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Stereological Studies in Prostate of Dietary Zinc Deficient Wistar Rats

KEYWORDS: Dorso-lateral prostate, ventral prostate, zinc deficiency, morphometry

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ABSTRACT *The study was conducted to evaluate the effect of dietary zinc deficiency on prostate.Prepubertal Wistar rats were divided into 3 groups: zinc control (ZC), pair fed (PF) and zinc deficient (ZD) and fed on 100µg / gm (zinc control and pair fed groups) and 1µg /gm (zinc deficient) diet. Dietary zinc deficiency after 2 and 4 weeks decreased weight of the dorso-lateral and ventral prostate .Morphometric study revealed a significant decrease in glandular epithelial cell height and nuclear diameter with consequent increase in absolute volume of lumen and fibromuscular stroma in dorso-lateral and ventral prostate. These observations revealed that the net secretory activity of the prostate is affected and that the growth of prostate during prepubertal period is regulated to a certain extent by zinc.*

Introduction

Zinc has myriad of functions in the biological systems being a component of numerous enzymes /proteins, with catalytic, structural as well as antioxidant role (Chasapis et al.2011; Hambidge and Krebs, 2007; Oteiza and Mackenzie, 2005), genomic integrity (Sharif et al. 2012 a) etc.Vallee and Falchuk (1993) reported that 30-40% of zinc is localized in the nucleus, 50% in the cytosol while remaining was found to be associated with the membranes. Zinc although a redox element acts as an antioxidant capable of replacing metals such as iron active in catalyzing free radicals (Oteiza et al. 2004; Zago and Oteiza, 2001). Zinc deficiency has been reported to cause degeneration in testes (Kumari et al. 2011a, 2011b, 2012) and prostate (Joshi et al. 2014) which would account for generation of oxidative stress (Nair et al. 2005; Bedwal et al. 2009). Intracellular zinc must be precisely regulated .Cells employs an intricate homeostatic mechanism and signaling for maintaining the cellular level of free zinc (Costello and Franklin, 2006; Rink and Hasse, 2007).A potent antioxidant – metallothionein has a significant role not only in scavenging the free radicals but is also involved in maintaining the homeostatic balance of zinc involving the zinc transporters (Luizzi and Cousins ,2004) thereby limiting the amount of chelatable free zinc in the cytoplasm under physiological conditions (Outten and O'Halloran 2001;Krezel and Maret 2006).

Prostate has the ability to accumulate high zinc level (Costello and Franklin, 1998) with human prostate containing ~ 209 µg /gm zinc (Costello and Franklin, 2006) .Contrary, authors (Cho et al. 2002; Park et al. 2007; Dubi et al. 2008) reported 1000 – 3000 µmol / kg zinc in human prostate tissue and fluid respectively. The normal peripheral zone of the prostate gland accumulates highest concentration of zinc as compared to other soft tissues (Costello et al. 2004; 2005).Moreover, the function of prostate are androgen dependent particularly 5α DHT which preferentially binds to androgen receptors and primarily arises from conversion of testosterone (Harris et al. 2009).Androgen receptors have been detected on prostate epithelial and stromal cells of the rats and humans (Pelletier et al. 2000; Banerjee et al. 2001).However, Sluzanowska-Glabowska et al. (2006) reported the presence of androgen receptor in columnar epithelial cells of all rat prostate lobe (lateral, dorsal and ventral).The present study aims to evaluate the effect of zinc deficiency on epithelial cell height, nuclear diameter and absolute volume of lumen and fibromuscular stroma of dorso –lateral and ventral prostate of Wistar rats.

Materials and Methods

Basal diet

The composition of diet (per kg) as per ICN Research Diet Protocol (1999) was as follows: egg albumin flakes (180 g), corn oil (100 g), corn starch (443 g), sucrose (200 g), cellulose (30 g), choline chloride (2 g), AIN-76 salt mixture (35 g), AIN-76C vitamin-antibiotic mixture (10 g) and DL-methionine (7 g) and D-biotin (20 mg). Diet was estimated at 213.9 nm in air-acetylene flame on GBC 902 atomic absorption spectrophotometer (AAS) (GBC Scientific Equipment Pty. Ltd., Dandenong, Victoria, Australia) and zinc concentrations were adjusted to 1 µg/g and 100 µg/g by addition of appropriate amounts of zinc sulfate.

