Effects of aqueous extract of *Salvia officinalis* on some parameters of sperms and histopathological changes in testes of rats treated with Doxorubicin

Khalid Al-Syaad
Biology Department, Faculty of Science, King Khalid University, Abha, P.O. Box 9004, Saudi Arabia.
alsayaad55@hotmail.com

**ABSTRACT**

Doxorubicin drug is an anti-cancer substance, but unwanted side effects result from its use. The objective of this research is to study the possibility of whether the Sage plant extract has the ability to reduce the harmful effects of the drug in question. Male albino rats (Rattus norvegicus) which are sexually mature were divided into four groups (8 each); the control group injected intraperitoneally (i.p.) with a saline solution, DOX group injected i.p. weekly with doxorubicin (DOX) drug at a dose of 4 mg/kg for 7 weeks; DOX and *Salvia* group were injected i.p. weekly with a dose of DOX of 4 mg/kg for 7 weeks and swallow daily oral Sage leaf extract at a dose of 85 mg/kg for the same period and *Salvia* group swallow daily Sage leaf extract rate of 85 mg/kg for 7 weeks. After completion of the experiment animals were dissected to get samples of testes to prepare textile sections for routine histopathological examination and the reproductive capacity of the adult rats were done. The histopathological examination in doxorubicin-treated rats, either with or without *Salvia* extract, revealed germ cell depletion, a significant ($p<0.05$) decrease in primary spermatocytes and spermatids, multinucleated formations of spermatids and germ cell showing apoptotic characteristics. Significant ($p<0.05$) reduction of seminiferous tubule volume was observed in all doxorubicin-treated subgroups and increased interstitial spaces as compared with control. In the *salvia* treated group there were a significant ($p<0.05$) increase in sperms motility, decreased percentage of dead and abnormalities of sperms and significant ($p<0.05$) increase in diameters of seminiferous tubules, primary spermatocytes and spermatids and decrease interstitial spaces compared with DOX-treated groups.

**Key words:** Doxorubicin; *Salvia*; plant extract; testis; histopathology.

**INTRODUCTION**

*Salvia officinalis* L. herbaceous, perennial plant of Fam. Lamiaceae, is a semi-shrub cultivated as aromatic, condiment, medicinal, melliferous and ornamental plant,
provided from South Europe (Mediterranean area), where it grows in the spontaneous flora. The genus includes more than 900 species spread within the whole world (Ambarus et al., 2005; Delamare et al., 2007).

The ethanol tinctures, decoctions from aerial plant parts, as well as the Salvia essential oils are used in treatment of wide range diseases; not only heart, nervous and circulator systems disturbances, respiratory, digestive diseases, but also metabolic and endocrine deregulations. Also, the Salvia preparations have many other therapeutic effects (Istudor, 1998; Capek et al., 2009).

The essential oils of Salvia officinalis have a high antibacterial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, cytotoxic activity against Vero cells and antiviral activity against herpes simplex 1 virus and virus of vesicular stomatitis (Tada et al., 1994; Sivropoulou et al., 1997) The ethanol extract of Salvia officinalis has antifungal properties against cells of Saccharomyces cerevisiae (Fârcășanu and Oprea, 2006).

The plant phytochemical investigations revealed the existence of numerous bioactive compounds about 160 kinds of polyphenols, flavonoids having various biological activities (Lu and Foo 2002). The polysaccharides extracted from Salvia officinalis exhibit immunomodulator and antitussive properties (Capek and Hribalova, 2004).

The Lamiaceae family includes a large number of plants, well known for their antioxidant properties. Among these, Salvia has been widely used and most of its antioxidant components have been identified. It has been established that the antioxidant effects of Salvia are mainly due to the polyphenolic compounds (Das and Pereira 1990; Pokorny, 1991; Schwarz and Ternes, 1992). The major phenolic compounds identified in the extracts of Salvia are rosmarinic acid, carnosic acid, salvianolic acid and its derivates carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate (Wu et al., 1982; Madsen and G. Bertelsen, 1995). Among these, the rosmanol is a major constituent of many Salvia species having strong antioxidant activities because these groups cause phenols to donate more easily the hydrogen atoms to activate free radicals, which interrupt the antioxidation chain reaction (Weng and Wang, 2000).

