Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Research Paper

Phytopreventive effect of *Salvia officinalis* L. on infertiliy induced by hypothyroidism in male albino rats

Biomedical

KEYWORDS : Phytopreventive , Salvia officinalis , infertiliy , hypothyroidism , rats

Hoda I. Bahr — Departments of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Abdelaziz E. Ibrahim — Departments of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

ABSTRACT

Overt hypothyroidism frequently affects male reproductive and fertility. Hypothyroidism claimed to increased ROS production with subsequent complications

The scope of this work is to highlights the mechanism of hypothyroidism induced production of ROS on male reproductive system and enumerate the benefits of SSalvia officinalis L. (S. officinalis) as an antioxidant in clinical and experimental settings. 30 male albino rats were equally divided into 3 groups (n=10 rats). Group 1 (control) have free access to water and food materials. Group II rendered hypothyroid by giving 0.1% (w/v) of propyl thiouracil (PTU) in drinking water, for 65 days. Group III rendered hypothyroid by giving 0.1% (w/v) of propyl thiouracil (PTU) in drinking water for 65 days in combination with Salvia officinalis L.extract in drinking water. The results indicated that hypothyroidism when compared with the control group significantly increase reproductive organs weight (testis, prostate and seminal vesicle glands), decrease sperm cell count, decrease sperm motility (%), and increase in dead and abnormal sperm count. also, hypothyroidism increase serum TSH with reduction in T3, T4 and testosterone. The reduced testicular GSH was accompanied with elevation in MDA level and the percentage of DNA fragmentation. Oral administration of sage extract significantly restored all parameters toward the normal values. In conclusion, this observation points to S. officinalis tea drinking improving the reproductive potency and fertility through antioxidant and thyroid regulating properties.

Introduction

Infertility is one of the major health problems in life, and approximately 30 % of this problem is due to male factors (1). Apart from the conventional causes for male infertility such as varicocoele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma, and tumors, a new and important cause has been identified: oxidative stress. Oxidative stress is a result of the imbalance between reactive oxygen species (ROS) and antioxidants in the body. It is a powerful mechanism that can lead to sperm damage, deformity by adversely affecting the quality of sperm DNA and eventually, male infertility (2).

Endocrine system is the second key regulator of organ system functions after nervous system in human body. Hormones are actual messengers in endocrine signaling. Thyroid gland holds a critical place in controlling brain and somatic development in infants and metabolic activities in adults. Upon stimulation by thyroid stimulating hormone (TSH), thyroid gland secretes thyroid hormones: triiodothyronine (T3) and thyroxin (T4). Although thyroid hormones have a central role in controlling basal metabolic rate, growth, as well as the development and differentiation of many cells in the body Thyroid gland holds a critical place in controlling brain and somatic development in infants and metabolic activities in adults. Upon stimulation by thyroid stimulating hormone (TSH), thyroid gland secretes thyroid hormones: triiodothyronine (T3) and thyroxin (T4). Although thyroid hormones have a central role in controlling basal metabolic rate, growth, as well as the development and differentiation of many cells in the body (3). Until very recent thyroid was thought not to affect spermatogenesis; however, research is now actively being pursued to understand the primary effects of thyroid hormones on spermatogenesis.

Spermatogenesis is generally divided into three distinct stages: (i) mitosis of spermatgonia (ii) meiosis to make haploid germ cells (iii) maturation of spermatids to spermatozoa. Disturbance at any step could affect the process of spermatogenesis and the spermatozoa may become defective (4). Spermatogonia give rise to mature spermatozoa under hormonal control of the gonadotropins such as luteinizing hormone (LH) and follicle stimulating hormone (FSH). Recent identification of thyroid hormone receptors (TRs) directly on the testes and finding that Thyroid hormone acts directly on Sertoli cells to inhibit proliferation while stimulating differentiation and maturation of the male testes (5-6).