Experimental Protocol

Sixty pre-pubertal male Wistar rats (30-40 days of age; 40-50 gm wt) were divided into three groups (ten animals for 2 weeks and 4 weeks subgroups of control, pair fed and zinc deficient): (i) Zinc control (ZC) groups were fed with diet containing 100 µg/g zinc. (ii) Pairfed (PF) groups received control diet but the amount of feed given was equal to the feed consumed (average) by zinc deficient group during the previous day. PF group was run so as to study the starvation stress effects caused by reduced intake of diet by zinc deficient group and (iii) Zinc deficient (ZD) groups were fed zinc deficient (1 µg /g) diet. All the groups were provided demineralized water ad libitum. The experiments were set for 2- and 4-weeks and were approved by Departmental Animal Ethics Committee, University of Rajasthan, Jaipur, India. The animals were housed individually in polypropylene cages with stainless steel grills. The polypropylene cages, grills and bottles were washed with detergent solution, demineralized water and finally rinsed in 10% EDTA solution so as to avoid any contamination and subsequent removal of zinc.

Animals were anesthetized under light ether anesthesia and prostate (dorso-lateral and ventral) were excised, trimmed off of extraneous tissue and weighed on Anamed electronic balance.

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Histology
Dorso-lateral and ventral prostate were fixed immediately in Bouin's fixative, dehydrated in graded series of alcohol and embedded in paraffin wax. Sections were cut at 5µ, stained with Ehrlich haematoxylene and alcoholic eosin.

Morphometric and absolute volume studies
The glandular epithelial cell height, nuclear diameter and absolute volume of lumen as well as fibromuscular stroma of dorso-lateral and ventral prostate was determined using calibrated ocular micrometer (Erma, Japan) according to the method of Weibel and Elais (1967).

Statistical Analysis
Data were expressed as mean ± SEM. Further, analyses of 2- and 4-weeks data was carried out separately using One way Analysis of Variance (ANOVA) and if the difference was found to be significant then post-hoc test (Duncan's Multiple Comparison Test) was applied. P<0.05 was considered to be significant. Statistical analyses were carried out using Sigma stat 3.5 software (Cranes Software International Ltd., Bangalore, India).

Results
A significant decrease (P<0.05) in ZD groups (2- and 4-weeks) was observed in dorso-lateral and ventral prostate weight after intake of synthetic diet when compared with their respective controls. Comparison of 2- and 4- weeks PF group with their respective controls revealed similar pattern. A significant (P<0.05) decrease in dorso-lateral and ventral prostate weight was observed when PF and ZD groups (2- and 4- weeks) were compared (Table 1).

Morphometric and absolute volume studies
Glandular epithelial cell height and nuclear diameter of dorso-lateral and ventral prostate of Wistar rats decreased significantly (P<0.05) when comparisons were carried out between the groups. However, absolute volume of the lumen and fibromuscular stroma of dorso-lateral and ventral prostate increased significantly in ZD groups (2- and 4-weeks) when compared with their respective controls (Table 2).

