

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Efficacy of Echinacea on the Action of Cyproterone Acetate in Male Rats

Nadia M.S. Arafa

Department of Physiology, National Organization for Drug Control and Research, Egypt

Abstract: The study aimed to evaluate the effect *Echinacea* extract (E) on the testicular antioxidants function in normal rats or that subjected to anti-androgenic compound, cyproterone acetate (CA). Rats were divided into 5 groups treated daily via an oral tube for two intervals 2 and 4 weeks, 1st control, 2nd E (*Echinacea* treated group in dose 63 mg kg⁻¹), 3rd CA (cyproterone acetate treated group in dose 25 mg kg⁻¹), 4th E+CA and 5th E as prophylactic one week before E+CA treatment with the same aforementioned E or CA doses. The body, testes, epididymis and vas deferens weights were recorded. Sperm count, Nitric Oxide (NO), calcium ion (Ca²⁺) and malondialdehyde (MDA) contents in addition to superoxide dismutase (SOD), glutathione S-transferase (GST) activities were determined in testicular tissues. CA exhibited direct negative effect on reproductive organs weight and significant reducing effect on sperm count and Ca²⁺ contents. SOD and GST activities significantly decreased in addition to significant increase in NO, MDA contents reflecting the oxidative status of testis in CA treated rats. The prophylactic effect of E treatment, in time related manner, showed significant improvement in the antioxidant status of the testicular tissue which is more pronounced as compared to E+CA treatment.

Key words: *Echinacea*, cyproterone acetate, testis, sperm count, oxidative status, antioxidants

INTRODUCTION

The antioxidant system plays an effective role in protecting testes and other biological tissues below a critical threshold of Reactive Oxygen Species (ROS), preventing testicular dysfunction (Ochsendorf, 1999). ROS mediated oxidative stress is one of crucial reasons of infertility, decreased sperm viability and increased level of free radicals which may cause degeneration of testicular tissue (Koksal *et al.*, 2002; Turner and Lysiak, 2008). The administration of antioxidants to normal animals, not suffering from induced oxidative stress, appears to improve testicular function, signifying oxidative stress a consistent feature of testicular physiology (Orozco *et al.*, 2003; Juan *et al.*, 2005). Antioxidants administered to infertile men advocate promising their use in male infertility treatment (Suleiman *et al.*, 1996; Keskes-Ammar *et al.*, 2003).

Cyproterone acetate is a synthetic steroidal drug which competes with testosterone and dihydrotestosterone for the androgen receptor. It is used to suppress male fertility in oral contraceptives formulations and also in the treatment of various sexual and metabolic disorders (Meriggiola *et al.*, 1996). Cyproterone also has progesterone like activity and reduces pituitary luteinizing hormone and plasma testosterone (McLeod, 1993) and suppresses the secretion of gonadotropins thus, interferes with testosterone production (Barradell and Faulds, 1994; Neumann, 1994; Honer *et al.*, 2003). Due to the direct

interaction with the cellular receptors CA inhibits the action of the androgens and due to this property it is used for the prostatic carcinoma treatment (Torri and Floriani, 2005).

Cyproterone is genotoxic and tumor initiating agent (Kasper, 2001) and the genotoxic effects postulated to be reduced by the use of antioxidants (Siddique *et al.*, 2008).

Echinacea (E) is herbaceous plant genus, consisting of nine species and the current study used one of them *Echinacea purpurea* or purple coneflower. *Echinacea* (E) preparations have been marketed as possible immune stimulators or enhancers worldwide. *Echinacea* became popular as remedies for the common cold and rhinovirus infection (Barrett *et al.*, 2006; Shah *et al.*, 2007; Simasek and Blandino, 2007). It has anti-tumor effect (Block *et al.*, 2002). The therapeutic effect of *Echinacea* has been assigned to the presence of caffeic acid derivatives such as cichoric acid, echinacoside, chlorogenic acid and lipophilic polyacetylene-derived compounds, such as alkylamides, constituting isobutylamides are parts of the active principles and suitable for standardization of *E. purpurea* raw material and finished products (Letchamo *et al.*, 1999). Pellati *et al.* (2005) reported that *Echinacea* roots are a good source of natural antioxidants possessing radical scavenging activity.

The objective of this study is to evaluate the physiological and antioxidant effect of *Echinacea* extract consumption on normal male rat reproductive system or that subjected to cyproterone acetate in addition to the prophylactic effect of *Echinacea* extract supplementation

by subjecting it to normal rat a week before cyproterone acetate treatment to explore its impact in reducing the postulated cyproterone related side effects.

MATERIALS AND METHODS

Experimental animals: This study was carried out on adult male albino rats (Wistar strain). Sixty animals were obtained from the farm of National Organization for Drug Control and Research (NODCAR) in 2008. Their weights were $(130.2 \pm 18.5 \text{ g})$. Male rats were housed in iron mesh cages, each cage contained six rats. Animals were kept under controlled temperature of $21 \pm 2^\circ\text{C}$ and 12 h light/12 h dark cycle throughout the experiment. A commercial pelleted diet was used during the experiment and allowed with water *ad libitum*. The animals were allowed to adapt to the laboratory conditions for two weeks before the beginning of the experiment.

Drugs: Cyproterone acetate ($\text{C}_{24}\text{H}_{29}\text{ClO}_4$) was obtained from Schering, Germany as ANDROCURE tablets each containing 50 mg active ingredient Cyproterone acetate (6-chloro-17-hydroxy-1 α ,2 α -methylene-pregna-4,6-diene-3,20-dione-acetate)(LOT. NO. 481A). *Echinacea purpurea* dry extract was supplied by MEPACO (Arab Company for Pharmaceutical and Medicinal Plants), Egypt as capsules each containing 175 mg from dray extract.

Experimental design: Animals divided into five groups using random selection each of twelve rats treated oral daily as 1st Normal control (C) received 0.1 mL of 0.5 g/100 mL carboxymethyl cellulose, (CMC) for each 100 g b.wt. 2nd Echinacea treated group (E) received 63 mg kg^{-1} , 3rd Cyproterone acetate treated group (CA) received 25 mg kg^{-1} , 4th Echinacea+Cyproterone acetate treated group (E+CA) received 63 mg kg^{-1} (E) +

25 mg kg^{-1} (CA) and 5th Echinacea (week) + Cyproterone acetate treated group (E w + CA) received 63 mg kg^{-1} Echinacea for 1 week before 63 mg kg^{-1} (E) + 25 mg kg^{-1} (CA) treatment. All groups were treated daily for either two or four consecutive weeks from the beginning of the experiment. These doses used calculated equivalent to the human therapeutic dose (Reagan-Shaw *et al.*, 2008). The groups treated with E+CA received cyproterone acetate first followed by *E.* after 3.5 h as the peak plasma concentration of CA is being achieved in 3 to 4 h from the gastrointestinal tract (Sweetman, 2005). The animal experiments in relation to maintaining and handling animals were strictly conducted in accordance with the internationally agreed guidelines.