LH and FSH are produced by the anterior pituitary. The production of these two hormones is stimulated by gonadotropin releasing hormone (GnRH) made by the hypothalamus.Testosterone is produced by the Leydig cells after receiving the signal from luteinizing hormone (LH) for its synthesis. This pathway is collectively known as the hypothalamus-pituitary-gonadal axis (HPG axis). Testosterone is required for successful completion of the spermatogenesis process. Without it, conversion of round spermatids to spermatozoa during spermiogenesis is impaired (7).

In cases of thyroid dysfunction, an increment of GNs levels in thyrotoxicosis and its decline in hypothyroidism (8).This decline is related to prolonged hypothyroidim induced pituitay hypogonadism. In men with hypothyroidism total androgen level is reduced due to multiple factors such as decreased SHBG, hypothyroidism induced pituitary hypogonadism and hyperprolactinemia (9). Men with post-pubertal hypothyroidism may experience a decreased libido, erectile dysfunction, and delayed ejaculation (10).

Free radicals causes reduction in GSH contents and alteration of reproductive hormones, oxidative DNA damages, genetic mutation, DNA strand breakage and chromosomal alterations (11-12), necrosis of spermatocytes/spermatids and degeneration in seminiferous tubules (13-14).

Medicinal plants are in high demand for application of functional food or biopharmaceuticals because of consumer preferences. Currently various medicinal plants has been investigated like Salvia officinalis L. (S. officinalis, common sage) which is a medicinal plant well known for its reputation of being a panacea and for its strong antioxidant properties attributed to its constitution in phenolic compounds (rosmarinic acid being the most representative) (15-16). Salvia extract has a prominent antioxidant effects, so it is conceivable that can improve male reproductive function (17). Administration of Salvia extracts caused to increased serum testosterone level and is effective on certain biochemical enzymes (18), by which can pose its effects on reproductive system.

The improving effects of Salvia on male reproductive system may come from the effects of Salvia components - in particular vi-

tamins C and E, flavonoids and antioxidants that can enhance Leydig cells normal function (19).

In a previous study, sage tea drinking significantly increased (rat and mouse) liver GST activity and protected against GSH depletion and lipid peroxidation induced by an oxidant agent (20).

Therefore the present study was arranged to evaluate the trational use of sage tea versus hypothyroidism induced oxidative stress associated reproductive hormonal changes and lipids peroxidation in rats.

Material and methods

1-Expermental materials:

1.1- Rats

30 adult male albino rats weighting 150-200 g were obtained from Animal House in Faculty of Vet. Medicine, Zagazig University. They were housed in separate well-ventilated cages, under standard conditions, with free access to the standard diet and water ad libitum. The experiment was conducted at the Animal house of Faculty of Veterinary Medicine, Suez canal University. The experiment was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (21).

2.1- Drugs

Thyrocil (Propyl thiouracil, 50mg) was used for hypothyroidism induction. It was obtained from Amoun pharmaceutical Co., Cairo, Egypt.

3.1-Plant

Salvia officinalis L. (common sage) was obtained from local market of Herbs and Medicinal plants, Egypt. Sage is used as a tea, an infusion of sage was routinely prepared by pouring 150 ml of boiling water onto 2 g of the dried plant material and allowing to steep for 5 min. Then filtered by Capron silica cloth 150 μ and the filtrate could be used as sage tea (20).

This preparation produced a 3.5 ± 0.1 mg of dry weight extract per ml of infusion, with rosmarinic acid (362 lg/ml of infusion) and luteolin-7glucoside (115.3 lg/ml of infusion) as a major phenolic compounds and 1,8-cineole, cis-thujone, trans-thujone, camphor and borneol as major volatile compounds (4.8 lg/ml of infusion)

***Salvia officinalis* L.**

4.1-Kits

a- ELISA Kit- MyBioSource for estimation of Mouse/Rat Triiodothyronine (T3) and Thyroxine (T4).

b- ELISA- ALPCO immunoassays for estimation of Mouse/Rat Thyroid Stimulating Hormone (TSH) and Testosterone.

c-Reduced GSH kits was obtained from Bio-diagnostic Co., Egypt.