The methanol extracts of eight Salvia species from Turkey – S. aethiopis, S. candidissima, S. limbata, S. microstegia, S. nemorosa, S. pachystachys, S. verticillata, S. virgata – exhibited different levels of antioxidant activity in all models studies (Tosun et al., 2009). The results from DPPH free radical-scavenging system revealed that Salvia had significant antioxidant and free radical-scavenging so these plants, notably S. verticillata and S. virgata, can be used as natural antioxidants. A positive linear correlation between total phenolic content and antioxidant activity of the extracts was observed (Tosun et al., 2009). The high antioxidant capacity correlated with an increased content of polyphenols was reported, on methanolic extracts, at two other sage species: Salvia officinalis and S. fruticosa (Pizzale et al., 2002; Pasias et al., 2010).

There were realized studies on leaf and roots extracts of Salvia przewalskii, S. miltiorrhiza, S. verticillata (Matkowski et al., 2008). The antioxidant capacity of the studied species is high, but differences between species and organs have been also revealed. Salvia przewalskii leaf extract was the strongest one in all tests, followed by S. miltiorrhiza root and S. verticillata leaf. Among the roots, the most active was S. miltiorrhiza extract, followed by S. verticillata. The antioxidant activity correlates to the total polyphenol and depending on the assay, to the hydroxycinnamic acids content. The high content of tanshinones in both S. miltiorrhiza and S. przewalskii
Effects of aqueous extract of S. officinalis on some parameters of sperms and histopathological roots is unlikely to contribute to the antioxidant activity (Matkowski et al., 2008). Membrane processes are modern techniques used for simple and efficient separation, purification and concentration of bioactive compounds from plant extracts. They presents several advantages: low cost, separation, purification and concentration of a specific compound in one phase, at cold, without the intervention of chemical reagents, the absence of phase changes, preserving the quality of preparations, the possibility of coupling with other conventional separation processes (Roman et al., 2008).

The antitumor Doxorubicin is composed of a tetracyclic ring system with quinone and hydroquinone moieties (Menna et al., 2007). Numerous NAD(P)H oxidoreductases catalyze an one-electron reduction of the quinone moiety, thereby forming a semiquinone that recycles to its parent compound by reducing oxygen to reactive oxygen species (ROS) such as superoxide anion (O2) and hydrogen peroxide (H2O2). The redox cycling of the quinone moiety does not alter the anthracycline chromophore, but it probably causes cardiotoxicity by forming ROS in excess of the detoxifying mechanisms of cardiomyocytes (Gewirtz, 1999; Minotti et al., 2004). The antitumor anthracycline doxorubicin causes a severe cardiotoxicity. Several lines of evidence indicate that cardiotoxicity correlates with the cardiac levels of doxorubicin; therefore, pharmacokinetic factors that increased or decreased the cardiac levels of doxorubicin were shown to aggravate or mitigate cardiotoxicity, respectively (Gewirtz, 1999).

The clinical use of anthracyclines in anti-cancer treatment is limited by their adverse cardiotoxic effects, which include cardiomyopathy and heart failure (Hershman et al., 2008). At high doses, cardiotoxicity often occurs within a few months, whereas low doses rarely cause deterioration of ventricular function in the first year after therapy (Von Hoff et al., 1979). However, there is now increasing evidence that, several years after therapy, one of three patients treated with low-dose anthracyclines develops hypokinetic cardiomyopathy (Hequet et al., 2004). A number of mechanisms have been proposed to explain anthracycline cardiotoxicity. Although the most accredited hypothesis states that anthracyclines induce myocyte loss through oxidative stress and apoptotic cell death (Arola et al., 2000; Spallarossa et al., 2004), there is controversy whether apoptosis contributes to late onset cardiotoxicity induced by low doses of doxorubicin (Arola et al., 2000). Maejima et al. (2008) recently showed that when cultured neonatal rat cardiomyocytes are exposed to low concentrations of doxorubicin, the cells do not enter apoptotic program but exhibit a senescence-like phenotype. The hallmark of cellular senescence is the cell cycle arrest that is accompanied by important changes in many aspects of cell morphology (Hayflick and Moorhead, 1961; Nishio et al., 2001). Senescence is the result of changes in the expression of many proteins that regulates cell cycle, cytoskeletal function, and cellular architecture and causes impairment of cell functions, including the regenerative capacity (Lin et al., 1998; Gonzalez et al., 2008; Urbanek et al., 2005; Vigneron et al., 2005).