Fingerprints of Echinacea extract: Chromatographic identification of Echinacea caffeic acid derivatives: one capsule was extracted with methanol: water (80: 20; v/v) with 15 min ultra-sonication at room temperature. Caftaric, chlorogenic and chichoric acid in addition to echinacoside active ingredients of *Echinacea* extract (Fig. 1) was determined by HPLC, UV detector at 330 nm wavelength according to the method of Liu and Murphy (2007).

Echinacea extract by GC-MS analysis according to the method described in Song *et al.* (2000). Phytosterols identified by their fragmentation pattern while scanning the mass range m/z 50-700 and retention times and the representation of the analysis included in Fig. 2 (B1, B2 and B3).

Biochemical analysis: Body weight of the rats recorded weekly throughout the duration of the experiment. After two and four weeks of treatment, rats sacrificed 12 h following the last dose by rapid decapitation. Testis, epididymis and vas deferens dissected out, cleaned and weighed. Testis tissues were used for the measurement of

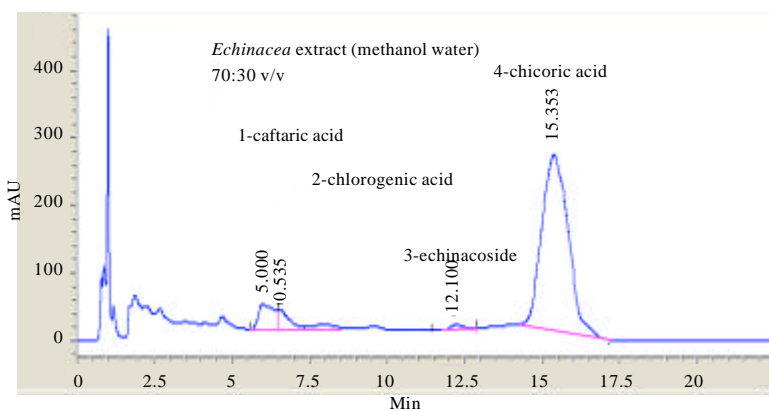


Fig. 1: HPLC chromatogram of *Echinacea purpurea* extracts active ingredients separation

enzyme activities of superoxide dismutase (SOD) according to Minami and Yoshikawa (1979) and Glutathione transferases (GST) according to the method of Habig *et al.* (1974) and Asmah *et al.* (1993). In addition to Malondialdehyde (MDA) was determined by HPLC according to the procedure of Karatas *et al.* (2002), nitric oxide (NO) (nitrites/nitrate) was determined according to the method of Papadoyannis *et al.* (1999) by HPLC and calcium ion was determined by colorimetric assay kit according to Ladenson (1980), Bradley and Schumann (1984) and Woo and Cannon (1984). Testicular sperm count was performed according to Blazak *et al.* (1993).

Statistical analysis: Statistical analysis was evaluated by one-way ANOVA. Once a significant F test was obtained, LSD comparisons were performed to assess the significance of differences among various treatment groups. Statistical Processor System Support SPSS for Windows software, Release 10.0 (SPSS, Chicago, IL) was used.

RESULTS

Figure 1 represents the HPLC analysis of the *Echinacea purpurea* extract and identified the active ingredients of the root extract which possesses the antioxidant potential. Figures 2a-c represent the gas chromatographic separation using the mass spectrum detector to identify the persistence of the vegetative sterols in the extract.

Testes weights in Table 1 showed significant decrement at $p < 0.05$ in CA, E+CA and E w+CA groups treated rats after two or four weeks in comparison with the corresponding control weights, while rats treated with E exhibited not statistically different decrease and increase in testes weights as compared to the control values throughout the experimental periods.

Testes/body weight ratio decreased significantly after 4 weeks of treatments in CA and E w+CA groups as compared to the control or the 2 weeks of treatment values. The ratio in E+CA groups decreased significantly throughout the 2 periods as compared to that of the