2-Experimental design

To study the antioxidant attributes of Sage, male albino rats were equally divided into 3 groups (n=10 rats). Group 1 (control) have free access to water and food materials. Group II rendered hypothyroid by giving 0.1% (w/v) of 6-n-propyl-2-thiouracil (PTU) in drinking water (22), for 65 days.

Group III rendered hypothyroid by giving 0.1% (w/v) of 6-n-propyl-2-thiouracil (PTU) in drinking water for 65 days in combination with sage tea in drinking water.

After 65 days (spermatogenesis period) of the last treatment, blood samples were collected for serum separation. Then the testes, prostate, seminal vesicle glands and epididymis from the rats were carefully dissected and weighed independently. From the epididymis, sperm were collected, mounted on a slide and their motility assessed immediately under the microscope at × 10 objective. The motility assessment was expressed as percentage motile forms. The slides were later stained with Carbol Fuschin and the sperm number and morphology were examined. After the process one testis of each rat was treated with liquid nitrogen for further enzymatic and DNA damage analysis.

2.1- Hormonal analysis

TSH, T3, T4 and testosterone hormone level were measured in serum using ELIZA kits.

2.2-Assessment of lipid peroxidation (MDA) and reduced glutathione assay.

1gm of tissue was homogenized in 5 volume of homogenized in phosphate buffer saline (pH7.4) and centrifuged at 3000 r.p.m for 30 min at 4°C. The supernatant was collected and used for assessment of malondialdhyde (MDA) and reduced glutathione (GSH) levels according to (23) and (24) respectively.

2.3- Evaluation of cell death by DPA assay

DNA fragmentation was used as indicator for cell death using DPA assay. The latter was conducted using the procedure of (27).

2.4- Semen analysis

Epididymal contents of the treated rats were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined according to the method described by (25). Microscopic examinations of the seminal smears stained with Eosin Nigrosin stain were carried out to determine the percentages of sperm viability (ratio of alive/dead) and sperm cell abnormality according to (26).

2.5. Statistical Analysis

Results are expressed as mean ± S.E. Data were analyzed using one-way analysis of variance (ANOVA). All statistical tests were done by using (SPSS Software, version 22, SPSS Inc., Chicago, USA) and the differences were considered significant at P< 0.05.

Results

Table (1): Effect of oral administration of sage extracts for 65 days on the weight of sexual organs of male hypothyroid rats.

Group	Weight of sexual organs (Mean ± S.E.)		
	testis	Prostate gland	Seminal vesicle gland
Normal control	2.52 ± 0.09	0.32 ±0.02	0.89 ± 0.04
Hypothyroid	3.94 ±0.17*	0.57 ±0.03*	1.80 ± 0.11*
Sage extract	3.06 ± 0.18*#	0.40 ±0.01*#	1.42 ± 0.10*#

Data having different superscript are significant at p< 0.05.

Table (2): Effect of oral administration of sage extracts for 65 days on sperm cell characteristics of male hypothyroid rats.

Group	Sperm cell characteristics (Mean ± S.E.)			
	Motility (%)	Count (10^6/epididymis)	Viability (%)	Abnormality (%)
Normal control	89.5±1.82	89.50 ± 2.35	90.30 ±2.09	2.27±0.12

Hypothyroid	27.5±1.85*	51.50 ±1.93*	32.20 ±2.05*	8.05±0.32*
Sage extract	63.30±2.22*#	68.20 ±1.69*#	75.70 ±1.98*#	4.28±0.27*#

Group	Hormones (Mean ± S.E.)			
	TSH (ng/ml)	T3 (ng/dl)	T4 (µg/dl)	Testosterone (ng/ml)
Normal control	1.89 ± 0.08	129.16 ± 2.57	5.77 ± 0.33	2.54 ± 0.22
Hypothyroid	4.35 ± 0.21*	75.59 ± 3.35*	1.60 ± 0.11*	0.54 ± 0.50*
Sage extract	2.66 ± 0.19*#	107.82 ± 2.11*#	2.61 ± 0.14*#	1.76 ± 0.40*#

Table (3): Effect of oral administration of extracts of sage for 65 days on TSH (ng/ml), T3 (µg/ml), T4 (µg/dl) and testosterone hormone (ng/ml) of male hypothyroid rats.