Studies performed on tumor cells indicate that low doses of doxorubicin, as well as several anti-cancer agents, induce mitotic catastrophe, a phenomenon that is characterized by chromosomal abnormalities and abnormal mitosis that leads to late cell death. It also has been shown that cells that ultimately die of mitotic catastrophe initially show a senescence-like phenotype (Eom et al., 2005). Therefore, the induction of senescence has been proposed as a novel mechanism of cardiotoxicity induced by low doses of doxorubicin (Maejima et al., 2008). A number of studies suggest that telomere dysfunction plays a main role in the stress-induced senescence
program and in apoptosis (Lechel et al., 2005). Telomeres are specialized, repetitive, noncoding sequences of DNA bound by several proteins, including telomere binding factors 1 and 2 (TRF1 and TRF2), which play a crucial role in telomere biology and govern chromosomal stability. The TRF1 multiprotein complex regulates telomere length and specifically affects mitotic progression, whereas TRF2 is critical for maintaining the telomere t-loop “endcapping” structure, whose function is to prevent chromosome end-to-end fusion and chromosome abnormalities (Stansel et al., 2001; van Steensel and de Lange, 1997). It is believed that telomeric dysfunction beyond a certain limit triggers a DNA damage response mediated by p53, a tumor suppressor protein, and by mitogen-activated protein kinases (MAPKs) (Smogorzewska and de Lange, 2002; Iwasa et al., 2003). MAPK are highly conserved serine/threonine kinases that are activated in response to a wide variety of stimuli and play a role in numerous cell functions including survival, growth, and proliferation (Valledor et al., 2000; Xia et al., 1995; Yujiri et al., 1998). Both p53 and MAPKs are involved in doxorubicin-induced cardiotoxicity (Liu et al., 2004; Spallarossa et al., 2006). The signal transduction pathways of doxorubicin–induced senescence and the mechanism by which different doses of the same stressing agent may induce either apoptosis or senescence and mitotic catastrophe are still poorly understood.

MATERIAL AND METHODS

Animals
All of the procedures involving animals in this study were approved by the institution’s animal welfare regulatory committee. Male Albino rats were maintained at King Khalid University animal house on a 14:10-hour light : dark cycle. Control and treated rats were provided with food and water ad libitum; there were no differences in food intake. One week after arrival, rats were randomly divided into 4 treatment groups, each composed of 8 rats.

Preparation of *Salvia officinalis* extract
Water extract of *Salvia officinalis* was done by soaking 10 g of leaves and stems of the plant in pure boiling water for 30 minutes with continuous stirring. The mixture was filtrated and the clear filtrate was kept in sterile dark containers at 4°C till use.

Treatment of animals
Adult Albino rat (*Rattus norvegicus*) were divided into four groups, containing 8 rats in each group, first group negative control received normal saline intraperitoneally (i.p.). While the second group was i.p. treated weekly with doxorubicin (DOX; 4 mg/kg) for seven weeks. The third group was weekly i.p. treated with doxorubicin (4 mg/kg) and orally extract of *salvia* (85 mg/kg) for seven weeks. The fourth group received *Salvia* extract (85 mg/kg) orally for seven weeks.

Organs collections
Males were anaesthetized, and the testes and epididymides were removed, trimmed of fat, and weighed in the end of the experiment and one epididymides was kept at -80°C for the determination of spermatozoal head counts and the other was used immediately for sperm viability and abnormality study.

Determination of motility, abnormality and viability of sperms
One epididymidis was cut into pieces in 2 ml of pre-warmed at 37°C TCM-199 medium. One drop of the resulted medium mixture was examined under light microscope on pre-warmed slide at 37°C. For abnormality study, one drop of the mixture was dried on microscope slide and then fixed in 95% alcohol, soaked in water.
Effects of aqueous extract of *S. officinalis* on some parameters of sperms and histopathological

and then stained in eosin solution (1%). For viability, live-dead sperms were counted on hemocytometer count slide.

