International Journal of Science and Engineering (IJSE)
Home page: http://ejournal.undip.ac.id/index.php/ijse

The efficacy of dutasteride and green tea combination towards angiogenesis and bleeding on BPH after TURP: Study the effect on VEGF, MVD, and Hb

Eriawan Agung Nugroho*, Rifki Muslim*, Ignatius Riwanto*, Soetojo¥

*Department of Surgery, Dr. Kariadi General Hospital, Medical Faculty, Diponegoro University, Indonesia
¥Department of Urology, Dr. Soetomo General Hospital, Medical Faculty, Airlangga University, Indonesia
Corresponding Author : Eriawan Agung Nugroho;
Email : eriawanspu_s3@yahoo.com

Abstract - *Trans urethral resection of prostate (TURP) is a standard procedure in benign prostate hyperplasia (BPH) management, however it may face with complication such as hemorrhage that may increase morbidity and mortality rate. This study is designed to find the efficacy combination dutasteride and green tea in reducing hemorrhage on TURP patients and to evaluate the anti-angiogenesis effect. Double Blind Randomized Controlled Trial Post Test Only Design with 80 subjects, randomized into 4 groups : three treatment groups (T1 with 0.5mg of dutasteride, T2 with 725mg of green tea extract and T3 with combination of 0.5mg dutasteride and 725mg of green tea extract and one control group (C) at least 14 days before TURP procedure. We compared the ΔHb (haemoglobin) to evaluate the hemorrhage status and define the VEGF expression and MVD count to evaluate the angiogenesis changes between four groups. The study revealed ΔHb (pg/mL) for T1(-0.20±0.067) T2(-0.18±0.081) T3(-0.14±0.092) and C(-0.40±0.246). VEGF expression were T1(12.90±15.509), T2(11.60±9.121), T3(3.60±1.667) and C(20.20±17.386). MVD count median were T1(33), T2(31.5), T3(24) and C(42). The result from between subject effect tests showed statistically significantly reduce ΔHb, VEGF expression and MVD count. Multivariate analysis showed that the combination of dutasteride and green tea significantly reduce the hemorrhage during TURP (ΔHb) by decreasing the MVD. Administration of oral combination of 0.5mg dutasteride and 725mg of green tea extract, for at least 14 days prior to undergoing TURP significantly reduce the hemorrhage during TURP by decreasing the MVD.*

Keywords*: VEGF; MVD; Hb; Dutasteride; Green Tea*

Submission: August 25, 2015 Corrected : September 15, 2015 Accepted: October 1, 2015

Doi: 10.12777/ijse.9.2.80-84

*[**How to cite this article**:* Nugroho, E.A., Muslim, R., Riwanto, I., Soetojo. *(2015).* The efficacy of dutasteride and green tea combination towards angiogenesis and bleeding on BPH after TURP: Study the effect on VEGF, MVD, and Hb*, International Journal of Science and Engineering, 9(2),80-84.* **Doi**: 10.12777/ijse.9.2.80-84*]*

I. INTRODUCTION

Benign prostate hyperplasia (BPH) is a disease in elderly. BPH is the second most frequent disease found in urological clinics in Indonesia, just behind urinary tract stone (Fadlol and Mochtar, 2005). Trans urethral resection of prostate (TURP) is a gold standard treatment to reduce the lower urinary tract symptoms and urinary retention in BPH patient that untreatable with medication. The most common complication, both during and post operation is hemorrhage that significantly affect the morbidity and mortality (Shanmugasundaram *et al*., 2007; Kim *et al*., 2015; Boccon *et al*., 2005; Hahn *et al*., 2007).