Discussion
Impaired growth is a cardinal feature of zinc deficiency and the mechanism involved appears to be multifactorial. In the present study a significant decrease in dorso-lateral and ventral prostate weight after dietary zinc deficiency was observed. Authors have reported zinc as a inhibitor of apoptosis (Anchordoquy et al. 2010).Histopathological studies indicated degeneration in dorso-lateral and ventral prostate after dietary zinc deficiency (Joshi et al. 2014) which is well supported by the present observation whereby there is decrease in glandular epithelial cell height as well as nuclear diameter. Moreover, lumen and fibromuscular stroma increased which are also indicative of the degeneration of the secretory luminal epithelial cells The reduction in intracellular zinc concentration in dorso-lateral and ventral prostate (Joshi et al. 2014) probably affected not only cell cycle promoting arrest, but may have enhanced programmed cell death leading to increased oxidative stress generating reactive oxygen species or reactive nitrogen species which is supported by increased nitric oxide level, impaired 17ß HSD as well as 3 ß HSD (Bostwick et al. 2000; Uzzo et al. 2002; Feng et al. 2003;Joshi et al. 2014)etc. Increased OGG1 expression involved in BER(base excision repair) pathway during marginal and severe zinc depletion indicative of oxidative damage has been reported (Song et al. 2009) leading to loss of cells capacity to repair damage (Song et al. 2010) via an increase in ROS. Intrinsic apoptotic pathway involving caspase 3 (Clegg et al. 2005), translocation of proapoptotic protein Bad to mitochondria ,cytochrome c as well as caspase 3 activation (Adamo et al. 2010) leading to high level of apoptotic cells as measured by CBMN-cyt assay (Sharif et al. 2012b) has been reported.Increased binding activity of transcription factors involved in regulating cell proliferation and apoptosis was reported (Yan et al.2008).Elevated expression may lead to imbalance of androgen metabolism. Prostate epithelial cells AR (androgen receptor) play an important role in homeostasis of the prostate (Wu et al. 2007). Zinc is localized in the Golgi complex or secretory vesicles of interstitiotrophs (IT), folliculotrophs (FT) (Thorlacius-Ussing, 1987) which regulate gonadotrophins (FSH, LH) synthesis and secretion (Hartoma et al. 1977; Habib, 1978). In zinc deficient rat decreased serum testosterone, follicle stimulating hormone and luetinizing hormone was observed after dietary zinc deficiency (Joshi et al. 2014) hence apparent changes in cell height, nuclear diameter, absolute volume of the lumen and fibromuscular stroma was noticed in the prostate. This is supported by studies of Om and Chung (1996) who reported that zinc deficiency reduces circulating luteinizing hormone and testosterone as well as modifies sex steroid hormone receptor levels which contribute to pathogenesis of male reproductive dysfunction. Steroid hormone receptors are zinc finger proteins. Dietary zinc deficiency must have effected the (a) expression of zinc transporters –Zip that mediates influx of Zn +2 with prostate epithelial cells (Huang et al.2006) and (b) metallothionein (St Croix et al. 2002).Zinc deficiency caused a decline in intracellular prostate zinc as well as serum zinc (Joshi et al. 2014).Similar decrease was also observed in testes after dietary zinc deficiency (Kumari et al. 2012) which has been associated with reduction in metallothionein level (Agarwal and Bedwal, 2003) indicative of homeostatic imbalance leading to increase in free zinc .Maret et al. (1999) observed that concentration of free zinc (Zn 2+) above 10^{-8} M inhibits several essential enzymes regulated by zinc. The increase in free zinc may perpetuate cytotoxicity (Maret, 2009).Hence, dietary zinc deficiency for 2- and 4-weeks causes changes in dorso-lateral and ventral prostate of Wistar rats which may in turn affect the fertility.

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Table 1: Dorso-lateral and ventral prostate weight (gm) of Wistar rats. (Mean ± SEM)

Groups	Dorso-lateral	Ventral
2 ZC	0.0491 ± 0.00276	0.0508 ± 0.00118
2 PF	0.0484 ± 0.00320	0.0404 ± 0.00167 a*
2 ZD	0.0382 ± 0.00240 b*c*	0.0320 ± 0.00208 b*c*
4 ZC	0.191 ± 0.0170	0.208 ± 0.0197
4 PF	0.183 ± 0.0128	0.181 ± 0.0133
4 ZD	0.124 ± 0.0139 b*c*	0.122 ± 0.0102 b*c*

a -ZC (zinc control) Vs PF (pair fed)
*** almost significant (P<0.05)**

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b - ZC (zinc control) Vs ZD (zinc deficient)
c - PF (pair fed) Vs ZD (zinc
Note: Multiple comparisons of means were performed separately for 2 weeks and 4 weeks sub groups

Table 2: Morphometric (µm) and Absolute volume (mm^3) measurements of dorso-lateral and ventral prostate of Wistar rats. (Mean ± SEM)