**Spermatozoal Head Counts**

The frozen epididymidis from each of the 7 rats per group were homogenized using tissue homogenizer in 5 ml of 0.9% NaCl, 2 drops of 1% eosin, 0.1% thimerosal, and 0.5% Triton X-100. To assess tissue content of spermatozoa, the heads of spermatozoa were counted hemocytometrically.

**Testicular Histology**

One testis was fixed in Bouin fluid overnight, and processed for routine paraffin embedding. The testes were cut into 5-mm sections. Three serial sections per testis were mounted on slides, deparaffinized, rehydrated, and stained with periodic acid Schiff (Sigma-Aldrich) following the manufacturer’s recommendations. Sections of the seminiferous tubules at all stages were examined by light microscopy.

**Statistical analysis**

The biochemical, weight and count data recorded were expressed as Mean ± SD and statistical and correlation analyses were undertaken using the One-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. A *p* value < 0.05 was statistically significant. A Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 15.0, Chicago, IL, USA).

**RESULTS**

Preparation of water extract of *Salvia officinalis* was done and the clear filtrate was kept in sterile dark containers at 4°C till use. All animals used in this experiment were weighed before and after treatment as shown in Table (1). Animals in control untreated group and *Salvia* alone treated group showed normal growth rate while growth rate was diminished in all of the other groups.

<table>
<thead>
<tr>
<th>Group (N=7)</th>
<th>Weight before treatment (g) (Mean ± SD)</th>
<th>Weight after treatment (g) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>375.66 ± 28.791</td>
<td>382.57 ± 19.33563</td>
</tr>
<tr>
<td>DOX</td>
<td>369.28 ± 33.798</td>
<td>335.5 ± 41.20968</td>
</tr>
<tr>
<td>DOX + Salvia</td>
<td>359.02 ± 49.687</td>
<td>333.6 ± 42.60561</td>
</tr>
<tr>
<td>Salvia</td>
<td>377.71 ± 19.867</td>
<td>385.83 ± 25.54548</td>
</tr>
</tbody>
</table>

Testes of all animals used in this experiment were collected and studied. The weight of these testes in different groups were recorded as shown in Table (2). From the table it was shown that there are no significant differences among groups after treatment.

<table>
<thead>
<tr>
<th>Group (N=8)</th>
<th>Weight of testes (g) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.98 ± 0.85</td>
</tr>
<tr>
<td>DOX</td>
<td>4.48 ± 0.48</td>
</tr>
<tr>
<td>DOX + Salvia</td>
<td>5.24 ± 0.99</td>
</tr>
<tr>
<td>Salvia</td>
<td>5.53 ± 0.39</td>
</tr>
</tbody>
</table>
One epididymidis was examined under light microscope for sperms abnormality, viability and motility (Table 3). The sperm mobility was significantly reduced ($p<0.05$) in rats treated when treated with DOX alone, but when treated with Dox in addition to Salvia there were no significant decrease in sperm motility. No significant changes were noticed when rats treated with Salvia alone. There was a visible reduction ($p<0.05$) in the sperm counts of the Dox treated group and there were no significant changes in sperm numbers in other treated groups. The sperm viability of the DOX alone and DOX and Salvia treated groups showed a significant reduction ($p<0.05$) from what was obtained in the control. Abnormal forms of sperms were significantly increased in DOX treated group than control and other groups.

<table>
<thead>
<tr>
<th>Group (N=8)</th>
<th>Total count (sperms/cm)</th>
<th>Viability (%)</th>
<th>Motility (%)</th>
<th>Abnormal forms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8,821,000±3,382</td>
<td>68.13</td>
<td>68.41</td>
<td>47.46627</td>
</tr>
<tr>
<td>DOX</td>
<td>7,807,000±5,826</td>
<td>51.36</td>
<td>47.02</td>
<td>77.34085</td>
</tr>
<tr>
<td>DOX + Salvia</td>
<td>8,485,000±6,322</td>
<td>60.61</td>
<td>59.68</td>
<td>57.98468</td>
</tr>
<tr>
<td>Salvia</td>
<td>8,742,000±6,051</td>
<td>75.36</td>
<td>70.94</td>
<td>47.84946</td>
</tr>
</tbody>
</table>

Histopathological studies

One testis was fixed in Bouin’s fixative and evaluated for any structural changes under a bright field microscope.

