Asian Journal of Innovative Research 1 (4) 29-36 (2016)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH

Available online at http://www.asianjir.com

Research article Siddha Medicine

Received 14th Nov.2016;
Accepted 17 Dec. 2016
Online Dec 2016

EFFECT *OF Evolvulus alsinoides* LINN. ON BIOCHEMICAL, ANTIOXIDANT AND HEMATOLOGICAL PARAMETERS IN ALUMINUM TOXICITY MALE RAT

Gomathi Rajashyamala L* and Elango V#

***Gomathi Rajashyamala L**
Research Scholar, Department of Siddha Medicine, Tamil University, Thanjavur, Tamil Nadu, India

Dr. Elango V#
Department of Siddha Medicine, Tamil University, Thanjavur, Tamil Nadu, India

ABSTRACT

The effect of consumption of leaves of Evalvulus alsinoides L. on the Biochemical, antioxidant status and hematological parameters in albino rats induced with prostatitis was studied. Twenty four animals divided into four experimental groups namely Normal Control group (NC-group), prostatitis group ($AlCl_3$-group), *Evlovulus Alsinoides* leaves extract group (EALE group) and $AlCl_3$ + EALE group were used in the study. The NC was not induced with prostatitis and fed a control diet. The $AlCl_3$-group was induced with prostatitis but EALE-group was treated with EALE and both were fed a control diet. $AlCl_3$ + EALE-group were induced with prostatitis by $AlCl_3$ and fed with EALE respectively. prostatitis was induced in the animals by daily injected $AlCl_3$ by oral for a period of 12 weeks and the animals were fed with respective diets throughout the duration. Hematological indices including hemoglobin, red blood cells, white blood cells, MCH, MCHC, MCV, ESR and PCV *in vivo* antioxidant markers such as Superoxide Dismutase (SOD), Glutathione-S-Transferase (GST), glutathione (GSH), catalase, Ascorbic acid and α- Tocopherol were determined. Superoxide dismutase (SOD), Glutathione-S-Transferase (GST) and glutathione (GSH) levels increased significantly ($p<0.05$) in the groups fed EALE. In the present results demonstrated that EALE treatment significantly attenuated the increased activities of Biochemical enzymes. These findings indicate that *Evolvulus Alsinoides Linn.* may prevent or suppress the development of prostatitis and be useful in its treatment and management.

Keywords: *Evolvulus Alsinoides*, prostatitis, Red blood cells, white blood cells, glutathione-S-transferase, superoxide dismutase, SGOT, SGPT, ALP, Antioxidant.

***Citation*:** Gomathi Rajashyamala L and Elango V (2016). Effect of *Evolvulus alsinoides* Linn. on biochemical, antioxidant and hematological parameters in aluminum toxicity male rat Asian Journal of Innovative Research 1(4) 29-36 (2016).

INTRODUCTION

Various studies demonstrated that Aluminum has possible to induce toxic effects in humans or laboratory animals exposed through inhalation, oral, or dermal exposure. It is widely accepted that nervous system is the most sensitive target of aluminum toxicity and it may induce cognitive deficiency and dementia when it enters the brain. Besides this Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals. deterioration in spermatogenesis and sperm quality; enhancement of freeradicals and alterations in antioxidant enzymes (Yousef et al., 2007, , Yousef et al., 2005, Yousef et al., 2009); are several of the aspects suggested that Aluminium exposure causes adverse impact on male reproduction.