The pathologic process on BPH involves the angiogenesis, increasing number and surface area of prostate vessels, that resulted in hemorrhage during TURP procedure. A number of studies has tried to find the factor to reduce angiogenesis (anti angiogenic therapy), one of them used 5-alpha reductase inhibitor, inhibits the conversion of testosterone into dehydotestosterone (DHT) that trigger the prostate enlargement (Chapple, 2004). 5-alpha reductase inhibitor also plays important role in androgen dependent angiogenic growth factor reduction, such as VEGF, sub urothelial microvessel density and blood vessels reduction, resulting prostate size reduction and apoptosis induction (Chapple, 2004; Clark *et al*., 2004). This type of drug consists of finasteride and dutasteride. Dutasteride selectively inhibits both 5-alpha reductase isoenzym (type 1 and 2). Both of finasteride and dutasteride are beneficial in reducing hemorrhage during TURP procedure. They have a distinct pharmacokinetic system, dutasteride has a longer half life and some studies has mentioned that dutasteride is more

effective in reducing DHT, more effective in reducing angiogenesis process and less adverse effect (Nickel, 2004). The possible side effects during long term 5-alpha reductase treatment are impotency, changes in libido, ejaculation disorder, and gynecomastia (Trost, 2013).

Green tea is a phytopharmaceutical with considerable anti angiogenesis agent. Epigallocatechin gallate (EGCG), one of active substances in green tea, has been proven to inhibit the angiogenesis process (Fassina *et al*., 2004). It has been reported to inhibit the urokinase and tyrosin kinase activation, therefore inhibit the activities of VEGF, IGF, EGF, and FGF (Adhami *et al*., 2009). After all, green tea inhibits the tumor growth by supressing the vascular growth. Administered together, it sound logically accepted that dutasteride and green tea has a synergism effect in angiogenesis inhibition.

It is suspected the combination off both agents, will reduce the hemorrhage as the risk of TURP procedure. This study was designed to evaluate the hemorrhage difference and VEGF activities that affect the reduction of MVD and inhibits the capillary endothelial cells growth. In the end, the angiogenesis will be reduced and prostatic vascularitation will be suppressed, finally, there will be less hemorragic blood volume during TURP procedure.

II. MATERIALS AND METHOD

This study was designed as an experimental double blind randomized controlled trial post test only. We tried to compare the angiogenesis changes by evaluating the VEGF expression, MVD count, haemoglobin (Hb) value, and blood transfusion rate in four groups of subjects: dutasteride only, green tea extract only, placebo, and combination of dutasteride and green tea groups, at least 14 days before TURP.

The subjects of the study were patients with TURP procedure to treat their BPH. The groups were pre-treated with 0.5 mg dutasteride capsule, 725 mg green tea extract capsule, combination of two former agents, and placebo in group T1, T2, T3, and C respectively.

The pre and post (2 hours after the procedure at the latest) procedural Hb values were obtained from all subjects along with another routine blood laboratory check as a prerequisite of the procedure. We determined the difference of pre and post procedure Hb value. The blood transfusion needs determined based on the post procedural Hb values, with less than 10 mg/dL as base value.

Every subjects underwent prostatic TRUS procedure to determine the exact volume of the prostate. All TURP procedures were commenced under spinal anesthesia. All TURP procedures were lead by an urologist surgeon using 24-F resectoscope (Karl Storz, Germany). We used aquadest as irigation fluid, and the volume in every procedure was recorded, as well as the duration of the procedure. The tissue subjects were obtained to evaluate the VEGF and MVD status by pathologist. VEGF expression were evaluated with immunohistochemistry staining under 100x microscopic magnification, Olympus BX-41. The MVD count were measured with CD34 surface marker under 100x microscopic magnification as well.

The results data were processed and presented in graphics. We used one way ANOVA test and Kruskall Wallis test to determine the variation of the groups when apropriate. We proceed with Mann Whitney U test and Post Hoc test to evaluate the statistical significance ($p<0.05$). To observe the correlation of each variables Pearson's product moment bivariate correlation test were performed. All statistical analysis were processed by computer using the SPSS. This study received its ethical clearance from Health Study Ethical Commision of Medical Faculty Diponegoro University and Kariadi General Hospital, number 292/EC/FK/RSDK/2012. This study was conducted from July 2012 until May 2013.