Control untreated group

The control groups (Figs. 1& 2) showed a thick fibrous capsule (tunica albuginea) enclosing a number of adjacent seminiferous tubules (ST) separated by interstitial cells. The seminiferous tubules appeared as rounded or oval surrounded by a thin basal lamina (BL). The tubules were lined by stratified germinal epithelium, which consisted of two distinct populations of cells: the spermatogenic cells and the Sertoli cells. Sertoli cells appeared as elongated cells, with irregular poorly defined outline and oval basal nuclei. The spermatogenic cells represented the different stages of spermatogenesis, with the spermatogonia resting on the basal lamina and having small and dark nuclei. Inner to spermatogonia, there were the primary spermatocytes that appeared as large cells with large oval nuclei. Inner to primary spermatocytes, there were the secondary spermatocytes with their relatively smaller size followed by spermatids and spermatooza (SP). The spermatids were detected at their different steps of spermiogenesis. The cells first appeared rounded with central rounded nuclei (round spermatids) and gradually, they became elongating spermatids that form the spermatozoa with their characteristic shape. In between the tubules, the interstitial tissue presents blood vessels with clusters of cells with ovoid or polygonal shape and spherical nuclei representing the Leydig cells.

Salvia alone treated Group

Rats treated with Salvia extract showed significant increase in diameters of seminiferous tubules, primary spermatocytes, spermatids and decrease in interstitial space when compared with control groups (Fig. 3).

DOX alone treated Group

H & E stained sections of the testis in the Dox treated group (Figs. 4, 5 & 6) showed histological changes that were at variance with those obtained in the control. There was disorganization of the normal appearance of the testis with overall different degrees of atrophy in the seminiferous tubules. At the same time a disorganization of the germinal epithelium, with loss of the spermatogenic cells specially spermatocytes
Effects of aqueous extract of *S. officinalis* on some parameters of sperms and histopathological

and spermatids and exfoliation of the germ cells was also observed. In the seminiferous tubular lumen and almost all tubules showed severe atrophy as they were devoid of epithelium, with only Sertoli cells and spermatogonia present within the depleted tubules. Sperms were hardly seen. The spermatogenic cells showed degeneration and/or necrosis. Necrosis was in the form of pale or vacuolated cytoplasm with condensation of chromatin (pyknotic nuclei), fragmentation of chromatin (karyorrhexis), chromatin migration and ghost nuclei (karyolysis). Vaculations within the lumen was also seen, which varied in size and number. The Leydig cells were markedly decreased in number.

**DOX and *Salvia* treated Group**

Rats treated with both doxorubicin and *Salvia* showed the same side effects of drug alone treatment but with a little improved picture (Fig. 7).

**DISCUSSION**

Doxorubicin is a very potent chemotherapeutic drug which has been used against a variety of cancers. Despite its efficiency, doxorubicin has been shown to cause death of healthy cells, especially those undergoing rapid proliferation. It has been shown that doxorubicin causes germ cell death and seminal alterations (Kang *et al.*, 2002; Kato *et al.*, 2001). In the present study doxorubicin was shown to harm the testes of animals under study either alone or in combination with *Salvia* extract. These results are in agreement with Brilhante *et al.* (2011) where they demonstrated that doxorubicin produces serious damage to the testis of adult rats treated during the early prepubertal phase, suggesting severe bad effects to the fertility and possible future sterility. Also, they demonstrated that rats of different ages in their study showed accentuated seminiferous epithelium depletion after treatment and they conveyed this to an increase of apoptosis rate. In the present study many germ cells were shown to be damaged. Similar results demonstrated that this drug causes male germ cell apoptosis (Shinoda *et al.*, 1999). The increase of germ cell death usually leads to a reduction of the morphometric and stereological parameters.

The results in the present work showed some reduction in seminiferous epithelium when treated with doxorubicin either alone or in combination with *Salvia* extract. Similar previous results were obtained by Vendramini *et al.* (2010) who demonstrated that a considerable reduction of the seminiferous epithelium height and volume density was observed in pubertal and adult rats that were treated with doxorubicin when they were 30 days old, that is, at a later stage of pre-puberty than that utilized in the current study. In current study an increase in lymphatic space was observed. This also was observed by Vendramini *et al.* (2010) as they demonstrated that the increase of lymphatic space volume density indicates the occurrence of testicular edema. This may be explained in that doxorubicin causes endothelial dysfunction and edema, as secondary effects of oxidative stress in the vascular wall. The vascular endothelium plays a fundamental role in the maintenance of organ function by forming a barrier regulating water and solute distribution between blood and tissues; however, this fluid control can be deregulated by oxidative stress (Wolf and Baynes, 2006 and 2007) resulting in movement of water and proteins from the vascular system into tissues and compromising organ function.

