Protective effect of *Salvia officinalis* against cypermethrin-induced reprotoxicity in male Wistar rats

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INTRODUCTION

Pesticides are toxic chemicals that are deliberately introduced into the environment to kill off pests as well as rodents, fungi, insects, and weeds⁴. The use of pesticides is beneficial in general, but the excessive and misuse of these chemicals leads to impairment of human and animals’ health⁵.

The widespread use of insecticides in agriculture to increase food production has become a major problem in developing countries, which is linked to various toxic activities⁶. Pyrethroids are among the most widely used insecticides⁷. Cypermethrin is a synthetic pyrethroid type (II) that is widely used in agriculture and other domestic applications⁸.

Recent studies have shown that exposure to cypermethrin may cause serious health impairments for humans and animals, including, nervous, immune, endocrine, hematological, cardiovascular, respiration, and reproductive system⁹. The reports of reproductive toxicity of cypermethrin has affected spermatogenesis leading to poor semen quality and reduced male fertility⁹. Other studies indicated that treatment of rabbit with cypermethrin caused a decrease in sperm concentration, sperm motility, and an increase in the number of abnormal and dead spermatozoa⁹. Furthermore, cypermethrin exposure has disrupted the pituitary receptor and provoked hormonal imbalance of FSH, LH and testosterone⁹.

Currently, there is an increased request for the use of herbal therapy, instead of using synthetic drugs, which could have harmful effects and therefore may perhaps be more dangerous than the disease itself⁹.

*Salvia officinalis* is an annual herb of the Lamiaceae (Labiatae) family, which grows in countries bordering the Mediterranean Sea and today it has been naturalized throughout the world¹⁰. For thousands of years, this plant has been used in many Countries as a spice and food preservative as well as a health remedy in traditional folk medicine for the treatment of numerous disorders¹¹,¹². *Salvia officinalis* is considered the most powerful source of antioxidants among plant herbs¹⁰, which is due to the complex mixture of monoterpenes, diterpene, sesquiterpenes, flavonoids and phenolic acids. It contains also other phytoconstituents such as cineol, borneol, rosmarinic acid, chlorogenic acid, salvianolic acid or vitamin C and E¹⁰. A review of the literature revealed that hydroethanolic leaf extract of *S. officinalis* showed the highest total flavonoid and phenolic content and antioxidant capacity¹⁰. It has been confirmed that those products also have multiple pharmacological effects including antibacterial¹⁰, anti-diarrhea¹⁰, anti-inflammatory¹⁰, anti-hyperglycemic¹⁰, anti-proliferative¹⁰, immunomodulatory¹⁰, anti-imutagenic¹⁰, and anti-cancer effect¹⁰. Indeed, the existence of these compounds presumably makes *S. officinalis* a potent source against different types of detrimental effects caused by xenobiotics¹⁰,¹⁵.

Hence, this study designed to evaluate the protective effect of the aqueous extract of *Salvia officinalis* (sage) against the reproductive toxicity associated with cypermethrin in male Wistar rats.

Insecticide

Technical grade of cypermethrin (Meghmani organics limited, Panoli, Bharuch, India) was obtained from agro store in Annaba, Algeria.

Plant

The aerial part of Salvia Officinalis L. was harvested in June, 2019 from the region of Annaba, Algeria. The fresh leaves were washed and then grounded in distilled water with a homemade mixer into small pieces; finally, the obtained aqueous extract was filtered using cotton gauze.

Animals

This study was carried out on 30 Wistar male rats procured from Pasteur institute of Algeria. Animals were maintained under standard conditions in polypropylene cages, offered commercial food pellets (Oued Fraga, Guelma, Algeria) and water ad libitum, and then they left to acclimatize for one week before the experiments.

Experimental design

Rats with a body weight of 240± g were divided into six equal groups as follows:

- **Control group (C):** received tap water
- **Positive control group (SLE):** received 500 mg.kg bw of sage leaves aqueous extract
- **Group (Cyp1):** received 1/30 LD50 of cypermethrin (8.33 mg/kg bw)
- **Group (Cyp2):** received 1/10 LD50 of Cypermethrin (25 mg/kg bw)
- **Group (SLE+Cyp1):** received a combination of SLE with Cyp1 (500mg + 8.33 mg/kg bw)
- **Group (SLE+Cyp2):** received a combination of SLE with Cyp2 (500g + 25 mg /kg bw).

The leaves aqueous extract and Cypermethrin were administrated orally for five days a week during one month.

Organ’s weight

At the end of the treatment period, the rats of the different groups are sacrificed by decapitation. Testis and epididymis were removed, trimmed of fat and weighed using a precision balance (K E R N PRS 320-3).