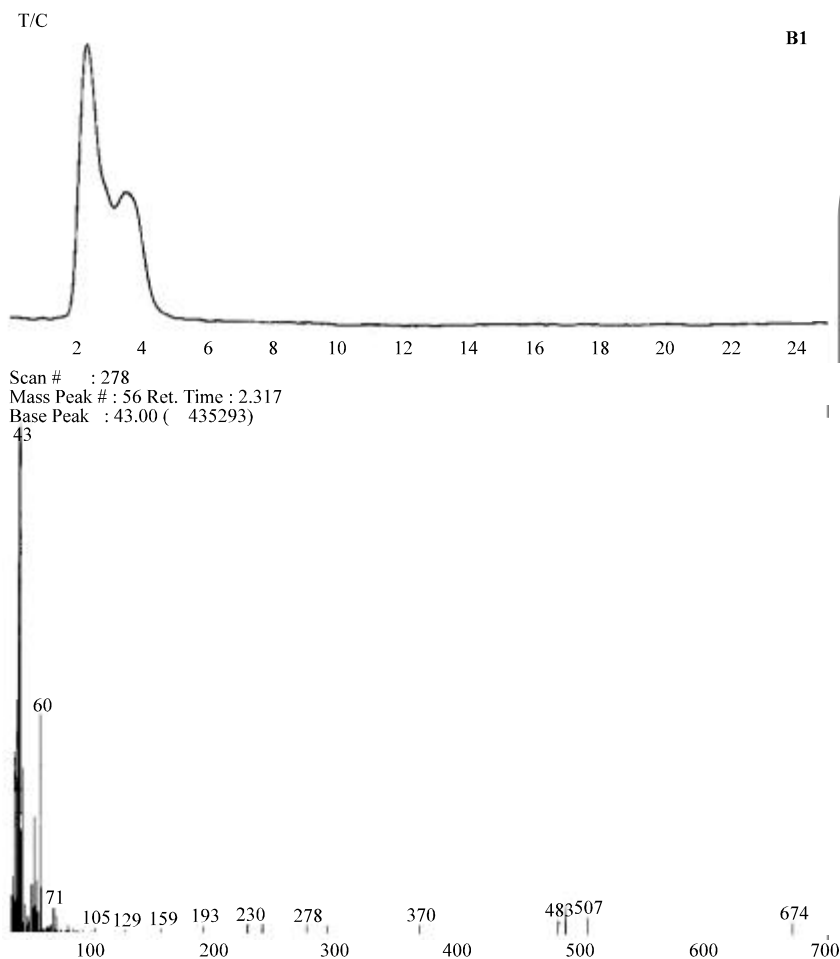
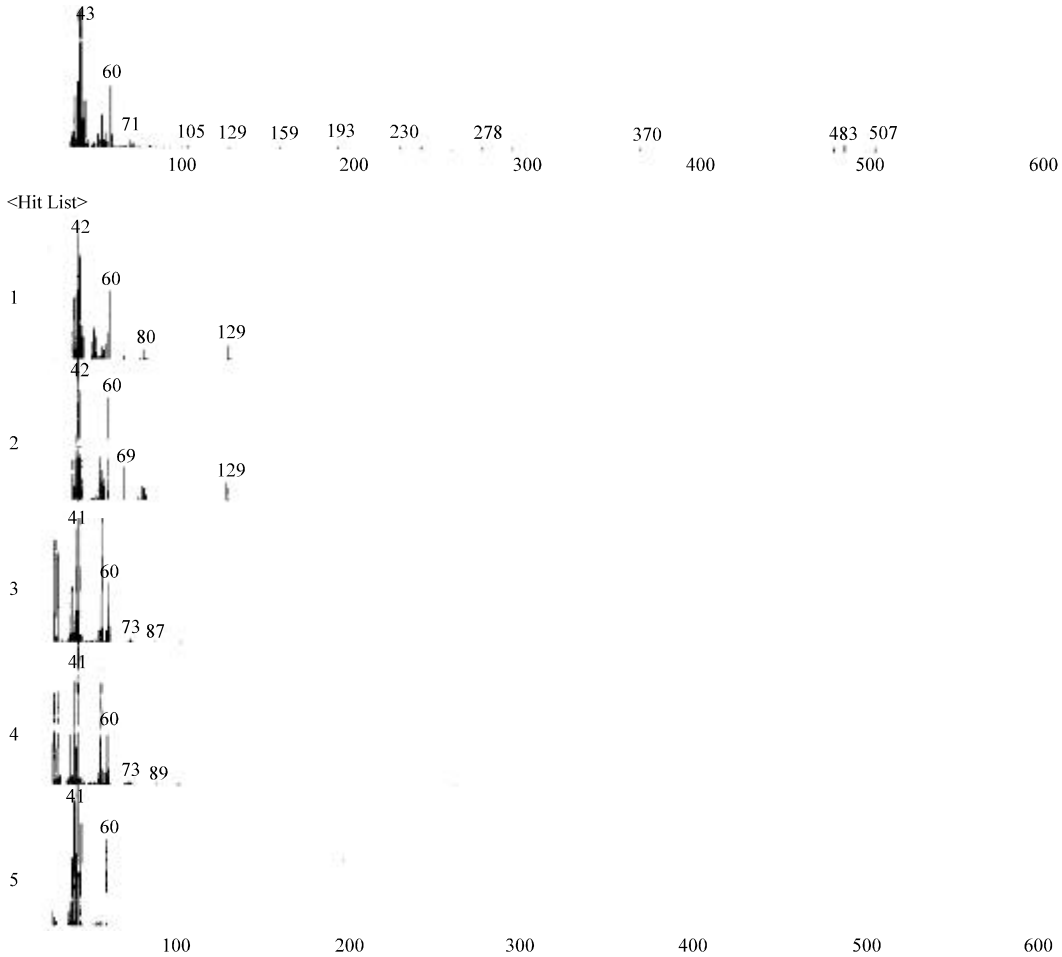


Fig. 2: Continued

Data : IMMULANT.D02
 Mass Peak # : 60 Ret. Time : 2.317
 Scan # : 278
 Base Peak : 43.00 (435293)

B2



No	SI	Mol. Wgt.	Mol. Form.	Compound Name	CAS No.	Entry	LIB#
1	82	129	C ₇ H ₁₃ N O	Isoxazolidine, 4-ethyl-2,5-dimethyl-, trans- (CAS) TRANS-2,5-DIMETHYL-4-ETHYLISOXAZOL	56728-13-3	15654	1
2	82	129	C ₇ H ₁₃ N O	Isoxazolidine, 5-ethyl-2,4-dimethyl-,trans- (CAS) TRANS-2,4-DIMETHYL-5-ETHYLISOXAZOL	56728-14-4	15656	1
3	81	102	C ₅ H ₁₀ N O ₂	Formic acid, 1-methylpropyl ester (CAS) sec-Butyl formate \$\$ 1-METHYLPROPYL FORMATE S	589-40-2	5985	1
4	81	102	C ₅ H ₁₀ N O ₂	Formic acid, 2-methylpropyl ester (CAS) Isobutyl formate \$\$ Tetryl formate \$\$ 2-Methy	542-55-2	5988	1
5	81	158	C ₂ H ₃ KN ₂ O ₄	POTASSIUM 1,1-DINITROETHANE \$\$	--0	33861	1