Table 1. Demographic and clinical characteristic of subjects included in curent study

	Mean ± SD				
Confounding Factors	**C**	**T1**	**T2**	**T3**	**p**
Age (year)	70.3 ± 6.626	69.9 ± 8.950	68.1 ± 7.732	69.5 ± 9.944	0.855
Prostatic weight (gram)	39.4 ± 12.923	45.3 ± 17.946	46.0 ± 17.096	43.7 ± 14.737	0.555
The duration of the treatment (day)	16.20 ± 1.704	15.95 ± 1.572	16.00 ± 1.414	15.85 ± 1.461	0.908
The duration of catheter placement (day)	17.53 ± 2.129	18.00 ± 2.152	17.60 ± 2.349	17.00 ± 1.622	0.532
The duration of the TURP (minute)	34.40 ± 3.979	34.65 ± 3.514	35.45 ± 2.762	34.90 ± 3.007	0.782
The irrigant fluid needed during the TURP (litre)	6.95 ± 0.780	7.04 ± 0.719	7.11 ± 0.596	6.97 ± 0.603	0.868
The time needed after the TURP procedure to obtain the blood subjects for Hb examination (minute)	85.00 ± 13.514	84.00 ±15.526	84.50 ±14.681	83.50 ± 9.473	0.807

T1 = group of 0.5 mg dutasteride / day, T2 = group of 725mg green tea extract/ day, T3 = group of the combination of 0.5 mg dutasteride and 725 mg green tea extract / day. C = the control group (placebo).

III. RESULTS AND DISCUSSION

We recruited 90 subjects with urinary retention, planned to undergo TURP pocedure, and all of them fulfilled the inclusion and exclusion criteria. The subject receive the treatment at least 14 days before TURP procedure.

Seven subjects were out of the analysis: 2 of them were unable to comply the treatment (both from T1), 3 subjects were aborted due to comorbid of New York Heart Association (NYHA) level III congestive heart failure (CHF) (2 from T2 and 1 from T3), 3 subjects were lost during follow-up period (2 from C group). We managed to have a data set from 83 subjects to be analyzed.

The average age of the subjects is 69.44 ± 8.287 years old with 48 and 87 as the youngest and the oldest respectively. The average prostatic weight is 43.62 ± 15.7 grams, with 18.6 and 85 grams as the minimum and maximum weight. The duration of the treatment are 16 + 1.5 14 and 19 days for the average the shortest and longes duration respectively. The duration of catheter placement are 17.53 ± 2.129 14 and 23 days for the average the shortest and longes duration respectively. The duration of the TURP are 34.85 ± 3.307 27 and 44 minutes in average the shortest and the longest duration respectively. The irrigant fluid needed during the TURP were 7.016 ± 0.669 litres, 5.8 litres and 8.8 litres in average minimum and maximum volume respectively. The time needed after the TURP procedure to obtain the blood subjects for Hb examination were 85 ± 13.36, 50 and 120 minutes for the average minimum and maximum values respectively. The statistical test for all variables in all groups showed p >0.05 therefore no statistically significant difference in age, prostatic volume, treatment duration, catheter placement duration, procedure time, irrigant volume, and time needed until blood collection. Therefore, the confounding factors were able to be controlled.