Semen analysis

Semen analysis was realized using the Computer-Assisted Sperm Analysis Method (CASA) using Sperm Class Analysis (SCA®, Microptic, Barcelona, Spain). The epididymal semen was obtained immediately after sacrifice, and then a drop of semen was diluted with NaCl 0.09%, and then 5μL of the mixture was put in a chamber slide (GoldCyto model). The slide was then placed on a Nikon Eclipse (E200-LED) microscope at the phase objective (×4). The concentration, motility and speed of sperm were calculated.

Histological study

The testes and epididymis samples were examined for histopathological changes. They were placed in 10% formol and dehydrated in 70–100% ethanol series, and then they placed in paraffin baths at 58°C. Sections of 4–6 mm were prepared from paraffin blocks using a rotary microtome. These sections were then stained with Hematoxylin–Eosin (H-E) according to the criteria of the method of 25, and then the slides were photographed using a Leica photomicroscope.

Data analysis

The obtained results were expressed as a Mean ± SEM. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA), followed by Tukey’s test using prism 5 software. The significant test was considered at p<0.05.

RESULTS

Organs absolute weight

The obtained results showed a highly significant decrease in testes weight in the group treated with the two doses of cypermethrin compared to the control group. On the other hand, the oral administration of SLE+Cyp1and SLE+Cyp2 to rats increased the weight of the testes in a highly significant way (tab.1) compared to group treated with cypermethrin only. Epididymal weight showed a significant decrease in animals of group Cyp2 compared to the control group (C). However, we recorded a highly significant increase in epididymis weight in group (SLE2) rats compared to group Cyp2.

Sperm concentration and motility

Table 2 demonstrates the results obtained after exposure to sage extract and cypermethrin on sperm parameters of adult male rats. Epididymal sperm concentration was reduced significantly in males that ingested 8.33 and 25 mg/kg bw of cypermethrin. On the other hand, the treatment with the combination of cypermethrin and leaves aqueous extract of Silvia (SLE1 and SLE2) increased in a highly significant way compared to group (Cyp1) and (Cyp2), respectively. For sperm motility, results represented by (Tab 2) showed a very highly significant decrease in the group of Cyp1 and Cyp2 groups compared to the control. Nevertheless, rats treated with the combination of sage aqueous extract and cypermethrin revealed a significant increase in sperm motility compared to rats treated with cypermethrin alone.

Sperm velocity

Results of rat sperm velocity of all groups are summarized in Figure 1. Rapid sperm velocity was significantly decreased in the rats of the (Cyp1) and (Cyp2) groups compared to the control. In contrast of that, there was a very highly significant increase in the groups treated with the combination of sage and cypermethrin (SLE+Cyp1 and SLE+Cyp2) compared with the groups treated only with cypermethrin.

Table 1: Variation in absolute testicular and epididymal weight (g) in the six groups of rats exposed to SLE and Cyp(Mean ± SEM, n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testis weight (g)</th>
<th>Epididymis weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.64 ± 0.03</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>SLE</td>
<td>1.56 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Cyp1</td>
<td>1.02 ± 0.20</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>Cyp2</td>
<td>0.60 ± 0.22</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>SLE+Cyp1</td>
<td>1.62 ± 0.02</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>SLE+Cyp2</td>
<td>1.62 ± 0.02</td>
<td>0.50 ± 0.03</td>
</tr>
</tbody>
</table>

Table 2: Variation of sperm concentration (10^6/mL) and motility (%) in the six groups of rats exposed to SLE and Cyp (Mean ± SEM, n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm concentration (10^6/mL)</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>62.33 ± 0.50</td>
<td>54.09 ± 3.40</td>
</tr>
<tr>
<td>SLE</td>
<td>63.48 ± 7.90</td>
<td>59.44 ± 7.16</td>
</tr>
<tr>
<td>Cyp1</td>
<td>19.33± 4.42</td>
<td>17.89± 6.71</td>
</tr>
<tr>
<td>Cyp2</td>
<td>21.93± 0.71</td>
<td>23.26± 4.35</td>
</tr>
<tr>
<td>SLE+Cyp1</td>
<td>51.83± 4.44</td>
<td>51.48± 4.43</td>
</tr>
<tr>
<td>SLE+Cyp2</td>
<td>56.87± 4.25</td>
<td>55.24± 9.11</td>
</tr>
</tbody>
</table>
Treatment of rats with Cyp1 and Cyp2 induced a significant decrease in mean sperm velocity compared to the control. In the other hand, sage aqueous extract combined group significantly increased the mean sperm velocity in SLE+Cyp1 group compared to Cyp1 and very highly significantly in the and SLE+Cyp2 group compared to the Cyp2 group.