Library Name
 (1) WILEY229.LIB

Fig. 2: Continued

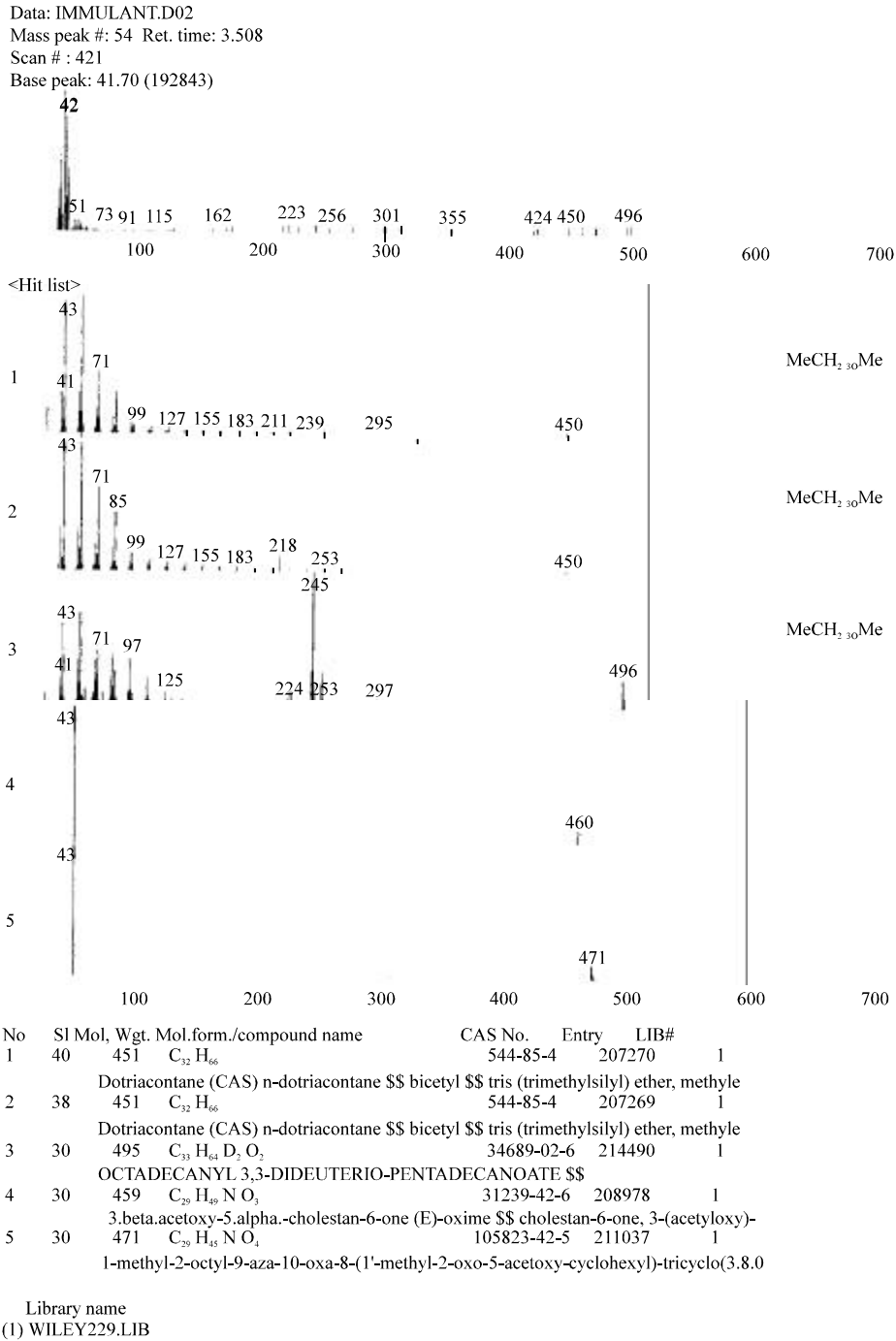


Fig. 2: (a-c): GC mass spectrums of Echinacea purpurea extract showing ingredients and identification of some library names

control. While in E treated groups in 4 weeks of treatment the ratio significantly increased in comparison with that of the 2 weeks of treatment without statistical difference comparing to control values.

The data depicted in Table 1 showed that epididymis weights decreased significantly at $p < 0.05$ in CA, E+CA

and E w+CA treated rats after two or four weeks in comparison with the corresponding control weights. In contrast, not statistically different decrease in epididymis weight in E treated rats after two weeks of treatment, followed by a significant decrease after the four weeks as compared with the corresponding control value.

A significant reduction in vas deferens weight reported in Table 1 in CA, E+CA and E w+CA treated groups of rats after two and four weeks of treatments in comparison with the corresponding control weights at the two periods. However rats treated with E showed a significant decrease after two weeks followed by not statistically different decrease in vas deferens weight after four weeks of treatment as compared with that of control.

The data in Table 2 revealed that CA treatment throughout the two experimental periods exhibited significant decrease in the testicular SOD and GST enzyme activities and sperm counts and calcium ions contents. In addition to the significant increase in the MDA and nitric oxide (nitrites/nitrates) concentrations as compared with the corresponding control or E treated groups' values.

Rats treated for two weeks in E groups showed significant increase in GST activity and nitric oxide values and significant decrease in sperm counts and calcium ions contents in comparison with the control values of the two week duration. While treatment with E for four weeks significantly affected the decrease in SOD and GST activities and the MDA, sperm counts and calcium ions contents comparing to four weeks control values.

E+CA treated group for two weeks showed significant decrease in the testicular SOD enzyme activity and sperm counts and calcium ions contents, moreover, significant increase in the MDA and nitric oxide levels as compared with their control values. E+CA treated group for four weeks exhibited significant decrease in the testicular GST enzyme activity and calcium ions contents and significant increase in the nitric oxide level comparing with the four weeks control values. E+CA treatment for four weeks significantly increased the testis SOD activity and the sperm count as compared to the corresponding CA treated group values. While the MDA and nitric oxide levels decreased in non-statistical difference as compared to that of the CA treated group values.

The groups of E w+CA treated rats showed no statistical difference in SOD activities and MDA and sperm count contents but the calcium contents decreased and nitric oxide increased significantly throughout the experimental periods comparing to the control values. Data showed significant increase in the SOD and GST activities and sperm counts values, while the MDA and nitric oxide contents decreased significantly as compared to the CA treated groups throughout the two experimental periods.

Table 1: Effect of *Echinacea* (E) and/or cyproterone acetate (CA) supplementation on the organs weights (g) of male rats

Parameter	Duration (weeks)	Groups				
		Control	E	CA	E+CA	E w+CA
Testes weight	2	2.05±0.07	1.87±0.12	1.59±0.04 ^{ab}	1.32±0.07 ^{bc}	1.70±0.13 ^{ad}
	4	2.11±0.12	2.13±0.11	0.95±0.10 ^{ab}	1.268±0.07 ^{abc}	1.38±0.04 ^{abc}
Testes/ b.wt. ratio	2	1.25±0.08	1.12±0.05	1.10±0.08	1.02±0.07 ^a	1.34±0.11 ^{bcd}
	4	1.19±0.08	1.35±0.07	0.68±0.08 ^{ab}	0.87±0.04 ^{ab}	0.97±0.02 ^{abc}
Epididymis	2	1.67±0.08	1.55±0.06	1.31±0.11 ^a	1.08±0.05 ^{ab}	1.41±0.03 ^{ad}
	4	1.90±0.14	1.50±0.10 ^a	0.94±0.05 ^{ab}	0.94±0.06 ^{ab}	1.38±0.11 ^{acd}
Vas deferens	2	0.15±0.02	0.09±0.01 ^a	0.08±0.01 ^a	0.06±0.01 ^a	0.06±0.004 ^a
	4	0.14±0.04	0.10±0.01	0.05±0.003 ^{ab}	0.06±0.01 ^a	0.071±0.01 ^a

The results are presented as Means±SE of 6 rats. a: Significant change from the corresponding control value, b: Significant change from the E group, c: Significant change from the CA group, d: Significant change from the E + CA treated group, t: Significant change between two and four weeks with the same treatment. Significance at 0.05 level