There was disorganization of the normal appearance of the testis with overall different degrees of atrophy in the seminiferous tubules after treating animals with doxorubicin in this study. This may be explained because it is known that doxorubicin causes death of intermediary and type B spermatogonia through its interaction with
topoisomerase II, an enzyme present in high quantity in intermediary and type B spermatogonia (Hou et al., 2005; Jahnukainen et al., 2000). Although type B and intermediary spermatogonia are not the only cell types of the rat seminiferous epithelium under continuous division, their localization in the base of the seminiferous tubules makes them more vulnerable to the action of cytotoxic drugs (Lu and Meistrich, 1979; Stumpp et al., 2004). In addition, the frequency of tubular sections containing primary spermatocytes was also significantly reduced in doxorubicin-treated rats, mainly in the adulthood. Although in pre-meiotic DNA synthesis the role of topoisomerase II can be less pronounced, it is possible that other topoisomerases can be involved in the process, as observed for etoposide (Freitas et al., 2002; Hakovirta et al., 1993).

Severe germ cell depletion was observed in groups treated with doxorubicin, with some tubular sections showing only Sertoli cells. This fact suggests that type A spermatogonia were damaged, hampering the repopulation of the seminiferous epithelium. Indeed, in rats, besides effective pre-mitotic DNA synthesis at stages II-III and IV-VI of the seminiferous epithelium cycle, in which B and intermediary spermatogonia are formed, there are other pre-mitotic peaks of DNA synthesis at stages IX, XII, XIV and I, involving A1, A2, A3 and A4 spermatogonia, respectively (Hakovirta et al., 1993). This fact agrees with the data observed in this study, which showed evident reduction of the frequency of seminiferous tubule sections containing type A spermatogonia in all doxorubicin-treated rat groups. The harm of spermatogonia could be a possible cause of the depletion of spermatocytes and spermatids, although it could be caused by a direct effect of doxorubicin on pre-meiotic DNA synthesis. Indeed, a drastic depletion of seminiferous epithelium and the large quantity of tubular sections containing only Sertoli cells can indicate that reserve and renewing spermatogonia were harmed. In the current experiment, the frequency of type A spermatogonia was significantly decreased in all doxorubicin-treated groups. Although the doxorubicin-treated adult rats showed a significant loss of weight, they did not show ascites, alteration of locomotion and signal of physical debility.

Improved results after treatment with *Salvia* could be due to the antioxidant property of *Salvia* as evidenced by restoration of glutathione and LPO levels. Restoration of acid phosphatase level pointed out the role of extract of *Salvia* in promoting the stability of cellular, nuclear and organelle membranes (Kumar et al., 2007). In the present study there was an increase in the viability and motility of sperms after treatment with plant extract. The same results was obtained by Ben Arush et al. (2000) and Sierens et al. (2002) and this may be explained by that this extract does not contain any mutagenic agents.

A number of classes of compounds possess estrogenic activity. All isoflavones are estrogenic to animals. Others are coumarins and diterpenoids steroids. *Salvia* is one of these plants containing steroid saponins. Also ethanolic extract with essential oil of *Salvia* was shown to have certain effect on fertility and that’s why many parameters under study gave a positive result for *Salvia* effects (Perry et al., 2000).

**CONCLUSION**

In conclusion *Salvia* leaves and stem extract has good effects in reducing the bad side effects of the drug Doxorubicin especially on testis.
Effects of aqueous extract of *S. officinalis* on some parameters of sperms and histopathological

**ACKNOWLEDGMENTS**

This research was supported by the King Khalid University, Project No. KKU-SCI-29. The author gratefully acknowledges the contribution of Laboratory Technicians at Biology Department, Faculty of Science, King Khalid University, Abha, KSA.