The prostate gland is a major secondary endocrine organ of males whose development and growth depends on androgen stimulation especially by dihydrotestosterone (DHT), an active metabolic product from the conversion of testosterone by steroid 5 "-reductase. It is documented that androgens and possibly estrogens constitute the primary factors responsible for prostate diseases (Shin *et al.*, 2012a; De Nunzio and Tubaro, 2011; Farley, 2011). There is an increased accumulation of DHT in the prostate with aging which results in increased cell growth and hyperplasia (Carson and Rittmaster, 2003). Prostatitis also involves increased adrenergic tone in prostate smooth muscle mediated by "1-adrenoceptors (Michel *et al.*, 1998). Prostatitis is not a known risk factor for prostate cancer but may increase the chance of its occurrence (Chang *et al*., 2012). The etiology of prostate inflammation is complex and not completely elucidated but involves age-related hormonal alterations, metabolic syndrome and inflammation (Thompson and Yang, 2000). Also, several studies have shown that other processes such as chronic inflammation and increased oxidative stress may play important roles in the development of prostatitis (Sciarra *et al*., 2008; Matsumoto *et al*., 2010). A positive association between consumption of vegetables and decreased incidence of diseases has been well documented. This is due to the antioxidant capacity and phytochemicals such as carotenoids, ascorbate, tocopherol, flavonoids and phenolics that are present in the vegetables (Liu, 2004; Hung *et al*., 2005). Free radicals generated under a number of conditions are involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing (Akinmoladun *et al*., 2007; Ziech *et al*., 2010). Humans are naturally protected against free radical damage by oxidative enzymes and proteins such as Superoxide Dismutase (SOD), Catalase (CAT) and glutathione as well as phytochemicals. Many plants have been identified as good sources of natural antioxidants which protect against degenerative diseases and cancer (Javanmardi *et al*., 2003; Arabshahi *et al*., 2007). Several phytomedicines are used for prevention and treatment of prostate disorders. This paper reviews the phytomedicines used in Africa, Western countries, India and China as treatment of BPH, prostatitis and prostate cancer. Herbals which hold potential promise are mentioned, although much research is still required. The ethanolic extract of *E. alsinoides* used to study the free radical scavenging activity, *In vitro* lipid peroxidation and FTIR analysis. The ability of plant extract to reduce ferric ions was determined in FRAP assay (Duraisamy Gomathi, Ganesan Ravikumar et al, 2014). In the present study *Evolvulus alsonoides* leaf extract has been selected to work for its the therapeutic effect of *Evolvulus alsonoides* Linn in experimental prostatitis.

MATERIALS AND METHODS

Collection of plants:

The fully mature *Evalvulus alsinoides* Linn. whole plants were collected from marungulam, Thanjavur District, Tamil Nadu, India from a single herb. The collected leaves were identified and authenticated by a Botanist Dr. S.John Britto S.J, The Director, The Rapinat Herbarium and Center for molecular systematic, st.Joseph's College, Tiruchirappalli, Tamil Nadu. A Voucher specimen has been deposited at Tamil University Herbarium. The plants were cut into small pieces and shade dried and powdered finely then used for extraction.

Animals

Male albino rats of Wistar strain approximately weighing 190-200g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals:

Nitroblue tetrazolium (NBT), ethylenediamine - -tetra acetic acid (EDTA), Sodium nitroprusside (SNP), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Potassium hexa cyano ferrate [K3Fe(CN)6], L-

ascorbic acid, Aluminum chloride, Sodium hydroxide and Trichloro Acetic acid (TCAs) and reduced glutathione were purchased for Sigma chemical company, Mumbai. All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Preparation of plant extract:

The *Evolvulus Alsinoides* leaves were first washed well and dust was removed from the plant. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Preparation of Aluminum chloride ($AlCl_3$)

Two grams of aluminum chloride was dissolved in 100 ml distilled water to prepare a stock solution (20 mg/ml). The solution was prepared weekly and kept in a plane bottle at 4ºC. $AlCl_3$ was daily administrated to rats (0.1ml (2mg) /100gm) orally.