Table 2: Effect of *Echinacea* (E) and/or cyproterone acetate (CA) supplementation on testicular SOD and GST activities, MDA, calcium ions and nitric oxide contents and sperm count

Parameter	Duration (weeks)	Groups				
		Control	E	CA	E+CA	E w+CA
SOD (µg g ⁻¹ wet tissue)	2	318.42±3.19	301.71±27.96	218.10±11.15 ^{ab}	209.38±6.84 ^{ab}	279.27±10.79 ^{cd}
	4	316.41±5.12	269.51±2.85 ^a	211.57±14.29 ^{ab}	287.73±16.11 ^c	310.52±19.41 ^{bc}
GST (µmol min/mg wet tissue)	2	1.38±0.09	1.73±0.14 ^a	0.85±0.04 ^{ab}	1.44±0.08 ^{bc}	1.17±0.11 ^{bcd}
	4	1.38±0.05	1.11±0.05 ^a	0.84±0.06 ^{ab}	0.88±0.12 ^a	0.96±0.05 ^a
MDA (µmol g ⁻¹ wet tissue)	2	114.07±6.36	98.22±5.30	150.21±9.11 ^{ab}	146.53±7.33 ^{ab}	119.29±2.87 ^{bcd}
	4	112.86±5.61	85.85±4.02 ^a	143.15±1.78 ^{ab}	129.14±6.22 ^b	112.91±8.94 ^{bc}
Sperm count (10 ⁶ g ⁻¹ wet tissue)	2	19.76±1.14	16.64±0.57 ^a	11.56±0.44 ^{ab}	11.14±1.20 ^{ab}	19.63±1.20 ^{bcd}
	4	20.19±1.10	17.41±1.15 ^a	8.88±0.79 ^{ab}	16.68±0.86 ^{bc}	18.81±0.56 ^c
Ca ²⁺ (mg g ⁻¹ wet tissue)	2	0.65±0.01	0.59±0.01 ^a	0.51±0.02 ^{ab}	0.54±0.02 ^{ab}	0.58±0.02 ^{ac}
	4	0.66±0.02	0.56±0.01 ^a	0.52±0.02 ^{ab}	0.55±0.03 ^a	0.56±0.02 ^a
Nitric oxide (mg g ⁻¹ wet tissue)	2	5.54±0.20	6.73±0.15 ^a	10.27±0.38 ^{ab}	11.14±0.27 ^{abc}	7.11±0.15 ^{acd}
	4	5.37±0.12	5.80±0.21	11.12±0.34 ^{ab}	11.09±0.41 ^{ab}	8.35±0.26 ^{bcd}

The results are presented as Means±SE of 6 rats. a: Significant change from the corresponding control value, b: Significant change from the E group, c: Significant change from the CA group, d: Significant change from the E + CA treated group, t: Significant change between two and four weeks with the same treatment. Significance at 0.05 level

DISCUSSION

In the present study treatment of male rats with cyproterone acetate reduced the testes, testes-body weight ratio, epididymis and vas deferens weights during the experimental periods as expected according to the anti-androgenic action of this drug in rat (O'Connor *et al.*, 2002; Campos *et al.*, 2003) and in line with Aleem *et al.* (2005) results about CA treatment in a dose of 20 mg kg⁻¹ for 15 days which reduced the fertility and weights of accessory sex glands of the male rats.

The oxidative stress in testis was discernible in terms of perturbations in the activities of antioxidant enzymes SOD and GST and nitric oxide level. SOD protects against spontaneous O₂ toxicity and lipid peroxidation. The reduction of SOD suggests that it is involved in antioxidant defense and it has been shown to act as an alternate regulatory switch in testicular steroidogenesis (Pradeep *et al.*, 1990). It is evident, that SOD plays an important role in scavenging of reactive oxygen species (ROS) in testes, because in comparison to rat liver, the activity of catalase and glutathione peroxidase is much lower in the testicular tissue (Peltola *et al.*, 1992). The GST conjugates GSH with compounds containing an electrophilic center and thereby provides critical protection against products of oxidative stress. Since the GSH-conjugate is transported out of the cell, intracellular GSH is consumed irreversibly in the conjugation and thus the maintenance of intracellular GSH levels is essential for the optimal function of GST (Zhang *et al.*, 1998). On the other hand GST significant reduced activity in CA treated groups may be explained through the tissue degeneracy, as the enzyme activities in the animal systems upon exposure to carcinogenic chemical have evolved various defense mechanisms to protect themselves from the oxidative damage. As GSTs form a part of adaptive response of germ cells to oxidative stress and are important constituents in detoxifying the products of lipid peroxidation that inhibition of GST leads to the augmentation of lipid peroxidation and resulting in germ cell apoptosis (Rao and Shaha, 2000; Yang *et al.*, 2001).

The testicular tissue nitrate/nitrite, as an index of NO production and MDA levels were significantly elevated in rat testes in CA treated groups. The tissue NO increase in CA group may be attributed to the injury of testicular tissue in relation to abundant ROS production and consequent migration of macrophages and polymorphonuclear leucocytes to the region (Sikka, 2001). The significant increase in lipid peroxidation marker (MDA) enhanced ROS generation in testis after two and four weeks of treatment suggesting cyproterone acetate potential to induce significant oxidative stress in the

reproductive milieu of rats. The results are consistent with the possible role of reactive oxygen species for the genotoxicity previously suggested by Siddique and Afzal (2005)

NO radicals have been found to regulate multiple biological functions in inflammation and in mediating many cytotoxic and pathological events. NO has a bimodal effect on sperm motility whereby low concentrations of NO enhance sperm motility, whereas high concentrations of NO decrease it. This effect might be due to the dual nature of NO, which is a signal transduction molecule at low concentrations, while being cytotoxic at higher concentrations (Sikka, 2001; Sheweita *et al.*, 2005). In general imbalance in peroxidant and antioxidant status could produce oxidative stress (Ong *et al.*, 2002).

Also, the formation of free radicals and oxidative stress by CA was involved in the destruction of Sertoli cells and the azospermia occurred and this result occurred indirectly via the androgen deprivation effect of CA causing diminution of antioxidant detoxification (Tam *et al.*, 2003).