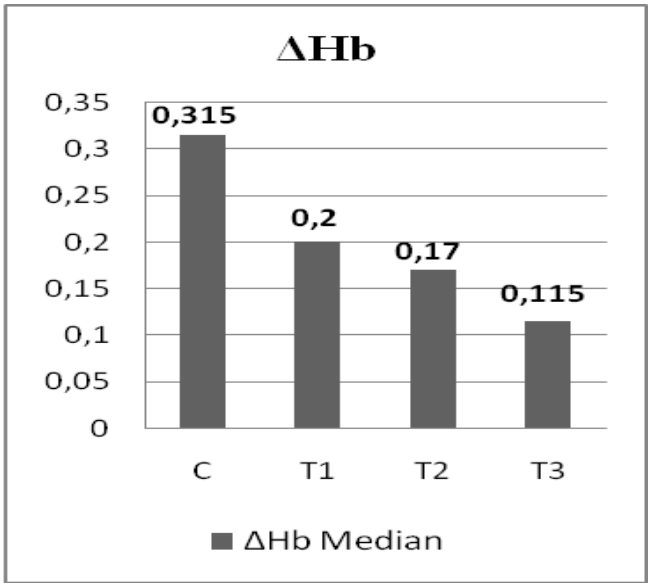

Figure 1. This diagram shows the difference Hb levels pre and post-TURP in four groups:
T1 = group of 0.5 mg dutasteride / day, T2 = group of 725mg green tea extract/ day, T3 = group of the combination of 0.5 mg dutasteride and 725 mg green tea extract / day. C = the control group (placebo).

T3 most effectively suppress the bleeding compared to other groups.

The Mann-Whitney test showed a significant difference of ΔHb between T2 and T3 groups and also T1 and T3 groups. There is no significant difference in ΔHb between T1 and T2. Therefore. ΔHb in T3 is significantly lower compared with T1 and T2.

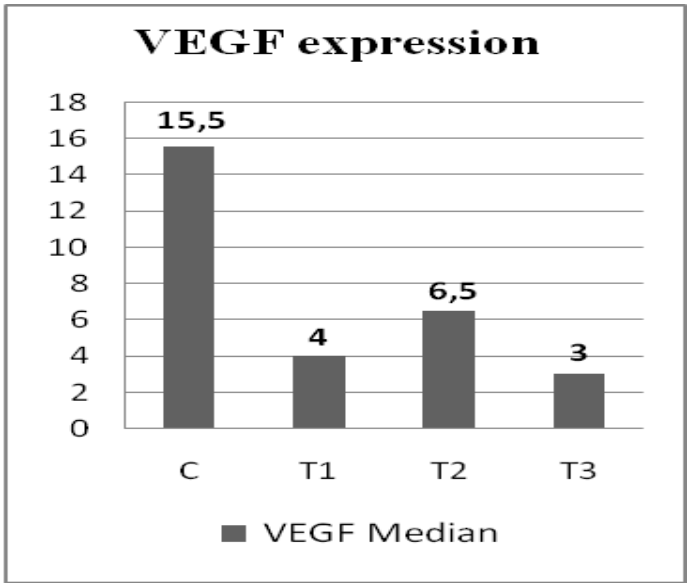

Figure 2. This diagram shows the difference VEGF expression after treatment in four groups :
T1 = group of 0.5 mg dutasteride / day, T2 = group of 725mg green tea extract/ day, T3 = group of the combination of 0.5 mg dutasteride and 725 mg green tea extract / day. C = the control group (placebo).
T3 lowest its VEGF expression compared to other groups.