Slow sperm velocity results illustrated a highly significant decrease in the group treated with the low dose of cypermethrin (Cyp1) compared to the control. However, a highly significant increase was recorded in the SLE+Cyp1 group compared to the Cyp1 group and a significant increase in the SLE+Cyp2 group compared to the Cyp2 group.

The percentage of immobile sperm significantly increased in groups exposed to cypermethrin compared to the control. Also, a highly significant decrease in immobile sperm is recorded in the rats of the group treated with the combined group SLE+Cyp1 compared to Cyp1 group, and a very highly significant increase in the group treated with SLE+Cyp2 compared to the Cyp2 group.

The testicular histology

The photomicrography of the transverse section in the seminiferous tubules of the testes of rats in the control and treated group is illustrated in figure 3. The control groups (c) showing seminal tubes with normal-looking spermatogenesis, mature spermatooza fill almost all the lumen of the seminal tubes that respect its diameter. Likewise, the positive control group (SLE) showed a normal tissue in good condition (defining all stages of spermatogenesis), similar to the control group. While the treated group (Cyp1) showed the presence of some vacuolization, circular empty zones, luminal spermatooza reduction, we can say that cypermethrin has affected the course of spermatogenesis. Furthermore, CYP2 treated group showed seminiferous tubules with low intensity compared to the control group. There was also an alteration in the first stage of spermatogenesis which may be due to cell necrosis or apoptosis caused by the toxic product, resulting in the absence of spermatogenetic cells. However, (SLE+Cyp1) group showed a return to the normal overall structure of the testis compared to (CYP1) group. In regards to (SLE+Cyp2) group, we noticed a decrease in spermatooza of seminiferous tubules compared to the CYP2 group. But it can be noted that the administration of the aqueous extract may have improved the overall structure of the testicle.

The epidydimal histology

The control (c) and positive control (SLE) groups showed normal tissue with an epididymal tubes lumen full of spermatozoa. While in treated groups (Cyp1) (Cyp2) we noticed an alteration and a decrease of spermatozoa in the lumen of the epididymal tubes compared to the control groups (c) and (SLE). Nonetheless, in (SLE+Cyp1) group we observed an increase in sperm density in the epididymal ducts compared to CYP1 group. We have also remarked a decrease in sperm density in (SLE+Cyp2) group, as compared to the CYP2 treated group.

DISCUSSION

Exposure to toxic substances from the environment may alter spermatogenesis, testicular morphology, and reduced reproductive organs weight. Our experimental results indicate a highly significant decrease in testis absolute weight accompanied with a significant reduction in epididymis absolute weight in rats treated with cypermethrin. These results correlate well with those of. There were several studies indicating that xenobiotics directly influence Sertoli cells function, hence spermatogenesis impairment and epithelial disorganization which may cause tubular atrophy. Thus, the decrease in testis weight may be caused by regressive and necrotic changes with germ cells number reduction induced by cypermethrin in the seminiferous tubules. The changes in epididymis weight could due to the alteration of tubules epithelial cells and reduction of sperm count in the epididymis lumen. In addition, the decrease in organs weight might also be to the oxidative stress induced by cypermethrin, leading to spermatogenic cells necrosis associated with sperm abnormalities.

The decrease in testicular and epididymal weights has also corresponded to the histopathological and cytopathological changes of the male reproductive system. The present study indicated that cypermethrin caused regressive histological changes in the seminiferous tubules, resulting in the suppression of spermatogenesis, expansion of interstitial spaces, vacuolization, and circular empty zones. Our results are in agreement with the results of who found significant decrease in the cell layers of testicular seminiferous tubules, which contained a large number of immature spermatids, congested blood vessels, vacuolization, and marked hemorrhage further significant thickening of the connective tissues surrounding the tubules in testes of...
Figure 2: Photomicrography of transverse section in the somniferous tubules on the testes of rats in the control and treated group (H.E.400X). 1-Seminal tube, 2- Spermatozoa, 3- Leydig cell, ( breve ) spz decrease, ( arrondi ) alteration ( circled ) Absence of spz .

Figure 3: Photomicrography of transverse section in the epididymis tubules on the testes of rats in the control and treated group (H.E.400X). 1'- The limiting membrane, 2'- The epithelium, 3'- Spermatozoa. ( breve ) spz decrease, ( arrondi ) alration, ( circled ) Absence of spz, ( circled right ) Increase of spermatozoa.
rat treated with cypermethrin. In support, a wide array of abnormalities represented in a degenerative changing in spermatogonia cells, necrosis, and separating of cells from basal region of somniferous tubules were seen in histological sections of the testes after cypermethrin exposure. Epididymis morphology was also altered by cypermethrin and this was demonstrated by the alteration and the decrease of sperm count in the lumen of the epididymal tubules with a reduction of their diameters. These changes may be due to a disturbance in testosterone biosynthesis, which may provoke germinal epithelium seminiferous tubular degeneration, which is in line with spermatogenesis suppression and decrease of spermatozoa in testis and epididymis.