Table (4): Effect of oral administration of extracts of sage for 65 days on testicular oxidative marker; reduced GSH content (mg/gm tissue), MDA level (µmol/ gm tissue) and the percentage of DNA fragmentation (%) of male hypothyroid rats.

Group	Oxidative marker (Mean ± S.E.)		
	reduced GSH (mg/gm tissue)	MDA level (µmol/ gm tissue)	DNA fragmentation (%)
Normal control	49.98 ±1.49	1.57 ± 0.09	1.85 ± 0.13
Hypothyroid	22.62 ±1.17*	4.57 ±0.16*	10.35 ± 0.52*
Sage extract	38.75 ± 0.50*#	2.82 ±0.17*#	4.82 ± 0.25*#

Hypothyroidism significantly increased testis, prostate and seminal vesicle glands weights compared to control group, while sage extracts Post treatment for 65 days significantly restored the weight table (1).

There was a marked reduction in sperm count, motility (%), with increase in dead and abnormal sperm count in hypothyroidism group as compared to control group which was significantly restored with Sage table (2).

Data in Table (3) show that induction of hypothyroidism in rats induced a significant increase in serum TSH with reduction in T3, T4 and testosterone versus to the normal control rats. Oral administration of sage extract significantly restored these hormonal alterations.

Hypothyroidism caused reduction in reduced GSH content that accompanied with elevation in MDA level and the percentage of testicular DNA fragmentation when compared to the control group, all these parameters were directed toward the normal values after oral administration of sage extract Table (4).

Discussion

Hypothyroidism is a clinical syndrome caused due to deficiency of thyroid hormones, characterized by decrease in serum T3 and T4 and increase in serum TSH concentration, and accompanied by increased ROS production and reproductive alterations.

Recently many investigations are now actively being pursued to understand the primary effects of thyroid hormones on male reproductive and fertility. The current study was carried out to determine the effect of extracts of Sage on fertility of male hypothyroid rats following oral administration for 65 days (period of spermatogenesis in the rat).

Our work depicts that, hypothyroidism significantly increased testis, prostate and seminal vesicle glands weights compared to control group, table (1). These finding maybe responsive to reduced thyroid hormones in a dose-dependent manner (28).

Concerning the elevation in testicular weight, (30-31) demonstrated that, hypothyroidism may result in a decrease in the sex hormone binding globulin (SHBG) levels and a decrease in total serum testosterone levels, as well as a decrease in the gonadotropins levels, specifically the luteinizing hormone (LH) and the follicle stimulating hormone (FSH) (29). In cases of prolonged pre-pubertal hypothyroidism due to drop in LH and FSH levels, the Leydig and Sertoli cells, respectively are less stimulated to differentiate into mature cells, negatively affecting spermatogenesis. This increases the number of cells in the testes but decreases the number of mature cells. Thus, in patients with hypothyroidism, increased testicular size is observed along with a significant drop in mature germ cells within the seminiferous tubules.

There was a marked reduction in sperm count, motility (%), with increase in dead and abnormal sperm count in hypothyroidism group as compared to control group table (2). Among the studies on human subjects, (32-33) concluded that hypothyroidism adversely affected semen quality by compromising semen volume and progressive sperm motility, abnormal sperm morphology and decreased motility in the patients.

The publications of (53) noted that hypothyroidism claimed to increased reactive oxygen species (ROS) production with subsequent elevated lipid peroxidation.

Antioxidant enzyme play key role in oxidative infertility so oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system. In view of these observations we hypothesised that sage tea would have protective effects in an in vivo situation of free radical-mediated testicular damage.