Groups	Dorso- lateral prostate				Ventral prostate			
	Glandular epithelial cell height (µm)	Lumen (mm^3)	Stroma (mm^3)	Nuclear di-ameter (µm)	Glandular epithelial cell height (µm)	Lumen (mm^3)	Stroma (mm^3)	Nuclear di-ameter (µm)
2 ZC	15.118 ± 0.272	11.66± 0.117	14.129 ±0.266	5.408 ± 0.0829	16.375 ± 0.253	13.859 ± 0.255	13.392 ± 0.157	5.156 ± 0.0568
2 PF	14.263 ± 0.221 a*	17.348 ± 0.174 a*	16.433 ± 0.147 a*	4.942 ± 0.0584 a*	15.959 ± 0.222	19.405 ± 0.141 a*	18.248 ± 0.191 a*	4.835 ± 0.0576 a*
2 ZD	12.647 ± 0.203 b* c*	24.695 ± 0 .351 b*c*	20.229 ± 0.228 b*c*	3.957 ± 0.0688 b* c*	13.333 ± 0.198 b*c*	28.361 ± 0.187 b*c*	21.3 ± 0.194 b*c*	4.583 ± 0.0574 b*c*
4 ZC	15.807 ± 2.82	20.369 ± 0.168	23.547 ± 0.147	5.378 ± 0.0605	16.724 ± 0.320	22.879 ±0.215	25.147 ± 0.173	5.355 ± 0.0745
4 PF	15.721 ± 0.255	21.736 ± 0.075 a*	24.263 ± 0.177 a*	5.248 ± 0.0666	14.336 ± 0.224 a*	31.371 ± 0.325 a*	26.743 ± 0.368 a*	5.294 ± 0.0632
4 ZD	9.573 ± 0.105 b* c*	26.441 ± 0.129 b* c*	35.334 ± 0.380 b*c*	4.958 ± 0.0520 b*c*	10.879 ± 0.164 b*c*	43.694 ± 0.304 b*c*	35.33 ± 0.186 b*c*	4.981 ± 0.0530 b*c*

a -ZC (zinc control) Vs PF (pair fed)
*** almost significant (P<0.05)**
b - ZC (zinc control) Vs ZD (zinc deficient)
c - PF (pair fed) Vs ZD (zinc deficient)
Note: Multiple comparisons of means were performed separately for 2 weeks and 4 weeks sub groups.