Experimental design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows.First group; was negative control administered 3 ml distilled water orally once daily. Second group; was positive control group ($AlCl_3$ group) administered aluminium chloride (20 mg/kg bw,), the LD 50 of $AlCl_3$ when administered orally to rats was reported to be (380 - 400 mg/kg bw (Krasovskii et al., 1979).Third group; was administered *Evolvulus alsinoides* leaves extract (EALE) (75 mg/kg bw) which dissolved in 3ml distilled water orally once daily according to Lekshmi and Reddy, (2011). Fourth group; was co administered with $AlCl_3$ and EALE in the same doses in 2nd and 3rd groups. Doses were given once daily via gavage for 70 consecutive days, for completion of the spermatogenic cycle and maturation of sperms in epididymis (Sarkar et al., 2003).

Collection of blood sample:

At the end of the experimental feeding period, the rats were fasted overnight and the Blood was collected by cervical dislocation into plain, heparinized and EDTA bottles, respectively for Biochemical, antioxidant and hematological determinations. The blood in the plain bottles was allowed to clot and the serum separated at 3500 rpm for 10 min was used for determination of Biochemical, antioxidant and hematological analysis.

Determination of antioxidant and hematological parameters:

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al* (1979). The level of ascorbic acid was estimated by the method of Omaye*et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980). Superoxide dismutase activity was determined by the procedure of Kakkar *et al* (1984). Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar *et al.* (1984) in plasma. The activity of glutathione peroxidase was assayed by the method of Rotruck*et al* (1973). Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968). RBC counted by the method of Ochei and Kolhatkar, (2000). WBC counted by the method of Ochei and Kolhatkar, (2000). ESR sedimentation rate measured by the method of Ochei and Kolhatkar,(2000).PCV counted by the method of Ochei and Kolhatkar, (2000).

Biochemical estimation

Urea was estimated by the method of Natelson (1957). The serum GOT was estimated by the method of Reitman and Frankel (1957). The serum GPT was estimated by the method of Reitman and Frankel *(*1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Serum creatinine was carried out by alkaline picrate method of Boneses and Taussk (1954). Protein was estimated by the method of Lowry *et al.* (1951). Acid phosphatase activity was measured by the method of Annon (1963).

Statistical analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons (Harvey and Paige, 1998). The results were statistically analyzed by using SPSS (Statistical Packages for Social Studies) version 20 was used and $p< 0.05$ was considered to be significant.

RESULTS

Antioxidant and detoxifying markers:

The $AlCl_3$ treated rats were administered *Evolvulus alsinoides* leaves (orally at the dose of 500mg per kg body weight). Malondialdehyde (MDA) was marked increased and also a distinct diminution in glutathione (GSH) content in the plasma of AlCl3 treated rats. In *Evolvulus alsinoides* leaves treated rats these biochemical parameters attained an almost normal level. The decreased activity of plasma antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-

transferase and non enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in $AlCl_3$ treated rats, and its retrieval towards near normalcy in EALE administered rats revealed the efficacy of *Evolvulus alsinoides* leaves in combating $AlCl_3$ induced oxidative stress. From these results, it was suggested that oxidative stress had been nullified due to the effect of *Evolvulus alsinoi*des leaves. Results were shown in Table.1

Effect of *Evolvulus alsinoides* leaves on heamotological changes in experimental rats

Hematological studies in rats can provide information on the effect of the external environment on the internal physiology of rats. Biochemical changes in blood values are particularly important to diagnose disease and stress in rat. The hematological results in group II treated with $AlCl_3$ group showed a significant alteration in RBC, WBC, packed cell volume, hemoglobin concentration MCHC and decreases in MCV and MCH. Statistical analysis showed the significant variation during the experimental period. Supplementation of EALE to $AlCl_3$ treated rats restored the hematological profile. Results were shown in Table.2.

Effect of *Evolvulus alsinoides* leaves on Biochemical markers in experimental rats

Hepatospecific enzymes were activated when hepatocellular damage gave rise to abnormalities of liver function and these enzymes are remarkably increased in prostatitis. Hepatic injury caused by $AlCl_3$ generally reflects instability of liver metabolism which leads to distinctive changes in the serum enzyme activities. Intracellular enzymes, such as transaminases, ALP, ACP and Protein are useful indicators for liver function and urea, creatinine, sodium and potassium are useful indicator for kidney function; their increased levels are indicators of liver and kidney damage. In the present results demonstrated that EALE treatment significantly attenuated the increased activities of these enzymes. EALE helps in regeneration in liver and kidney, thus protecting membrane integrity and thereby minimizing enzyme leakage. This result suggested that EALE possess potential hepatoreno protective activity. Results were shown in Table 3 & 4.