(35) Found in their experiments on rat testis a decrease in the ratio of reduced Glutathione to oxidized state of Glutathione (GSH/GSSG) following hypothyroid state, suggesting induction of oxidative stress in the testis. This might be the key factor in contributing towards oxidative stress in testicular mitochondria, reflected in higher levels of oxidatively damaged membrane lipids and proteins ultimately leading to tissue injury and dysfunction.

Oxidative stress, in turn, can damage all intracellular macromolecules (glutathione, DNA, RNA, proteins, lipids and ATP). Any changes in the level of these substances are of key importance for cell viability and great deviations cause cell damage and death (36-37).

Oxidative stress impedes spermatogenesis, resulting in the generation of spermatozoa with poorly remodelled chromatin. These defective cells have a tendency to default to an apoptotic pathway associated with motility loss, caspase activation, phosphatidylserine exteriorization and the activation of free radical generation by mitochondria. The latter induces lipid peroxidation and oxidative DNA damage, which leads to DNA fragmentation and cell death. The physical architecture of spermatozoa prevents any nucleases, activated as a result of this apoptotic process, from gaining access to the nuclear DNA and inducing its fragmentation. Simultaneously, oxidative stress is a key event which starts nonprogrammable cell death. Differences in DNA fragmentation in experimental and control groups may be caused by activation of different sets of nucleases (38) and different rates of lipid peroxidation (39). Depending on the quality and quantity of nucleases, the levels of DNA oxidative dam-

Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

age DNA fragmentation results in high or low molecular weight fractions only or in high and lower molecular weight fractions simultaneously (38-39). The DNA damage in male germ cells can be accompanied with poor fertilization rates, defective pre-implantation embryonic development, high rates of miscarriage and morbidity in the offspring (40).

Some studies indicated the presence of high-inducible cytochrome P-450 2E1 isoform in male gonads (41-42). CYP 2E1 generates reactive oxygen intermediates, such as superoxide radicals, which in turn could rapidly react with organic molecules generating secondary free radicals and reactive oxygen radical species (43). Such cascades may alter the reducing milieu of testis and epididymis, producing conditions for sperm oxidative damage. Excessive free radicals generation often involves errors in spermiogenesis and as a result the release of spermatozoa from the germinal epithelium with abnormally high levels of cytoplasmic retention (44). Lipid peroxidation can profoundly affect sperm quality, including the percentage of motility and specific motility parameters (45).

Glutathione (GSH) is the most abundant non-protein thiol in mammalian cells. Cellular GSH plays a key role in biological processes, including proteins and DNA synthesis and amino acid transport. However, its most important role is the protection of cells against oxidation, including control of male fertility (46). The sulfhydric group (SH) is a strong nucleophilic group which confers protection against damage by oxidants, electrophilic agents and free radicals. High concentrations of GSH have been observed in rat and mouse testes. A 3-fold increase in the concentration of GSH in rat testis was observed during the onset of spermatogenesis (47).

Testicular oxidative stress appears to be a common feature in infertility, which suggests that, there may be benefits to develop better antioxidant therapies for relevant cases of hypo spermatogenesis (48-49).

The results of our present investigation showed that, reduction of antioxidant enzymes activity in testicular tissue are might be due to accumulation of free radicals leads to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. The reduction in GSH activity might be due to the decreased availability of GSH resulted during the enhanced lipid peroxidation.

(11) investigated that oxidative damage can occur in DNA during the peroxidative breakdown of membrane polyunsaturated fatty acids. DNA damage affects homeostasis of various cells leading to induced signal transductions associated with apoptosis and cell proliferation (50).

Our data showed that, oral administration of sage extract significantly increase thyroid hormone when compared to hypothyroid group. This result came in accordance with result of (51) who recorded that, Sage has long been recognized as a very rich source of the antioxidant carnosic acid, which as noted above, can increase T3 activity through improved RXR/TR heterodimerization.