As regard to Echinacea treatment the data in this study showed not statistically different decrease in testes and vas deferens weights but significant decrease in epididymis weight after four weeks of treatment with Echinacea. Echinacea treatment for 2 to 4 weeks showed gradual antiandrogenic activity through the effect on male sexual hormone testosterone producing organ which may be associated with the vegetative sterols which are from the constituents of Echinacea extract (Skaudickas *et al.*, 2003; 2004). The chemical structure of these compounds is very similar to cholesterol. In the digestive duct vegetative sterols reduce the absorption of cholesterol, creating a certain competition between sterols and cholesterol thus dietary phytosterols may reduce the risk of prostate cancer by lowering the activities of the enzymes of testosterone metabolism (Awad and Fink, 2000; Trautwein and Demonty, 2007).

In Mishima *et al.* (2004) study, they assumed that SOD activity in peripheral blood was increased because of antioxidants such as echinacocide and caffeine acid in *Echinacea purpurea* (identified by HPLC) which eliminate superoxide (O⁻²) by a free radical scavenging effect. Pellati *et al.* (2004) indicate that E roots are a good source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects, capable of scavenging hydroxyl radicals, nitration inhibition and to suppress the oxidation of human low-density lipoprotein, particularly at the molecular level and also transition metal chelating (Hu and Kitts, 2000; Weiss and Landauer, 2003; Masteikova *et al.*, 2007).

These results are agreed with the results in this study as treatment with E caused a significant reduction in MDA content in testes and not statistically different change in SOD in the first two weeks. Weiss and Landauer (2003) documented a protective effect of polyphenols from E against free radical damage and a class of specific antioxidants known as caffeoyl derivatives in appreciable amounts. SOD remove (O^{-2}) generated by NADPH-oxidase in neutrophils and can play an important role in protecting spermatozoa during genitourinary inflammation (Baker *et al.*, 1996).

In the present results CA induced a significant decrease in sperm count and this reduction was improved by using treatment with E+CA. this improvement may be due to improvement of hemoglobin levels and number of erythrocytes by Echinacea treatment (Aboueilla *et al.*, 2007).

Cyproterone acetate treatment alone or with E induced a significant reduction in testicular NO. Lue *et al.* (2003) suggested NO physiological role in regulation of germ cell number and in determining testicular size. Concomitant treatment with CA + E improves but did not inhibit the significant reduction of testicular NO. Endothelial production of NO is at least partially regulated by the inositol trisphosphate (IP3)-dependent calcium (Ca^{2+}) signalling pathway, which may be activated by ligand-receptor binding and/or wall shear stress. The elevated levels of free Ca^{2+} in the cytosol reversibly bind with calmodulin; the resulting Ca^{2+} -calmodulin complexes activate endothelial nitric oxide synthase enzyme, thus causing an increase in synthesis and release of NO. In addition, NO leads to increased levels of cyclic guanosine monophosphate (cGMP), this activates protein kinase. This is thought to inhibit Ca^{2+} influx, thus providing a negative feedback mechanism and limiting the concentration of Ca^{2+} in the cytosol. (Shen *et al.*, 1992; Lin *et al.*, 2000; Plank *et al.*, 2007). Ca^{2+} can directly activate antioxidant enzymes, increase the level of SOD in animal cells (Gordeeva *et al.*, 2003) and induce mitochondrial GSH release (Brookes *et al.*, 2004). The result in this study in the same trend, there is a significant reduction in testicular SOD and intra cellular testicular Ca ions. Yan *et al.* (2006) studies indicated that Ca^{2+} plays dual roles in regulating ROS homeostasis.

The net Ca^{2+} effects on ROS generation and annihilation appear to be tissue-specific and context-sensitive, and, within a given cell, are differentially regulated in local subcellular compartments. These results give an explanation for intracellular testicular calcium ion reduction. Also, Lyng *et al.* (2000) suggested that the calcium signals are probably coupled to the regulation of gap junctional efficiency between Sertoli cells.

The low-affinity receptors may convey complementary androgen signals at elevated local levels such as in the testis, when nuclear receptors are (over) saturated. Androgens can also induce rapid calcium fluxes in a variety of cell types, including human prostatic cancer cells and rat sertoli cells (Steinsapir *et al.*, 1991; Gorczynska and Handelsman, 1995; Gorczynska-Fjalling, 2004). This suggestion explains the effect of CA treatment on decreasing the testicular Ca ion content.

The study suggests that *Echinacea* supplementation especially a week before cyproterone acetate shows improvement in the oxidative stress induced by cyproterone acetate treatment. That may be due to the *Echinacea* antioxidant activity. The anti-androgenic effect of E may in addition to the anti-inflammatory and immune enhancing activity add valuable for using it as a co-treatment for male contraceptives or in treatment of prostate cancer which needs more investigations.

ACKNOWLEDGMENTS

The author gratefully acknowledge the help of NODCAR (The national organization for drug control and research), Department of Physiology, for facilities in the HPLC analysis. As regard to conflicts of interest, the author declares that there is no conflict of interest associated with this study. And the Author contributed to the design of the study, elaboration of bench work and analytical procedures in addition to interpretation of the data, writing the manuscript.

REFERENCES

- Aboueilla, A.M.K., Y.E. Shahein, S.S. Tawfik and A.M. Zahran, 2007. Phytotherapeutic effects of *Echinacea pupurea* in gamma-irradiated mice. *J. Vet. Sci.*, 8: 341-351.
- Aleem, M., V. Padwal, J. Choudhari, N. Balasinor, P. Parte and M. Gill-Sharma, 2005. Cyproterone acetate affects protamine gene expression in the testis of adult male rat. *Contraception*, 71: 379-391.
- Asmah, R., W.Z. Wan-Ngah, A. Gapor and B.A.K. Khalid, 1993. Long-term tocotrienol supplementation and glutathione-dependent enzymes during hepatocarcinogenesis in the rat. *Asia Pacific. J. Clin. Nutr.*, 2: 129-134.
- Awad, A.B. and C.S. Fink, 2000. Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J. Nutr.*, 130: 2127-2130.
- Baker, H.W.G., J. Brindle, D.S. Irvine and R.J. Aitken, 1996. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes *Fertil. Steril.*, 65: 411-419.