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REFERENCE

1. Adamo, A.M. Zago, M.P. Mackenzie, G.G. Aimo, L. Keen, C.L. Keenan, A. & Oteiza, P.I. (2010).The role of zinc in the modulation of neuronal proliferation and apoptosis.Neurotoxicity Research, 17, 1-14. | 2. Agarwal, R. & Bedwal, R.S. (2003). Effect of dietary zinc deficiency on metallothionein concentration of epididymal luminal fluids of weanling Wistar albino rats. Indian Journal of Experimental Biology, 41,118-122. | | | 3. Anchordoquy, J. M. Picco, S.J. Seoane, A. Anchordoquy, J.P. Ponzinibio, M.V. Mattioli, G.A. Peral, G.P. & Furnus, C.C. (2011). Analysis of apoptosis and DNA damage in bovine cumulus cells after exposure in vitro to different zinc concentrations. Cell Biology International, doi: 10.1042/CBI20100507. | 4. Banerjee, P.P. Banerjee, S. & Brown, T.R. (2001). Increased androgen receptor expression correlates with development of age-dependent, lobe-specific spontaneous hyperplasia of the brown Norway rat prostate. Endocrinology, 142, 4066-4075. | 5. Bedwal, S. Prasad, S. Nair, N. Saini, M. R. & Bedwal, R. S, (2009). Catalase in testes and epididymidis of Wistar rats fed zinc deficient diet. Indian Journal of Pharmaceutical Sciences, 71(1), 55-58. | 6. Bostwick, D.F. Alexander, E.E. Singh, R. Shan, A. Oian, J. Santella, R.M. Oberley, L.W. Yan, T. Zhong, W. Jiang, X. & Oberlay, T.D. (2000). Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. Cancer, 89:123-134. | 7. Chasapis, C.Loutsidou, A.Spiliopoulou, C. & Stefanidou, M.(2011). Zinc and human health: An update.Archives of Toxicology, 86, 1-14. | 8. Cho, Y.H. Lee, S.J. Lee, J.Y. Kim, S.W. Kim, J.J. Lee, W.Y. & Yoon, M.S.(2002). Changes in serum and prostatic zinc concentrations in rats after intraprostatic injection of zinc: Comparison of two forms of zinc delivery. International Journal of Urology, 9,681-687. | 9. Clegg, M.S. Hanna, L.A. Niles, B.J. Momma, T.Y. & Keen, C.L. (2005). Zinc deficiency –induced cell death. International Union of Biochemistry and Molecular Biology, Life, 57, 661-669. | 10. Costello, L.C. & Franklin, R.B. (1998). Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. The Prostate, 35,285-296 | 11. Costello, L.C. & Franklin, R.B. (2006). The clinical relevance of the metabolism of prostate cancer, zinc and tumor suppression: Connecting the dots. Molecular Cancer, 5, 5-17. | 12. Costello, L.C. Franklin, R.B. & Feng, P. (2005). Mitochondrial function, zinc and intermediary metabolism relationships in normal prostate and prostate cancer. Mitochondrion, 5,143-153. | 13. Costello, L.C. Guan, Z. Kukoyi, B. Feng, P. & Franklin, R.B. (2004). Terminal oxidation and the effects of zinc in prostate versus liver mitochondria. Mitochondrion, 4,331-338. | 14. Dubi, N. Gheber, C. Fishman, D. Sekler, I. & Hershfinkel, M. (2008). Extracellular zinc and zinc citrate, acting through a putative zinc-sensing receptor, regulate growth and survival of prostate cancer cells. Carcinogenesis, 29, 1692-1700. | 15. Feng, P. Li, T.L. Guan, Z.Y. Franklin, R.B. & Costello, L.C. (2003). Effect of zinc on prostatic tumorigenicity in nude mice. Annals New York Academy Sciences (USA), 1010, 316-320. | 16. Habib, F.K. (1978). Zinc and the steroid endocrinology of the human prostate. Journal of Steroid Biochemistry, 9,403-407. | 17. Hambidge, K.H. & Krebs, N.F. (2007). Zinc deficiency: A special challenge. Journal of Nutrition, 137, 1101-1105. | 18. Harris, W.P. Mostaghel, E.A. Nelson, P.S. & Montgomery, B. (2009). Androgen deprivation therapy: Progress in understanding mechanisms of resistance and optimizing androgen depletion. Nature Clinical Practice Urology, 6, 76-85. | 19. Hartoma, T.R. Nahoul, K. & Netter, A. (1977). Zinc, plasma androgens and male sterility. The Lancet, 2, 1125-1126. | 20. Huang, L. Kirschke, C.P. & Zhang, Y. (2006). Decreased intracellular zinc in human tumorigenic prostate epithelial cells: A possible role in prostate cancer progression. Cancer Cell International, 6, 10. doi: 10.1186/1475-2867-6-10. | 21. Joshi, S. Nair, N. & Bedwal, R.S. (2014). Dietary zinc deficiency effects dorso-lateral and ventral prostate of Wistar rats: Histological, biochemical and trace element study. Biological Trace Element Research , doi 10.1007/s12011-014-0053-1 | 22. Krezel, A. & Maret, W. (2006). Zinc –buffering capacity of a eukaryotic cell at physiological pZn. Journal of Biological Inorganic Chemistry, 11, 1049-1062. | 23. Kumari, D. Nair, N. & Bedwal, R.S. (2011a). Effect of dietary zinc deficiency on testes of Wistar rats: Morphometric and cell quantification studies. Journal of Trace Element in Medicine and Biology, 25, 47-53. | 24. Kumari, D. Nair, N. & Bedwal, R.S .