The present study pointed that, administration of sage extract to hypothyroid rats can ameliorate the testicular oxidative stress. Sage able to restore Sperm cell characteristics toward normal value. These results can be related to the elevation of T3, T4. (52) Found that, thyroid hormone is known to be one of the stimulating factors for the synthesis and secretion of Glyceryl phosphoryl choline (GPC). The latter is a major constituent of epididymal secretion and maintains the osmatic balance in epididymal fluid and sperm membrane stability and serves as an energy source through inhibition of phospholipase A2 activity. GPC is reduced and degraded in hypothyroidism which in turn leading to the decreased sperm count as observed in the present study.

The increased testicular GSH content and reduction of lipid peroxidation, percentage of DNA fragmentation induced by sage administration in our experiment came in harmony with results of (20). The improving effects of S. officinalis on male reproductive system may come from the effects of its components in particular vitamins C, E, flavonoids and reflecting the strong antioxidant properties of phenolic compounds (rosmarinic acid) that can enhance Leydig cells normal function (15,16,19)

The protective potential may either involve antioxidant; signal transduction, gene expression and effective involvement in the metabolic pathways. Antioxidant enzyme play key role in oxidative infertility so oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system. In view of these observations we hypothesized that sage tea would have protective effects in an in vivo situation of free radical-mediated testicular damage. The current work highlights the protective potential of S. officinalis that may involve antioxidant properties and effective involvement in the thyroid and testosterone regulation.

Conclusion

This study recommends that intake of sage as a drink may be useful for hypothyroid patients who suffer from sexual impotency as their extracts produce antioxidant activity and exhibit fertility enhancing properties in male hypothyroid rats. So, S. officinalis can use to rectify the fertility in patient suffering from impotency either from hypothyroidism or from other causes.

