. Nancy G, Monique T, Luc A, Jacques D, Inhibitory effects of proanthocyanidins from Ribes nigrum leaves on carrageenin acute inflammatory reactions induced in rats, BMC Pharmacology, 4, 2004, 25.
52. Déziel BA, Patel K, Neto C, Gottschall-Pass K, Hurta RA, Proanthocyanidins from the American Cranberry (Vaccinium macrocarpon) inhibit matrix metalloproteinase-2 and matrix metalloproteinase-9 activity in human prostate cancer cells via alterations in multiple cellular signaling pathways, J Cell Biochem, 111(3), 2010, 742-54.
53. Martína MA, Sonia R, Raquel M, Jannie P.J, Laura B, Christina K, Luis G. Chemical characterization and chemo-protective activity of cranberry phenolic powders in a model cell culture, Response of the antioxidant defenses and regulation of signaling pathways, Food research international, (71), 2015, 68-82.
54. Denis MC, Desjardins Y, Furtos A, Marcil V, Dudonné S, Montoudis A, Prevention of oxidative stress, inflammation and mitochondrial dysfunction in the intestine by different cranberry phenolic fractions, Clin Sci (Lond), 128(3), 2015, 197-212.
55. Mettler F, Upton A, Medical Effects of Ionizing Radiation, Philadelphia, PA: Saunders Elsvier, 2008.
56. Holley AK, Xu Y, St Clair DK, St Clair WH, RelB regulates manganese superoxide dismutase gene and resistance to ionizing radiation of prostate cancer cells, Ann N Y Acad Sci, 1201, 2010, 129–136.
57. Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, Thompson RG, Robinson SC, Balkwill FR, Reeducating tumor-associated macrophages by targeting NF-kappa B, J Exp Med, 205, 2008, 1261–1268.
58. Li F, Sethi G, Targeting transcription factor NF-kappa B to overcome chemoresistance and radioresistance in cancer therapy, Biochim Biophys Acta, 1805, 2010, 167–180.
59. Gupta-Elera G, Garrett AR, Robison RA, O'Neill KL, The role of oxidative stress in prostate cancer, Eur J Cancer Prev, 21, 2012, 155–162.
60. AJ Russo, Increased Serum Cu/Zn Superoxide Dismutase in Individuals with Anxiety, Nutr Metab Insights, 3, 2010, 37–42.
61. Liochev SI, Fridovich I, Mechanism of the peroxidase activity of Cu, Zn superoxide dismutase, Free Radic Biol Med, 48, 2010, 1565–1569.
62. Qing G, Tienan F, Han C, Yiting T, Xin G, Judong L, HIV-TAT mediated protein transduction of Cu/Zn-superoxide dismutase-1 (SOD1) protects skin cells from ionizing radiation, Radiation Oncology, 8, 2013, 253.
63. Pérez-Hernández J, Víctor J, David V, Elisa V, Anahí C, A Potential Alternative against Neurodegenerative Diseases: Phytodrugs, Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity, 2016, 19.
64. Elberry AA, Abdel-Naim AB, Abdel-Sattar EA, Nagy AA, Mosli HA, Mohamadin AM, Ashour OM, Cranberry (Vaccinium macrocarpon) protects against doxorubicin-induced cardiotoxicity in rats, Food Chem Toxicol, 48(5), 2010, 1178-84.
65. Liao S, Kao YH, Hiipakka RA, Green tea: biochemical and biological basis for health benefits, Vitam Horm, 62, 2001, 1.
66. Duferesne CJ, Farnworth ER, A review of latest research findings on the health promotion properties of tea, J Nutr Biochem, 12, 2001, 404.
67. Meunier MT, Duroux E, Bastide P, Free radical scavenger activity of procyanidolic oligomers and anthocyanosides with respect to superoxide anion and lipid peroxidation, Plantes medicinales et phytotherapie, 23, 1989, 267.
68. Facino RM, Carini M, Procyanidines from Vitis vinifera seeds protect rabbit heart from ischemia reperfusion injury: Antioxidant intervention and/or iron and copper sequestering ability, Planta medica, 62, 1996, 495.

Source of Support: Nil, **Conflict of Interest:** None.