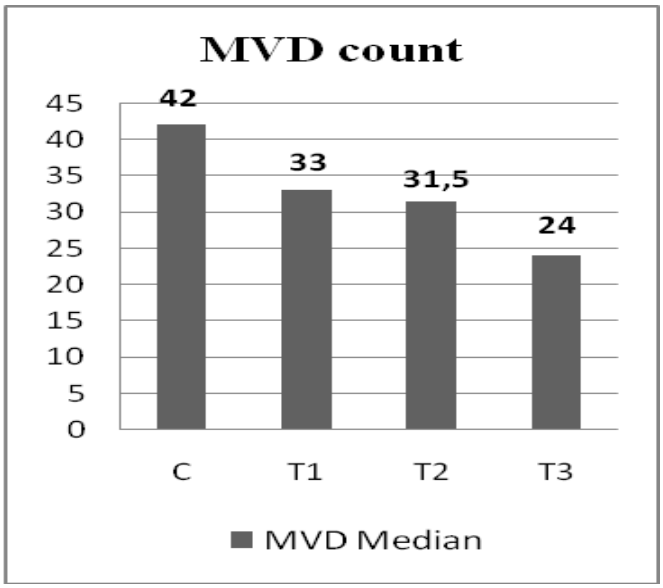

Figure 3. This diagram shows the difference MVD count after treatment in four groups :
T1 = group of 0.5 mg dutasteride / day, T2 = group of 725mg green tea extract/ day, T3 = group of the combination of 0.5 mg dutasteride and 725 mg green tea extract / day. C = the control group (placebo).
T3 lowest its MVD count compared to other groups.

The VEGF expression in treatment groups (T1. T2. T3) was significantly different compared to control group. There is significant difference in VEGF expression reduction between T2 and T3 groups and T1 and T3 groups. There was no significant difference between T1 and T2. Therefore. the VEGF expression in T3 were significantly lower compared to T1 and T2.

The post hoc test analysis showed significant reductions in group T1. T2. T3 compared with K. There were significant MVD count between T2 and T3. and also between T1 and T3. but not in T1 and T2. Therefore MVD count in T3 was much more significant compared with T1 and T2.

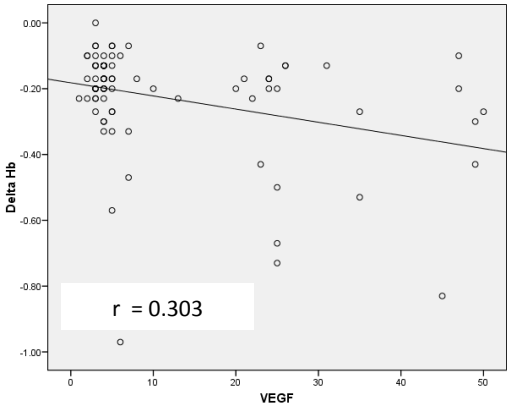

Figure 4. Scatter Graph VEGF correlation test against ΔHb, obtained value of r = 0.303 showed a weak association between VEGF with Δ Hb

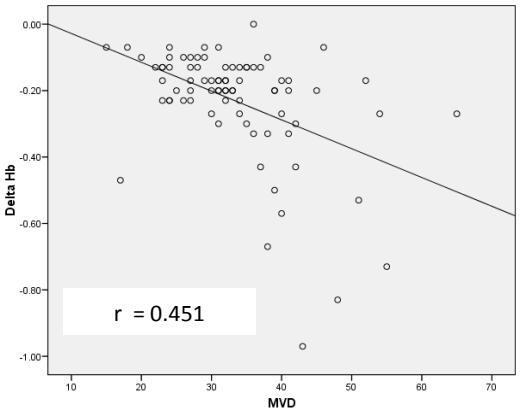

Figure 5. Scatter Graph MVD against ΔHb correlation test, a score of r = 0.451 showed moderate correlation between MVD with Δ Hb

Multivariate analysis showed that the combination of dutasteride and green tea significantly reduce the number of bleeding (Δ Hb) by decreasing the MVD. The possible factors that affect the hemorrhage volume during TURP procedure in all study subjects are : age, prostate volume, medical treatment duration, Catheter placement duration, operation time, irrigant fluid volume, and time until blood sample drawn after TURP procedure. All off them are well controlled and unable to affect the validity of study results (Walsh and Retik, 1998).

The study showed that the lowest amount of bleeding complication (ΔHb) is in the T group. The study also showed that combination of dutasteride and green tea reduced the hemorrhage count due to MVD count supression.