(2011b). Testicular apoptosis after dietary zinc deficiency: Ultrastructural and TUNEL studies. Systems Biology in Reproductive Medicine, 57, 233-243. | 25. Kumari, D. Nair, N. & Bedwal, R.S. (2012). Protein carbonyl, 3 β -, and 17 β- hydroxysteroid dehydrogenases in testes and serum FSH, LH, and testosterone levels in zinc deficient Wistar rats. Biofactors, 38(3), 234-239. | 26. Luizzi ,J.P. & Cousins, R.J. (2004). Mammalian zinc transporters. Annual Review of Nutrition, 24, 151-172. | 27. Maret, W. (2009) Molecular aspects of human cellular zinc homeostasis: Redox control of zinc potentials and zinc signals.BioMetals, 22(1), 149-57. doi: 10.1007/s10534-008-9186-z | 28. Maret, W. Jacob, C. Vallee, B.L. & Fischer, E.H. (1999). Inhibitory sites in enzymes :Zinc removal and reactivation by thionein.Proceedings of National Academy of Sciences, USA 96: 1936-1940. | 29. Nair, N. Bedwal, R.S. Prasad, S. Saini, M. R. & Bedwal, R. S. (2005). Short term zinc deficiency in diet induces increased oxidative stress in testes and epididymis of rats. Indian Journal of Experimental Biology, 43,786-794. | 30. Om, A.S. & Chung, K.W. (1996). Dietary zinc deficiency alters 5 alpha-reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. Journal of Nutrition, 126,842-848. | 31. Oteiza, P.I. & Mackenzie, G.G. (2005). Zinc, oxidant –triggered cell signaling and human health. Molecular Aspects of Medicine, 26, 245-255. | 32. Oteiza, P.I. Mackenzie, G.G. & Verstraeten, S.V. (2004). Metals in neurodegeneration: Involvement of oxidants and oxidant –sensitive transcription factors. Molecular Aspects of Medicine, 25,103-115. | 33. Outten, C. E. & O'Halloran, T.V. (2001). Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science, 292, 2488-2492. | 34. Park ,S.E. Park, J.W. Cho, Y.S. Ryu, J.H. Paick, J.S. & Chun, Y.S. (2007). HIF-Iα promotes survival of prostate cells at a high zinc environment. The Prostate, 67, 1514-1523. | 35. Pelletier, G. Labrie, C. & Labrie, F. (2000). Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. Journal of Endocrinology, 165,359-370. | 36. Rink, L. & Hasse, H. (2007) Zinc homeostasis and immunity. Trends in Immunology, 28, 1-4. | 37. Sharif, R. Thomas, P. Zalewski, P. & Fenech, M. (2012a). The role of zinc in genomic stability. Mutation Research, 733,111-121. | 38. Sharif, R. Thomas, P. Zalewski, P. & Fenech, M. (2012 b). Zinc deficiency or excess within the physiological range increases genome instability and cytotoxicity respectively in human oral keratinocyte cells. Genes Nutrition, 7, 139-154. | 39. Słuczanowska-Głabowska, S. Laszcyńska, M. Glabowski, W. & Wylot, M. (2006) Morphology of the epithelial cells and expression of androgen receptor in rat prostate dorsal lobe in experimental hyperprolactemia. Folia Histchemica et Cytobiologica, 44, 25-30. | 40. Song, Y. Chung, C.S. Bruno, R.S. Traber, M.G. Brown, K.H. King ,J.C. & Ho, E, (2009) Dietary zinc restriction and repletion affects DNA integrity in healthy men. American Journal of Clinical Nutrition, 90(2), 321-328. | 41. Song, Y. Elias, V. Loban, A. Scrimgeour, A.G. & Ho, E. (2010). Marginal zinc deficiency increases oxidative DNA damage in the prostate after chronic exercise. Free Radical Biology and Medicine ,48(1) 82-88. | 42. St. Croix ,C.M. Wasserloos, K. Dineley, K. Reynolds, I. Levitan, E.S. & Pitt, B.R. (2002). Nitric oxide mediated changes in zinc homeostasis are regulated by metallothionein/thionein. American Journal of Physiology- Lung Cellular and Molecular Physiology., 282, L185-L193. | 43. Thorlacius-Ussing, O. (1987). Zinc in the anterior pituitary of rat: A histochemical and analytical work. Neuroendocrinology, 45,233-242. | 44. Uzzo, R.G. Leavis, P. Hatch, W. Gabai, V.L. Dulin, N. Zvartau, N. & Kolenko, V.M. (2002). Zinc inhibits nuclear factor-kappa B activation and sensitizes prostate cancer cells to cytotoxic agents. Clinical Cancer Research, 8, 3579-3583. | 45. Vallee, B.L. & Falchuk, K.H. (1993). The biochemical basis of zinc physiology. Physiology Reviews, 73, 79-118. | 46. Weibel, E.R. & Elais, H. (1967). Introduction to stereological principles. In: Quantitative methods in morphology. Springer –Verlag.Berlin, Heidelberg, New York, 91-98. | 47. Wu, C-T. Altuwaijri ,S. Ricke, W.A. Huang, S-P. Yen, S. Zhang, C. Niu, Y. Tsai, M-Y. & Chang, C.(2007). Increased prostate cell proliferation and loss of cell differentiation in mice lacking prostate epithelial androgen receptor. Proceedings of National Academy Sciences ,USA, 104, 12679- 12684. | 48. Yan, M. Song, Y. Wong, C.P. Hardin, K. & Ho, E. (2008) Zinc deficiency alters DNA damage response genes in normal human prostate epithelial cells. Journal of Nutrition, 138, 667-673. | 49. Zago, M.P. & Oteiza, P.I. (2001). The antioxidant properties of zinc interaction with iron and antioxidants. Free Radical Biology Medicine, 31, 266- 274. |