Table 1: Effect of *Evolvulusalsinoides*.L on antioxidant in serum of experimental rats

Animal Groups	MDA (nmol MDA formed/l)	GSH (mg/dl)	SOD (U/ml)	Catalase (U/ml)	GPX (U/ml)	Vitamin-E (mg/dl)	Vitamin-C (mg/dl)
Group- I (Normal)	10.76±0.75	0.50±0.35	5.52±0.38	9.33±0.65	4.76±0.33	6.62±0.46	7.36±0.51
Group-II ($AlCl_3$)	16.03±1.12 [a]	0.32±0.02 [a]	3.19±0.22 [a]	6.0±0.42 [a]	3.49±0.24 [a]	3.4±0.23 [a]	5.63±0.39 [a]
Group-III (Plant)	9.12±0.63 [b]	0.43±0.03 [b]	5.65±0.39	8.0±0.56 [b]	4.31±0.30 [b]	6.52±0.45 [b]	7.98±0.55 [b]
Group-IV $AlCl_3$+Plant	11.90±0.83 [b]	0.47±0.03 [b]	5.39±0.37	8.26±0.57 [b]	4.95±0.34 [b]	6.24±0.43 [b]	6.87±0.48 [b]

Table 2: Effect of *Evolvulus alsinoides* leaves on heamotological changes in experimental rats

Animal Groups	Hemoglobin (gm/dl)	RBC (Million/cu.mm)	WBC (cu.mm)	ESR (mm)	PCV (%)
Group- I (Normal)	15.62±1.09	11.32 ±0.79	4.23 ±0.29	12.23±0.85	38±2.66
Group-II (Induction)	8.53±0.59 [a]	8.65 ±0.60 [a]	3.02±0.21[a]	19.65±1.37[a]	59±4.13[a]
Group-III (Plant)	16.21±1.13[b]	12.35 ±0.86 [b]	4.18±0.29[b]	11.25±0.78[b]	40±2.8 [b]
Group-IV ($AlCl_3$+Plant)	14.49±1.01[b]	11.05 ± 0.77 [b]	4.11±0.28[b]	13.56±0.94[b]	42±2.94[b]

Values are expressed as mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

Table 2a: Effect of *Evolvulus alsinoides* leaves on heamotological changes in experimental rats

Animal Groups	MCH (pg/cell)	MCHC (%)	MCV (cubic micron)
Group- I (Normal)	13.79±0.96	41.10±2.87	33.56±2.34
Group-II (Induction)	9.86±0.69[a]	14.45±1.01[a]	68.20±4.77[a]
Group-III (Plant)	13.12±0.91[b]	40.52±2.83	32.38±2.26
Group-IV ($AlCl_3$+Plant)	13.11±0.91[b]	34.5±2.41[b]	38.00±2.66[b]

Values are expressed as mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

Table 3: Effect of *Evolvulus alsinoides* leaves on liver markers in serum of experimental rats

Animal Groups	Liver biomarker				
	Protein (gm/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)
Group- I (Normal)	7.38±0.51	41.39±2.89	25.45±1.78	71.56±5.00	32.41±2.26
Group-II (Induction)	6.46±0.45[a]	69.70±4.87[a]	45.11±3.15[a]	95.36±6.67[a]	47.25±3.30[a]
Group-III (Plant)	7.43±0.52[b]	45.75±3.20[b]	28.00±1.96[b]	72.63±5.08[b]	33.56±2.34[b]
Group-IV ($AlCl_3$+Plant)	7.07±0.49[b]	52.28±3.65[b]	32.66±2.28[b]	73.02±5.11[b]	33.62±2.35[b]