- Barradell, L.B. and D. Faulds, 1994. Cyproterone. A review of its pharmacology and therapeutic efficacy in prostate cancer. *Drugs Aging*, 5: 59-80.
- Barrett, B., R. Brown, R. Volland, R. Maberry and R. Turner, 2006. Relations among questionnaire and laboratory measures of rhinovirus infection. *Eur. Respir. J.*, 28: 358-363.
- Blazak, W.F., K.A. Treinen and P.E. Juniewicz, 1993. Application of Testicular Sperm Head Counts in the Assessment of Male Reproductive Toxicity. In: *Male Reproductive Toxicology*, Chapin, R.E. and J.J. Heindel (Eds.). Vol. 3A. Academic Press, New York, pp: 86-94.
- Block, K.I., D.B. Boyd, N. Gonzalez and A. Vojdani, 2002. Point-counterpoint: The immune system in cancer. *Integr. Cancer Ther.*, 1: 294-316.
- Bradley, M. and G.B. Schumann, 1984. Examination of Urine. In: *Clinical Diagnosis and Management by Laboratory Methods*, Henry, J.B. (Ed.). 17th Edn., W.B. Saunders, Philadelphia, pp: 380.
- Brookes, P.S., Y. Yoon, J.L. Robotham, M.W. Anders and S.S. Sheu, 2004. Calcium, ATP and ROS: A mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.*, 87: 817-833.
- Campos, M., P.L. Morais and A.S. Pupo, 2003. Effect of cyproterone acetate on alpha1-adrenoceptor subtypes in rat vas deferens. *Braz. J. Med. Biol. Res.*, 36: 1571-1581.
- Gorczyńska, E. and D.J. Handelsman, 1995. Androgens rapidly increase the cystolic calcium concentration in Sertoli cells. *Endocrinology*, 136: 2052-2059.
- Gorczyńska-Fjalling, E., 2004. The role of calcium in signal transduction processes in Sertoli cells. *Reprod. Biol.*, 4: 219-241.
- Gordeeva, A.V., R.A. Zvyagil'skaya and Y.A. Labas, 2003. Cross-talk between reactive oxygen species and calcium in living cells. *Biochem. (Moscow)*, 68: 1077-1080.
- 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.
- Honer, C., K. Nam, C. Fink, P. Marshall and G. Ksander *et al.*, 2003. Glucocorticoid receptor antagonism by cyproterone acetate and RU486. *Moll. Pharmacol.*, 63: 1012-1020.
- Hu, C. and D.D. Kitts, 2000. Studies on the antioxidant activity of Echinacea root extract. *J. Agric. Food Chem.*, 48: 1466-1472.
- Juan, M.E., E. Gonzalez-Pons, T. Munuera, J. Ballester, J.E. Rodriguez-Gil and J.M. Planas, 2005. Trans-Resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J. Nutr.*, 135: 757-760.
- Karatas, F., M. Karatepe and A. Baysar, 2002. Determination of free malondialdehyde in human serum by high performance liquid chromatography. *Anal. Biochem.*, 311: 76-79.
- Kasper, P., 2001. Cyproterone acetate: A genotoxic carcinogen. *Pharmacol. Toxicol.*, 88: 223-231.
- Keskes-Ammar, L., N. Feki-Chakroun, T. Rebai, Z. Sahnoun and H. Ghazzi *et al.*, 2003. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch. Androl.*, 49: 83-94.
- Koksal, T., T. Erdogru, B. Toptas, K.H. Gulkesen, M. Usta, A. Baykal and M. Baykara, 2002. Effect of experimental varicocele in rats on testicular oxidative stress status. *Andrologia*, 34: 242-247.
- Ladenson, J.H., 1980. Clinical Chemistry of Disorders of Mineral Homeostasis. In: *Clinical Laboratory Methods and Diagnosis*, Sonnenwirth, A.C. and L. Jarrett (Eds.). 8th Edn., CV Mosby Company, St. Louis (MO), pp: 337.
- Letchamo, W., J. Livesey, T.J. Arnason, C. Bergeron and V.S. Krutilina, 1999. Cichoric Acid and Isobutylamide Content in Echinacea Purpurea as Influenced by Flower Developmental Stages. In: *Perspectives on New Crops and New Uses*, Janick, J. (Ed.). ASHS Press, Alexandria, VA., pp: 494-498.
- Lin, S., K.A. Fagan, K.X. Li, P.W. Shaul, D.M.F. Cooper and D.M. Rodman, 2000. Sustained endothelial nitric-oxide synthase activation requires capacitative Ca²⁺ entry. *J. Biol. Chem.*, 275: 17979-17985.
- Liu, Y. and P.A. Murphy, 2007. Alkamide stability in echinacea purpurea extracts with and without phenolic acids in dry films and in solution. *J. Agric. Food Chem.*, 55: 120-126.
- Lue, Y., A.P. Sinha Hikim, C. Wang, A. Leung and R.S. Swerdloff, 2003. Functional role of inducible nitric oxide synthase in the induction of male germ cell apoptosis, regulation of sperm number and determination of testes size: Evidence from null mutant mice. *Endocrinology*, 144: 3092-3100.
- Lyng, F.M., G.R. Jones and F.F. Rommerts, 2000. Rapid androgen actions on calcium signaling in rat sertoli cells and two human prostatic cell lines: Similar biphasic responses between 1 picomolar and 100 nanomolar concentrations. *Biol. Reprod.*, 63: 736-747.
- Masteikova, R., J. Muselik, J. Bernatoniene and R. Bernatoniene, 2007. Antioxidative activity of *Ginkgo*, *Echinacea* and *Ginseng* tinctures. *Medicina (Kaunas)*, 43: 306-309.
- McLeod, D.G., 1993. Antiandrogenic drugs. *Cancer*, 71: 1046-1049.