Exposure to cypermethrin caused a significant reduction in sperm concentration in a dose dependent manner. The results are in agreement with those reported that ingestion of 34,286 ppm cypermethrin for 12 weeks per male rats significantly reduced sperm count. Thus, cypermethrin may produce testicular steroidogenic alterations by affecting indirectly hypothalamus-ovary axis or by acting directly on Leydig and Sertoli cells, leading to a decrease in testosterone biosynthesis; which is the key hormone of spermatogenesis regulation. Diminishing in sperm concentration may also be due to necrosis and loss of germ cells. Demonstrated a degeneration and depletion of spermatocytes and spermatids in adult pheasants exposed to cypermethrin. The capacity of sperm production depends on the number of Sertoli cells which serve as support to germ cells’ development.

Regarding the drop in sperm mobility and velocity in the groups treated with the different doses of cypermethrin of the actual study, a reduction of net weight of testes was reported after oral administration of Cyp for 14 days. Moreover, Cypermethrin can inhibit mitochondrial ATP production, which influences fructose synthesis, or spermatozoa microtubule structure. Consequently, perturbations in mitochondrial activity can reduce the sperm motility and velocity. Moreover, the detrimental effects of cypermethrin on reproductive performance can be associated to excessive free radical formation, which may cause sperm DNA damage and inhibit the activities of antioxidant enzymes leading to spermatogenic cells degeneration that is associated with sperm abnormalities. Likewise, oxidative stress is likely to produce membrane integrity damage therefore, decreased sperm motility and viability.

Recently, reported that non-occupational environmental pyrethroid exposure could have a negative impact on sperm DNA integrity and semen quality in Chinese males. Thus, the excessive ROS generation can induce oxidation of polysaturated fatty acids in membranes of sperm lipids, which alters the structure of lipid matrix that is associated with rapid loss of intracellular ATP leading to axonemal damage, increased mid-piece morphological defects, and inhibited spermatozoa activity.

On the other side, sage aqueous extract improved reproductive organs weight and sperm parameters when administrated simultaneously with cypermethrin. indicated that hydroalcoholic leaf extract of Salvia officinalis has excitatory effects on male reproductive system leading to increase in serum testosterone level and spermatogenesis. Such effect was perhaps linked to officinalis extract by improving the antioxidant defense system and suppressing the oxidative stress. In addition, has demonstrated that aqueous extract of S. officinalis had a positive effect on some fertility parameters and pituitary-testicular hormone axis. The aqueous extract of sage had high content of flavonoids, and was rich in cineol, borneol, pinene, saponin, glycoside, resin, vitamin C and E, rosmarinic acid, chlorogenic acid, caffeic acid, methyl rosmareinate, cinnamic acid, quinic acid, ferulic acid, apigenin, luteolin, quercetin steroids and tannins, endowed with a strong antioxidant power. It has been reported that flavonoids have a series of biological effects such as lipid peroxidation reduction because of their antioxidant properties and their ability of removing free radicals and chelating divalent cations. On the other hand, quercetin as an important dietary flavonoid has a prominent pharmacological effects, such as free radical scavenging and the TNF-α inhibition which is an extensive factor that stimulates inflammatory pathway resulting of oxidative damage. It is also proved that quercetin has enhanced rats’ sperm viability, motility, and serum total testosterone. Furthermore, Vitamins C and E have a beneficial effect on male infertility treatment and sperm function.

CONCLUSIONS

Based on these findings, we conclude that cypermethrin toxicity affected reproductive function of adult male wistar rats, which is manifested in reduced organs weight, decreased sperm concentration, motility and velocity. Salvia officinalis has a beneficial effect on preventing cypermethrin-induced testicular damages by enhancing sperm quality, and this perhaps is linked to its phenolic and flavonoid compounds, which has free strong radicals’ scavenging activity.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest with this work.

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AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration among all authors.

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CHOUABIA A, et al.: Protective effect of Salvia officinalis against cypermethrin-induced reprotoxicity in male Wistar rats


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GRAPHICAL ABSTRACT
CHOUABIA A, et al.: Protective effect of *Salvia officinalis* against cypermethrin-induced reprotoxicity in male Wistar rats

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