**REFERENCES**


Effects of aqueous extract of *S. officinalis* on some parameters of sperms and histopathological

![Image](image1.png)  
Fig. 1: Histological morphology of testis of untreated control group showing normal histology. H&E stained (X100).

![Image](image2.png)  
Fig. 2: Histological morphology of testis of untreated control group showing normal histology of seminiferous tubules and spermatogenic cells. H&E stained (X200).

![Image](image3.png)  
Fig. 3: Histological morphology of testis of *Salvia* treated group showing normal histopathology of the testis. H&E stained (X 400).

![Image](image4.png)  
Fig. 4: Histological morphology of testis of DOX treated group showing severe damage, necrosis and congestion with inflammatory cells infiltrations. H&E stained (X100).

![Image](image5.png)  
Fig. 5: Histological morphology of testis of DOX treated group showing severe damage, necrosis and congestion with inflammatory cells infiltrations. H&E stained (X200).

![Image](image6.png)  
Fig. 6: Histological morphology of testis of DOX treated group showing desquamation in tubules lining cells leading to filling of the tubules with damaged cell. H&E stained (X400).

![Image](image7.png)  
Fig. 7: Histological morphology of testis of DOX and *Salvia* treated group showing severe damage, necrosis and congestion with inflammatory cells infiltrations. H&E stained (X200).
آثار المستخلص المائي لنبات الميرامية على بعض صفات الحيوانات المنوية والتغيرات التشريحية المرضية

خالد بن مشيب آل صيد
قسم الأحياء - كلية العلوم - جامعة الملك خالد - المملكة العربية السعودية

بعد عقار الدوكسوروبسينين من المواد المضادة للسرطان لكن ينتج عن استخدامه بعض الآثار الجانبية غير المرغوب فيها. الهدف من هذا البحث هو دراسة إمكانية ما إذا كان مستخلص نبات الميرامية المقدّرة على تقليل الآثار الضارة الناجمة عن استخدام هذا العقار. قُسمت الذكور الجرذان البيضاء (الجرذ الرومي) الناضجة جنسياً إلى أربع مجموعات (8 كل منها). المجموعة الضابطة والتي تم حقنها داخل الغشاء البريتوني بجلول ملحي. مجموعة الدوكسوروبسينين تم حقنها داخل الغشاء البريتوني أسبوعياً بجرعة 4 ملغ / كغ لمدة 7 أسابيع. مجموعة الميرامية والدوخسوروبسينين والتي تم حقنها داخل الغشاء البريتوني أسبوعياً بجرعة الدوكسوروبسينين 4 ملغ / كغ لمدة 7 أسابيع. وجرعة الدوكسوروبسينين 4 ملغ / كغ لمدة 7 أسابيع وتجريبياً يومياً بجرعة 4 ملغ / كغ لمدة 7 أسابيع وتجريبياً يومياً بجرعة 4 ملغ / كغ لمدة 7 أسابيع. بعد الانتهاء من التجربة تم تشيريج الحيوانات وأخذت عينات من الخصيتين لعمل قطاعات نسيجية ومن ثم فحصها لاختبار القدرة الإنجابية في الجرذان البالغة. وقد بين فحص الأسناة في الجرذان المعالجة بالدوخسوروبسينين فقط أو مستخلص الميرامية اختلاف في عدد الخلايا الجرمومية، كما لوحظ انخفاض معنوي (P<0.05) في عدد الخلايا المنوية الأولية والطلاع المنوي، ووجد تشكيلات متعددة نوى في الطلاع المنوي وعلامات موت الخلايا المبرمج في الخلايا الجرمومية. لوحظ أيضاً انخفاض معنوي (P<0.05) في حجم الأنابيب المنوية في جميع المجموعات المعالجة بالدوخسوروبسينين زيادة المسافات البينية مقارنة بالمعالجة الضابطة. أما في مجموعة الميرامية فقد سجلت زيادة معنوية (P<0.05) في حركة الحيوانات المنوية، وانخفاض في نسبة موت وتلوثات الحيوانات المنوية وزيادة معنوية (P<0.05) في أقطار الأنابيب المنوية والخلايا المنوية الأولية والطلاع المنوي وانخفاض المسافات البينية مقارنة بالمعالجات المعالجة بالدوخسوروبسينين.