- Meriggiola, M.C., W.J. Bremner, C.A. Paulsen, A. Valdiserri and L. Incorvaia *et al.*, 1996. A combined regimen of cyproterone acetate and testosterone enanthate as a potentially highly effective male contraceptive. *J. Clin. Endocrinol. Metab.*, 81: 3018-3023.
- Minami, M. and H. Yoshikawa, 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clin. Chim. Acta*, 92: 337-342.
- Mishima, S., K. Saito, H. Maruyama, M. Inoue, T. Yamashita, T. Ishida and Y. Gu, 2004. Antioxidant and immuno-enhancing effects of *Echinacea purpurea*. *Biol. Pharm. Bull.*, 27: 1004-1009.
- Neumann, F., 1994. The antiandrogen cyproterone acetate: Discovery, chemistry, basic pharmacology, clinical use and tool in basic research. *Exp. Clin. Endocrinol.*, 102: 1-32.
- Ochsendorf, F.R., 1999. Infections in the male genital tract and reactive oxygen species. *Human Reprod. Update*, 5: 399-420.
- Ong, C.N., H.M. Shen and S.E. Chia, 2002. Biomarkers for male reproductive health hazards: Are they available?. *Toxicol. Lett.*, 134: 17-30.
- Orozco, T.J., J.F. Wang and C.L. Keen, 2003. Chronic consumption of a flavanol- and procyanidin-rich diet is associated with reduced levels of 8-hydroxy-2-deoxyguanosine in rat testes. *J. Nutr. Biochem.*, 14: 104-110.
- O'Connor, J.C., S.R. Frame and G.S. Ladics, 2002. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicol. Sci.*, 69: 92-108.
- Papadoyannis, L.N., V.F. Samanidou and C.C. Nitsos, 1999. Simultaneous determination of nitrite and nitrate in drinking water and human serum by high performance anion-exchange chromatography and UV detection. *J. Liq. Chrom. Rel. Technol.*, 22: 2023-2041.
- Pellati, F., S. Benvenuti, L. Magro, M. Melegari and F. Soragni, 2004. Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. *J. Pharm. Biomed. Anal.*, 35: 289-301.
- Pellati, F., S. Benvenuti, L. Magro and T. Lasseigne, 2005. Variability in the composition of antioxidant compounds in *Echinacea* species by HPLC. *Phytochem. Anal.*, 16: 77-85.
- Peltola, V., I. Huhtaniemi and M. Ahotupa, 1992. Antioxidant enzyme activity in the maturing rat testis. *J. Androl.*, 13: 450-455.
- Plank, M.J., D.J.N. Wall and T. David, 2007. The role of endothelial calcium and nitric oxide in the localisation of atherosclerosis. *Mathematical Biosci.*, 207: 26-39.
- Pradeep, K.G., N. Seerwami, M. Laloraya, M. Nivsarkar, S. Verma and A. Singh, 1990. Superoxide dismutase as a regulatory switch in mammalian testicular steroidogenesis. *Biochem. Biophys. Res. Commun.*, 173: 302-308.
- Rao, A.V. and C. Shaha, 2000. Role of glutathione S-transferases in oxidative stress-induced male germ cell apoptosis. *Free Radical Biol. Med.*, 29: 1015-1027.
- Reagan-Shaw, S., M. Nihal and N. Ahmad, 2008. Dose translation from animal to human studies revisited. *FASEB J.*, 22: 659-661.
- Shah, S.A., S. Sander, C.M. White, M. Rinaldi and C.I. Coleman, 2007. Evaluation of echinacea for the prevention and treatment of the common cold: A meta-analysis. *Lancet Infectious Dis.*, 7: 473-480.
- Shen, J., F.W. Luscinikas, A. Connolly, C.F. Dewey and M.A. Gimbrone, 1992. Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. *Am. J. Physiol.*, 262: 384-390.
- Sheweita, S.A., A.M. Tilmisany and H. Al-Sawaf, 2005. Mechanisms of male infertility: Role of antioxidants. *Curr. Drug Metab.*, 6: 495-501.
- Siddique, Y.H. and M. Afzal, 2005. Genotoxic potential of cyproterone acetate: A possible role of reactive oxygen species. *Toxicol. In vitro*, 19: 63-68.
- Siddique, Y.H., G. Ara, T. Beg, M. Faisal, M. Ahmad and M. Afzal, 2008. Antigenotoxic role of *Centella asiatica* L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. *Toxicol. In vitro*, 22: 10-17.
- Sikka, S.C., 2001. Relative impact of oxidative stress on male reproductive function. *Curr. Med. Chem.*, 8: 851-862.
- Simasek, M. and D.A. Blandino, 2007. Treatment of the common cold. *Am. Fam. Physician.*, 75: 515-520.
- Skaudickas, D., A.J. Kondrotas, K. Baltrusaitis and G. Vaitiekaitis, 2003. Effect of *Echinacea Purpurea* L. Moench preparations on experimental prostate gland. *Medicina*, 39: 761-766.
- Skaudickas, D., A. Kondrotas and K. Baltrusaitis, 2004. The effect of *Echinacea purpurea* extract on sexual glands of male rats. *Medicina (Kaunas)*, 40: 1211-1218.
- Song, Y.S., C. Jin and E.H. Park, 2000. Identification of phytosterols in rat feces using GC/MS. *Arch. Pharm. Res.*, 23: 599-604.
- Steinsapir, J., R. Socci and P. Reinach, 1991. Effects of androgen on intercellular calcium of LNCaP cells. *Biochem. Biophys. Res. Commun.*, 179: 90-96.
- Suleiman, S.A., M.E. Ali, Z.M. Zaki, E.M. El-Malik and M.A. Nasr, 1996. Lipid peroxidation and human sperm motility: Protective role of vitamin E. *J. Androl.*, 17: 530-537.

- Sweetman, S.C., 2005. Martindale, The Complete Drug Reference. 34th Edn., Pharmaceutical Press, Chicago, London, pp: 1544.
- Tam, N.N., Y. Gao, Y.K. Leung and S.M. Ho, 2003. Androgenic regulation of oxidative stress in the rat prostate: Involvement of NAD(P)H oxidase and antioxidant defense machinery during prostatic involution and regrowth. *Am. J. Pathol.*, 163: 2513-2522.
- Torri, V. and I. Floriani, 2005. Cyproterone acetate in the therapy of prostatic carcinoma. *Arch. Ital. Urol. Androl.*, 77: 157-163.
- Trautwein, E.A. and I. Demonty, 2007. Phytosterols: Natural compounds with established and emerging health benefits. *Oléagineux Corps Gras Lipides*, 14: 259-266.
- Turner, T.T. and J.J. Lysiak, 2008. Oxidative stress: A common factor in testicular dysfunction. *J. Androl.*, 29: 488-498.
- Weiss, J.F. and M.R. Landauer, 2003. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicology*, 189: 1-20.
- Woo, J. and D.C. Cannon, 1984. Metabolic Intermediates and Inorganic Ions. In: *Clinical Diagnosis and Management by Laboratory Methods*, Henry, J.B. (Ed.). 17th Edn., W.B. Saunders, McPherson, RA., Philadelphia, pp: 133.
- Yan, Y., C.L. Wei, W.R. Zhang, H.P. Cheng and J. Liu, 2006. Cross-talk between calcium and reactive oxygen species signaling. *Acta Pharmacol. Sin.*, 27: 821-826.
- Yang, Y., J.Z. Cheng, S.S. Singhal, M. Saini, U. Pandya, S. Awasthi and Y.C. Awasthi, 2001. Role of glutathione S-transferases in protection against lipid peroxidation: Overexpression of hGSTA2-2 IN K562 cells protects against hydrogen peroxide-induced apoptosis and inhibits Jnk and caspase 3 activation. *J. Biol. Chem.*, 276: 19220-19230.
- Zhang, K., P. Mack and K.P. Wong, 1998. Glutathione-related mechanisms in cellular resistance to anticancer drugs. *Int. J. Oncol.*, 12: 871-882.