REFERENCE

1. Isidori, A.M.; Pozza, C.; Gianfrilli, D. and Isidori, A. (2006) Medical treatment toimprove sperm quality. J. Reproduc. Biomed., 12: 704 -714. | 2. Makker, K.; Agarwal, A. and Sharma, R. (2009) Oxidative stress & male infertility. Review Article. Indian J Med Res., 129: 357-367. | 3. Singh, R.; Hamada, J. and Agarwal, A. (2011) Thyroid Hormones in Male Reproduction and Fertility. The Open Reproductive Science Journal, 3: 98-104. | 4. De Kretser DM, Loveland KL, Meinhardt A, Simorangkir D and Wreford N. (1998) Spermatogenesis. Hum Reprod., 13:1-8. | 5. Cooke, P. S. (1991) Thyroid hormones and testis development: a model system for increasing testis growth and sperm production. Ann N Y Acad Sci., 637: 122-132. | 6. Wagner MS, Wajner SM, Maia AL. (2008) The role of thyroid hormone in testicular development and function. J Endocrinol., 199: 351- 65. | 7. Sun YT, Wreford NG, Robertson DM, de Kretser DM. (1990) Quantitative cytological studies of spermatogenesis in intact and hypophysectomized rats: identification of androgen-dependent stages. Endocrinology; 127: 1215-1223. | 8. Rojdmark S, Berg A, Kallner G. (1988) Hypothalamic-pituitary-testicular axis in patients with hyperthyroidism. Horm Res; 29: 185-90. | 9. Donnelly P and White C. (2000) Testicular dysfunction in men with primary hypothyroidism; eversal of hypogonadotropic hypogonadism with replacement thyroxine. Clin Endocrinol; 52: 197-201. | 10. Carani C, Isidori AM, Granata A, et al (2005) Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. J Clin Endocrinol Metab; 90: 6472- 6479. | 11. Jia, X.; Han, C. and Chen, J. (2002) Effect of tea on preneoplastic lesions and cell cycle regulators in rat liver. Cancer Epidemiology Biomarkers and Prevention, 11:1663–1667. | 12. Khan MR, Rizvi W, Khan GN, Khan RA and Shaheen, S. (2009) Carbon tetrachloride induced nephrotoxicity in rat: Protective role of Digera muricata. J Ethnopharmacol 2009, 122:91–99. | 13. Guo, C.; Lu, Y. and Hsu, G.S. (2005) The influence of aluminum exposure on male reproduction and offspring in mice. Environ Toxicol Pharmacol., 20:135–141. | 14. Horn, M.M.; Ramos, A.R.; Winkelmann, L. et al (2006) Seminiferous epithelium of rats with food restriction and carbon tetrachloride-induced cirrhosis. Intl Brazilian J Urol, 32(1): 94–99. | 15. Cuvelier, M.E., Berset, C. and Richard, H. (1994) Antioxidant constituents in sage (Salvia officinalis). J. Agric. Food Chem. 42, 665–669. | 16. Baricevic, D. and Bartol, T. (2000) The biological/pharmacological activity of the Salvia genus. In: Kintzios, S.E. (Ed.), SAGE – The Genus Salvia. Harwood Academic Publishers, Amsterdam, pp. 143–184. | 17. Capek P, Hribalova V.(2004). Water-soluble polysaccharides from salvia officinalis L.possessing immunomodulatory activity. Phytochemistry; Vol. 65, Issue 13, 1983-1992. | 18. Mercier B, Prost J, Prost M. (2009) The essential oil of turpentine and its major volatile fraction (α- AND α-PINENES): A REVIEW. Int J Occup Environ Health;22(4):331 – 342. | 19. Lindi L, Haolin C, Michael A, Trush M.D, Show M, Anway D, Barry Zirkin R. (2005) Aging and the Brown Norway Rat Leydig Cell Antioxidant Defense System. J Androl; 22: 32-37. | | 20. Cristovao F. Limaa, Paula B. Andradeb, Rosa M. Seabrab et al (2005) The drinking of a Salvia officinalis infusion improves liver antioxidant status in mice and rats. Journal of Ethnopharmacology 97: 383–389. | | 21. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C, pp 21-55. | 22. Grattagliano I.; Lauterburg, H. B. and Portincasa, P. et al (2003) Mitochondrial glutathione content determines the rate of liver regeneration after partial hepatectomy in eu- and hypothyroid rats Journal of Hepatology, 39: 571–579. | 23. Yoshioko, T. N.; Kawada, T.; Shimoda, M. and Mori, T. (1979) Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in the blood. Am.J. Obestetric. Gynecology, 135: 372 – 376. | 24. Beutler, E.; Duran, O. and Kelly, B. (1963) Improved method for the determination of blood glutathione. J. of lab. And clinic. Med. 61: 882. | 25. Bearden, H. J. and Fluquary J. (1980) In Applied Animal Reproduction, Restore Publishing Co. Inc., Reston, Virginia, USA, Page 158-160. | 26. Amann, R. P. (1982) Use of animal models for detecting specific alteration in reproduction. Fund. Appl. Toxicol., 