Overt hypothyroidism frequently affects male reproductive and fertility. Hypothyroidism claimed to increased place in controlling brain and somatic development in infants. 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 to play a critical role 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, 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, 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).

In conclusion, this observation points to Salvia 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 varicocele, 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).

The scope of this work is to highlight the mechanism of hypothyroidism induced production of ROS on male reproductive system and enumerate the benefits of Salvia 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 Salvia officinalis tea drinking improving the reproductive potency and fertility through antioxidant and thyroid regulating properties.
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 tritional use of sage tea versus hypothyroidism induced oxidative stress associated reproductive hormonal changes and lipids peroxidation in rats.

Material and methods
1-Experimental 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-7-glucoside (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 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 x 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 malondialdehyde (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.

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

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm cell characteristics (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Motility (%)</td>
</tr>
<tr>
<td>Normal control</td>
<td>89.5 ±1.82</td>
</tr>
</tbody>
</table>
Hypothyroidism significantly increased tests, prostate and seminal vesicle glands weights compared to control group, table (1). These finding may 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 chromatins. These defective cells have a tendency to default to an apopotic 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-

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.
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 cytoplasmatic 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 sulfhydryl 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 antioxidant 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 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