Angiogenesis is an adaptation process by creating a new blood vessel to respond the environmental changes that endanger the tissue. Angiogenesis itself can be pathological or physiological. The physiologic type occurs during growth of the tissue healing and menstrual cycle in women. The pathological type occurs during malignancy or infectious disease, vascular formation, and other hypoxic-induced condition (Carmeliet and Jane, 2000). The angiogenesis starts with trigerring factors, and it most case it will be hypoxic status. In tumor, the distant between the vessel and the cell affects the oxygen level diffusing into the cells. This condition, known as hypoxia, it will trigger the hypoxic-indicible factor 1α (HIF-1α) and increase the transcription process of angiogenic factor genes. HIF-1α also plays role on wether the cell move into apoptotic mode or not. The mechanical stress (intratumoral high pressure), inflammation/immune response, and genetic mutation on oncogene or tumor supressor gene, also play their part in angiogenesis (Hicklin and Ellis, 2005).

Hipervascularisation in prostate ussualy accounts for hemorrhage in BPH patient. The angiogenesis supression can be tried by manipulating the hormone that inhibits the testosterone. The blood flow into the prostate will be very affected by the testosterone (Lee, 1996). Testosterone triggers the growth and maintain prostate epithelium. In this study, green tea has a role in supressing the hemorrhage, reducing need of tranfusion, reducing VEGF expression and MVD count into the level that more significant with the combination of dutasteride and green tea.

Combination of both dutasteride and green tea extract, given as anti angiogenic therapy in TURP procedure for BPH patient. Green tea inhibits the Pl3k/Akt pathway, reduce the VEGF production, and finally reduce the angiogenesis (Steel *et al*., 2000; Yang *et al*., 1998; Lill *et al*., 2003). Active component in green tea, EGCG; inhibits cell growth and endothelial cell growth. EGCG is a strong antioxidant and effectively inhibits the carsinogenic activity. EGCG inducing the phase-II detoxification enzymes, such as peroxidase, stop the cycle cell, induce apoptosis and prostate growth (Robb and Brown, 2001). EGCG also inhibits another growth factors in prostate, for example basic fibroblast growth factor (bFGF); epidermal growth factor (EGF), insulin-like growth factor (IGF). Dutasteride reduces the growth factors by inhibiting the conversion testosterone into DHT, therefore reducing the androgen activity (Mc Conenel *et al*., 1992; Ku *et al*., 2009). Both mechanism reduce the prostatic VEGF and MVD, minimize hemmorhage during TURP, reduce the haemoglobin

decrease during operation, and reducing tranfusion rate after TURP procedure.

IV. CONCLUSIONS

It can be concluded that administration of oral combination of 0.5 mg dutasteride and 725 mg extract green tea once daily, for at least 14 days prior to undergoing TURP, significantly reduce the hemorrhage during TURP procedure, by decreasing MVD

ACKNOWLEDGMENT

The author would like to say thank you to management board and staffs Department of Surgery and Pathology Anatomy, Medical Faculty, Diponegoro University, Dr. Kariadi General Hospital, for the support during the research.

REFERENCES

Adhami, V.M., Siddiqui, I.A., Ahmad, N., Gupta, S., Mukhtar, H. (2004). Oral consumption of green tea polyphenols inhibits insulin-like growth factor-induced signaling in autochthonous mouse model of prostate cancer. Cancer Res, 64(23) : 8715-8722

Boccon, G.L., Valton, M., Ibrahim, H., Comenduci, A. (2005). Effect of dutasteride on reduction of intraoperative bleeding related to transurethral resection of the prostae. Prog Urol, 15 : 1085-1089

Carmeliet, P., and Jain R.K. (2000). Review article Angiogenesis in cancer and other diseases. Nature, 40(6801) : 249-257

Chapple, CR. (2004). Pharmacological therapy og benign prostate hyperplasia/lower urinary tract symptoms : an overview for the practising clinician. BJU Int, 94(5) : 738-744