Values are expressed as mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

Table 4: Effect of *Evolvulus alsinoides* leaves on kidney markers in serum of experimental rats

Animal Groups	Kidney biomarker			
	Urea (mg/dl)	Creatinine (mg/dl)	Sodium (Meq/dl)	Potassium (Meq/dl)
Group-I	30.00±2.1	0.98±0.06	5.38±0.37	148.32±10.38
Group-II	41.42±2.89[a]	1.85±0.12[a]	7.15±0.50[a]	120.75±8.45[a]
Group-III	32.85±2.29[b]	0.86±0.06[b]	5.61±0.39[b]	142.58±9.98[b]
Group-IV	31.42±2.19[b]	1.02±0.07[b]	5.26±0.36[b]	145.65±1.19[b]

Values are expressed as mean ± SD for six rats in each group.[a]Significantly different from group I ($p < 0.05$), [b]Significantly different from group II ($p < 0.05$)

CONCLUSION:

In conclusion, consumption of *Evolvulus Alsinoides* leaves appear to be protective against oxidative stress and tissue damage and are a promising candidate for further laboratory and clinical research on prostate related diseases including prostate cancer.

REFERENCES

. Akinmoladun, A.C., E.O. Ibukun, E. Afor, B.L. Akinrinlola and T.R. Onibon *et al*., 2007. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol., 6: 1197-1201.

Annon, K. (1963). The colorimetric determination of phosphatase- Sigma Technical Bulletin, Sigma chemicals co., St Louis, USA. 104-108.

Arabshahi, S., D.V. Devi and A. Urooj, 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chem., 100: 1100-1105.

Arruzazabala, M.L., R. Mas, V. Molina, M. Noa, D. Carbajal and N. Mendoza, 2006. Effect of D-004, a lipid extract from the Cuban royal palm fruit, on atypical prostate hyperplasia induced by phenylephrine in rats. Drugs R&D, 7: 233-241.

Aydin, A., Z. Arsova-Sarafinovska, A. Sayal, A. Eken and O. Erdem *et al*., 2006. Oxidative stress and antioxidant status in non-metastatic prostate cancer and benign prostatic hyperplasia. Clin. Biochem., 39: 176-179.

Babu, S.V.V., B. Veeresh, A.A. Patil and Y.B. Warke, 2010. Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in rats. Eur. J. Pharmacol., 626: 262-265. Barnes, J., 2002. Benign prostatic hyperplasia. Pharm. J., 269: 250-252.

Baker H., Frank O., De Angeles B and Feinglod S. (1980). Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutrition Reports International 21: 531.

Beers , R., and Sizer, I. (1952). A spectrophotometric method for measuring the down of hydrogen peroxide by catalase. Journal of Biological Chemistry 195: 133.

Belostotskaia, L.I., I. Nikitchenko, O.N. Gomon, L.A. Chaika, V.V. Bondar and V.N. Dziuba, 2006.Effect of biologically active substances of animal and plant origin on prooxidant-antioxidant balance in rats with experimental prostatic hyperplasia. Eksperimental'naia Klinicheskaia Farmakologiia, 69: 66-68.

Beuge, J.A., and Aust, S.D. (1978). The thiobarbituric acid assay. Methods in Enzymology 52: 306-307.

Boneses RN and Taussk HA (1945). On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem. 158: pp 581-591.

Bub, A., B. Watzl, M. Blockhaus, K. Briviba and U. Liegibel *et al*., 2003. Fruit juice consumption modulates antioxidative status, immune status and DNA damage. J. Nutr. Biochem., 14: 90-98.

Bullock, T.L. and G.L. Andriole Jr., 2006. Emerging drug therapies for benign prostatic hyperplasia. Expert Opin. Emerg. Drugs, 11: 111-123.