2: 1336. | 27. Perandones, C.E.; Illera, V.A.; Peckham, D.; Stunz, L.L. and Ashman, R.F. (1993): Regulation of apoptosis in vitro in mature murine spleen T cells J. Immunol., 151: 3521-3529. | 28. Mahmoud Saadi M. Taha Mohammed N.. and Yahia A. K Gaida (2013) Effect of Carbimazol-induced Hypothyroidism on Male Rat Reproductive System. Ibn Al-Haitham Jour. for Pure & Appl. Sci. Vol. 26 (1). | 29. Krassas GE and Perros P. (2003) Thyroid disease and male reproductive function. J Endocrinol Invest; 26: 372-80. | 30. Krassas GE and Pontikides N. (2004) Male reproductive function in relation with thyroid alterations. Best Pract Res Clin Endocrinol Metab; 18: 183-95. | 31. Wajner SM, Wagner MS and Maia AL. (2009) Clinical implications of altered thyroid status in male testicular function. Arq Bras Endocrinol Metabol; 53: 976-82. | 32. Hernández Corrales JJ, García Miralles JM, Diez García LC. (1990) Primary hypothyroidism and human spermatogenesis. Arch Androl; 25: 21-27. | 33. Krassas GE, Papadopoulou F, Tziomalos K, Zeginiadou T, Pontikides N. (2008) Hypothyroidism has an adverse effect on human spermatogenesis: a prospective, controlled study. Thyroid, 18: 255-259. | 34. Peltola V, Huhtaniemi I and Ahotupa M. (1992) Antioxidant enzyme activity in the maturing rat testis. J Androl; 13: 450-455. | 35. Choudhury S, Chainy GB, Mishro MM. (2003) experimentally induced hypo- and hyper-thyroidism influence on the antioxidant defence systemin adult rat testis. Andrologia; 35: 131-40. | 36. Cooke, M.S.; Evans, D.M.; Dizdaroglu, M and Lunec, J. (2003) Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal. 17: 1195–1214. | 37. Jones, D.P. (2008) Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 295: 849–868. | 38. Hakansson, A.; Zhivotovskyt, B. Orrenius, S et al. (1995) Apoptosis induced by a human milk protein. Proc. Natl. Acad. Sci. USA 92: 8064–8068. | 39. Aitken, RJ, Koppers AJ. (2011) Apoptosis and DNA damage in human spermatozoa. Asian Androl 13(1): 36–42. | 40. Aitken, RJ, De Iuliis GN. (2007) Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online 14(6): 727–733. | 41. Oropeza-Hernandez LF, Quintanilla-Vega B, Reyes-Mejia RA et al. (2003) Trifluoroacetylated adducts in spermatozoa, testes, liver and plasma andCYP2E1 induction in rats after subchronic inhalatory exposure to halothane. Toxicol Lett 144(1): 105–116. | 42. Quintans LN, Castro GD, Castro JA. (2005) Oxidation of ethanol to acetaldehyde and free radicals by rat testicular microsomes. Arch Toxicol 79 (1): 25– 30. | 43. Lieber, C.S. (1997): Cytochrome P-4502E1: Its physiological and pathological role. Physiol Rev 77: 517–544. | 44. Sanocka, D. and Kurpisz, M. (2004) Reactive oxygen species and sperm cells. Reproductive Biology and Endocrinology, 2: 12–26. | 45. Bansal, A.K. and Bilaspuri, G.S. (2011) Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int., 7: 1–7. | 46. Luberda, Z. (2005) The role of glutathione in mammalian gametes. Reprod Biol 5(1): 5–17. | 47. Donnelly, E.T.; Mcclur, N. and Lewis, S.E. (2000) Glutathione and hypotaurine in vitro: effects on human sperm motility, DNA integrity and production of reactive oxygen species. Mutagenesis 15(1): 61–68. | 48. Turner, T.T. and Lysiak, J.L. (2008) Oxidative stress: A common factor in testicular dysfunctions. J Androl. 29:488–498. | 49. Yousef, M.I. and Salama, A.F. (2009) Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem Toxicol., 47:1168–1175. | 50. Khanna, K.K. and Jackson S.P. (2001) DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet., 27:247–254. | 51. Rutherford, D.M.; Nielsen, M.P.; Hansen, S.K. et al (1992) Isolation and identification from Salvia officinalis of two diterpenes which inhibit t-butylbicyclophosphoro [35S] thionate binding to chloride channel of rat cerebrocortical membranes in vitro. Neurosci Lett. 3; 135(2):224- 226. | 52. Anbalagan, J.; Kanagaraj, P.; Srinivasan, N.; Aruldhas, M.; and Arunakaran, J. (2003) Effect of polychlorinated biphenyl, Aroclor1254 on rat epididymis. Indian J Med Res 118: 236-242. | 53. Baskol, G.; Atmaca, H.; Tanrıverdi, F.; Baskol, M.; Kocer, D.; Bayram, F. (2007): Oxidative Stress and Enzymatic Antioxidant Status in Patients with Hypothyroidism before and after Treatment Exp Clin Endocrinol Diabetes; 115(8): 522-526