Clark, R.V., Hermann, D.J., Cunningham, G.R., Wilson, T.H., Morrill, B.B., Hobbs, S. (2004). Marked supression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5-alpha reductase inhibitor. J Clin Endocrinol Metab, 89 : 2179-2184

Fadlol, A., Mochtar, C.A. (2005). Prediksi Volume Prostat pada Penderita Pembesaran Prostat Jinak. JIBI, 33(4) : 139-145

Fassina, G., Vene, R., Morini, M., Minghelli, S., Benelli, R., Noonan, D.M., et al. (2004). Mechanism of inhibition of tumor angiogenesis and vascular tumor growth by epigallocatechin-3-gallate. Clin Cancer Res 10(14) : 4865-4873

Hahn, R.G., Fagerstrom, T., Tammela, T.L., Van Vierssen TO., Beisland, H.O., et al. (2007). Blood Loss and Postoperative Complications Associated with Trans Urethral Resection of The Prostate After Pretreatment with Dutasteride. BJU Int, 99(3) : 587-594

Hiclin, D.J., and Ellis, L.M. (2005). Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. Journal of Biochemistry and Molecular Biology, 23(5) : 1011-1012

Kim, K.S., Jeong, W.S., Park, S.Y., Kim, Y.T., Moon, H.S. (2015). The effect of two weeks of treatment with dutasteride on bleeding after transurethral resection of the prostate. World J Mens Health, 33(1) : 14-19

Ku, J.H., Shin, J.K., Cho, M.C., Myung, J.K., Moon, K.C., Paick, J.S. (2009). Effect of dutasteride on the expression of hypoxia-inducible factor-1 alpha, vascular endothelial growth factor and microvessel density in rat and human prostate tissue. Scand J Urol Nephrol, 43(6) : 445-453

Lee, C. (1996). Role of Androgen in Prostate Growth and Regression : Stromal Epithelial Interaction. Prostate, 6 : 52-56

Mc Connel, J.D., Wilson, J.D., George, F.W., Geller, J., Pappas, F., Stoner, E. (1992). Finasteride, an inhibitor of 5 alpha reductase, supresses prostatic dehydrotestosterone in man with benign prostate hyperplasia. J Clin Endocrinolog Metab, 74(3) : 505-508

Nickel, J.C. (2004). Comparison of Clinical Trials with Finasteride and Dutasteride. Rev Urol, 6(9) : 31-39

Robb, C.S., Brown, P.R. (2001). Catechin in Tea : Chemistry and Analysis. In : Advanced in Chromatography, Brown, P.R and Grushka, E (Eds), Marcel Dekker Inc, New York, NY, pp : 379-390

Shanmugasundaram, R., Singh, J.C., Kekre, N.S. (2007). Does Dutasteride Reduce Perioperative Blood Loss and Postoperative Complications After Transurethral Resection of The Prostate. Indian J Urol, 23(3) : 334-335

Steel, V.E., Kellof, G.J., Balentine, D., Boone, C.W., Mehta, R., Bagheri, D., et al. (2000). Comparative chemopreventive mechanism of green tea, black tea and selected polyphenol extracts measured by in vitro bioassays. Carcinogenesis, 21(1) : 63-67

Trost, L., Saitz, T.R., Hellstrom, W.J.G. (2013). Side Effects of 5-Alpha Reductase Inhibitors : A Comprehensive Review. Sex Med Rev, 1(1) : 24-41

Walsh, P.C., Retik, A.B. (1998). Transurethral Surgery. In : Campbell's Urology, Walsh, P.C., Retik, A.B., Vaughan, E.D., Wein, A (Eds), WB Sauners, Philadelphia, pp : 1581-1528

Yang, F., de Villiers, W.J., McClain, C.J., Varilek, G.W. (1998). Green tea polyphenol block endotoxin-induced TNF production and lethality in a murine model. American Society for Nutritional Sciences. J Nutr, 128(12) : 2334-2340