Chapple, C.R. and D. Smith, 1994. The pathophysiological changes in the bladder obstructed by benign prostatic hyperplasia. Br. J. Urol., 73: 117-123.

Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.

Dacie, J.V. and S.M. Lewis, 1991. Practical Hematology. 11th Edn., Churchill Livingstone,London, UK .

Disbrey, B.D. and J.H. Rack, 1970. Histological Laboratory Methods. Livingstone, Edinburgh, ISBN-13: 9780443006944, pp: 56-128.

Emeka, E.J.I. and O. Obidoa, 2009. Some biochemical, haematological and histological responses to a long term consumption of *Telfairia occidentalis*-supplemented diet in rats. Pak. J. Nutr., 8: 1199-1203.

Farley, S.J., 2011. BPH: Lift and separate to relieve LUTS. Nat. Rev. Urol., 8: 352-352.

Ferlay, J., H.R. Shin, F. Bray, D. Forman, C. Mathers and D.M. Parkin, 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer, 127: 2893-2917.

Geavlete, P., R. Multescu and B. Geavlete, 2011. *Serenoa repens* extract in the treatment of benign prostatic hyperplasia. Therapeut. Adv. Urol., 3: 193-198.

Gravas, S. and M. Oelke, 2010. Current status of 5"-reductase inhibitors in the management of lower urinary tract symptoms and BPH. World J. Urol., 28: 9-15.

Greenwald, R.A., 1985. CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, ISBN-13: 9780849329364, pp: 267-278.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hung, S.H., C.W. Yu and C.H. Lin, 2005. Hydrogen peroxide functions as a stress signal in plants.Bot. Bull. Academia Sinica, 64: 1-10.

Javanmardi, J., C. Stushnoff, E. Locke and J.M. Vivano, 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem., 83: 547-550.

Jemal, A., R. Siegel, E. Ward, Y. Hao, J. Xu and M.J. Thun, 2009. Cancer statistics, 2009. CA: Cancer J. Clin., 59: 225-249.

Kakkar, P., Das, B., and Viswanathan, P.N. (1984). A modified spectrophotometric assay of SOD. Indian Journal of Biochemistry and Biophysics 21: 130-132.

King, R.P.N., and Kind, E.J. (1954). Determination of alkaline phosphatase activity by colorimetric method.J. Clin. Path. 7: 322.

Kolonel, L.N., J.H. Hankin, A.S. Whittemore, A.H. Wu and R.P. Gallagher *et al*., 2000. Vegetables,fruits, legumes and prostate cancer: A multiethnic case-control study. Cancer Epidemiol.Biomark Prev., 9: 795-804.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with the Folin's reagent. Journal of Biological Chemistry 193, 265-276.

Mansour, S.A., A.H. Mossa and T.M. Heikal, 2007. Haematoxicity of a new natural insecticide Spinosad on male Albino rats. Int. J. Agric. Biol., 9: 342-346.

Mchedlidze, M.G. and T.I. Shioshvili, 2006. [Influence of antioxidants on the development of benign prostatic hyperplasia]. Georgian Med. News, 140: 23-27, (In Russian).

Moron, M.S., DsePierre, J.W., and Manerwik, K.B. (1979). Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochimica et Biophysica Acta 582: 67-68.

Natelson, S. (1957). Micro-techniques of clinical chemistry for the routine laboratory. C.C.Thomas, Spring-Field, Illinois: 381.

Nickel, J.C., D. Shoskes, C.G. Roehrborn and M. Moyad, 2008. Nutraceuticals in prostate disease:The urologist's role. Rev. Urol., 10: 192-206.

Novara, G., A. Galfano, R.B. Berto, V. Ficarra, R.V. Navarrete and W. Artibani, 2006. Inflammation, apoptosis and BPH: What is the evidence? Eur. Urol. Suppl., 5: 401-409.

Odukoya, O.A., S.I. Inya-Agha, F.I. Segun, M.O. Sofidiya and O.O. Ilori, 2007. Antioxidant activity of selected Nigerian green leafy vegetables. Am. J. Food Technol., 2: 169-175.

Olajire, A.A. and L. Azeez, 2011. Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. Afr. J. Food Sci. Technol., 2: 22-29.

Omaye, S.T., Tumball, J.D., Sauberlich, H.E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology .62: 1-11.

Palozza, P., S. Luberto, S. Calviello, P. Ricci and G.M. Bartoli, 1997. Antioxidant and prooxidant role of $-carotene in murine normal and tumor thymocytes: Effects of oxygen partial pressure. Free Radical Biol. Med., 22: 1065-1073.

Prasad, S., N. Kalra, M. Singh and Y. Shukla, 2008. Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice. Asian J. Androl., 10: 313-318.

Reitman, S., and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Path 25: 56.

Romieu, I., F. Castro-Giner, N. Kunzli and J. Sunyer, 2008. Air pollution, oxidative stress and dietary supplementation: A review. Eur. Respir. J., 31: 179-197.

Rotruck JT., Pope AL., Ganther HE., Swanson AB., Hafeman DG and Hoekstra WG. (1973) Selenium: biochemical roles as component of glutathione peroxidase. Science. 179: pp588-590.

Ruela de Sousa, R.R., K.C.S. Queiroz, A.C.S. Souza, S.A. Gurgueira and A.C. Augusto *et al*., 2007.Phosphoprotein levels, MAPK activities and NF6B expression are affected by fisetin. J. Enzyme Inhib. Med. Chem., 22: 439-444.

Sciarra, A., G. Mariotti, S. Salciccia, A.A. Gomez, S. Monti, V. Toscano and F. Di Silverio, 2008. Prostate growth and inflammation. J. Steroid Biochem. Mol. Biol., 108: 254-260.

Shin, I.S., M.Y. Lee, D.Y. Jung, C.S. Seo, H.K. Ha and H.K. Shin, 2012a. Ursolic acid reduces prostate size and dihydrotestosterone level in a rat model of benign prostatic hyperplasia. Food Chem. Toxicol., 50: 884-888.

Thompson, T.C. and G. Yang, 2000. Regulation of apoptosis in prostatic disease. Prostate, 45: 25-28.

Torricelli, P., P. Ferorelli, A. de Martino, F. Antonelli and S. Beninati, 2013. Preventive effects of A mixture of micronutrients with antioxida-tive properties on experimentally induced prostate hyperplasia. Am. J. Life Sci., 1: 22-26.

Traish, A.M., J. Hassani, A.T. Guay, M. Zitzmann and M.L. Hansen, 2011. Adverse side effects of 5"-reductase inhibitors therapy: Persistent diminished libido and erectile dysfunction and depression in a subset of patients. J. Sexual Med., 8: 872-884.

Yousef, M.I., Kamel, K.L., ElGuendi,M.I. and El-Demerdash, F.M. An in vitro study on reproductive toxicity of aluminium chloride on rabbit sperm: The protective role of some antioxidants. Toxicology. 2007; 239: 213-223.

Yousef,M.I. and Salama,A.F. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food and Chem. Toxicology. 2009; 47: 1168-1175.

Yousef,M.I., El-Morsy,A.M.A. and Hassan,M.S. Aluminium induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: protective role of ascorbic acid. Toxicology. 2005; 215: 97-107.

Source of support: Nil;
Conflict of interest: None declared

Zhang, K., P. Mack and K.P. Wong, 1998. Glutathione-related mechanisms in cellular resistance to anticancer drugs. Int. J. Oncol., 12: 871-882.

Ziech, D., R. Franco, A.G. Georgakilas, S. Georgakila and V. Malamou-Mitsi *et al*., 2010. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chem. Biol. Interact., 188: 334-339.

Zou, G.L., X.F. Gui, X.L. Zhong and Y.F. Zhu, 1986. Improvements in pyrogallol autoxidation method for the determination of SOD activity. Prog. Biochem. Biophys., 4: 71-73.