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Histological, ultrastructural and molecular studies on the effect of *Coustus speciosus* extract on raising the efficiency of fertility in the testes of male rats treated with risperidone (antipsychotic drug)

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ABSTRACT

Psychiatric disorders affect a high percentage of the world`s population. Schizophrenia represents more than 90% of the inpatients of mental health hospitals. Risperidone is an important drug in the treatment schizophrenia. The study was designed to assess efficiency of Costus extract in limiting side effects occur as a result of the use of risperidone. Rats were divided into six groups G1 control animals. G2 treated with Costus extract. G3+5 treated with risperidone at low & high doses. G4 +6 treated with both risperidone at two dose and Costus extract. Bioassay results of blood`s rat in G3,5 showed below-average levels of testosterone, high average levels in rat in G2,G4,G6. Histological and ultrastructural observations in testes of rats in G3, 5 showed: atrophy of several seminal tubules, necrosis of spermatogenic cells, pyknotic nuclei and loss of sperms. Rat in G4, 6 showed: Seminiferous tubules had a regular architecture more or less similar to control. Risperidone induced increase of *CYP*3A, *CYP*2E1, *CYP*2C6 and a decrease of *CYP*1A1 levels of P450 In testis. High-dose risperidone with Costus induced a decrease of all previs gene levels. Costus extract was raising the efficiency of fertility in the testes of male rats treated with risperidone.

Keywords: costus extract, risperidone, testis, ultrastructural, molecular, fertility.

1. INTRODUCTION

Psychiatric disorders affect large numbers of population in different age groups, and of various economic and social strata. Statistics indicate substantial increase in the prevalence of mental disorders in the world because of many interrelated psychiatric, biological, and social factors (Washington, 1994). Schizophrenia represents more than 90% of the inpatients of mental health hospitals and care facilities. With accurate diagnosis, the incidence of

schizophrenia appears to be stable in all parts of the world in the past half-century, i.e. 24 million people in the world, as of 2011 (Burada, 2009). Risperidone is atypical "second generation" and a derivative Benziasul is used to treat acute and chronic psychiatric conditions. Risperidone is important drug that is widely used in the treatment of schizophrenia and other psychiatric diseases, for a long period, mostly for life, (Lin et al., 2006a). Due to the side effects of using antipsychotics for long periods, it was not easy to assess the effects of antipsychotics on sexual function in patients with schizophrenia (Collins & Kellner, 1986). But it was observed that the great majority of these patients have diminished sexual activities, in particular, patients treated with typical antipsychotic (Ghadirian et al., 1982; Lingjaerde et al., 1987). It was known that antipsychotic drugs have the potential to cause sexual dysfunction through several direct or indirect mechanisms, especially in men, because it is associated to receptors of dopamine, histamine, choline and adrenaline which may affect sexual function directly (Culter, 2003; Knegtering et al., 2003; Haddad & Wieck, 2004). The sexual dysfunction associated with risperidone and the percentage was 43.2% that it related to the therapeutic dose of the drug (Bobes et al., 2001). Sexual dysfunction rate increased in male rats after treatment antipsychotic (risperidone), where he noted the low rate of testosterone in the serum, decrease in the weight of the epididymis, seminal vesicles and prostate gland Zhang et al. (2007 & 2011). This study aims to explore natural extracts to limit these effects. The study was designed to assess efficiency of Costus extract, as Prophetic medical treatment, and its role in limiting side effects expected to occur as a result of the use of risperidone.

Costus

Kingdom: Plantae.
Division: Spermatophyta
Sub- Division: Angiospermae
Class: Monocotyledoneae
Sub- Class: Commelinids
Order: Zingiberaeceae
Family: Costaceae
Genus: Costus
Species: *Speciosus*
(Specht & Stevenson, 2006)

Several studies have confirmed that the extract of the Costus roots was very rich in bio-compound Sesquiterpene lactones and containing Germacrenes, So was selected to prevent or reduce side effects and raise the efficiency of fertility in testicular tissue, it use to improve the level of blood sugar and lose fat therefore the possibility of its use for the treatment of diabetes Mellitus (Elizaa *et al.*, 2008; Ranjitha *et al.*, 2013). Detecting the presence of Filavonat (Anti-inflammatory) in costus extract explained effectiveness of its roots as a high anti-oxidants and thus be used as a treatment for many diseases and disorders (El sawi *et al.*, 2010). Costus extracts methanol and methanol dichloride in the subfamily Zingibeeraceae Including costus speciosus showed an anti-microbial activity and antioxidant (Habsah et al., 2000). The fruits of subfamily Aframomum (Zingiberacea) used as an aphrodisiac at the People's tribal in southern Nigeria (Lwu, 1993). Several vital compounds of Costus plant the most important Costunolide (antioxidant and antibacterial), Cynaropicrin which contains Lactons (anti-inflammatory) and dehydrocostus (which causes to stop cell cycle and apoptosis) this has encouraged researchers to Costus -manufacturing as a medicine (Pandey et al., 2007). It proved the effectiveness of the methanol extract of C.speciosus in inhibition of liver cancer cells, and apoptosis after using different concentrations of it (Nair et al., 2014). The present study aims to highlight the effectiveness of using the costus-extract in reducing the side effects expected to occur on testicular tissue after use of risperidone (anti-psychotic) and raise it to the level of efficiency of fertility in male rats.

2. MATERIALS AND METHODS

Risperidone

Risperidal is a brand name in the form of an oral solution of a concentration of 1 mg / mL, and molecular formula C23H27Fn4O2. Molecular weight is 410.4845, obtained from the manufacturer Janssen. The animals were treated with risperidone once daily by oral injection by stomach tube.

Costus Extract

Costus roots was Obtained from herbs shops in Jeddah, Saudi Arabia, then washed, crushed and preparation extracted by adding 100 ml of boiling water to 10 grams of powdered roots in cups dark and covered at 24 hours then filter the solution (Domiaty, 2009), the supernatant was put in a dark bottles and saved at a temperature of 4°C, then injected the animals once daily orally by Stomach tube.

Experimental Animals

Experiments of this research was conducted on 30 male albino rats of the Wister strain and ranged in age from 40-45 days (6 weeks) and weights between 68-122jm, rats were obtained from the Animal House, Faculty of Pharmacy, King Saud University. Breeding animals are kept in a private room, at a temperature of 2 ± 24 m º relative humidity at 5 ± 55%, periods of light and darkness at 12 hours each. Rats supplied the feed and water. Experimental duration is from Jan 2018 to Jun 2020.

Treatment Schedule

Divided the experiments animals into six main groups as follows:

1- First Group: composed of 5 rats of control animals that were given distilled water orally throughout the experiment.

2- Second Group: composed of 5 rats that were treated orally with only Costus extract equal at a dose similar high-dose of the drug (2 mg/kg b.w).

3- Third Group: composed of 5 rats that were treated orally with risperidone at a low dose level equal to 1 mg/kg b.w.

4- Fourth Group: composed of 5 rats that were treated orally with both risperidone at the low dose and Costus extract.

5- Fifth group: composed of 5 rats that were treated orally with risperidone at a high dose level equal to 2 mg/kg b.w.

6- Sixth Group: composed of 5 rats that were treated orally with high-dose risperidone at dose level 2 mg/kg b.w and Costus extract (2 mg/kg b.w).

Samples Collection

The animals were treated for ninety days, which is the duration of the experiment, and were dissected after 90 days. Blood samples were collected in special EDTA anticoagulant tubes. Blood tests were done to determine testosterone. The testes were excised, placed in a suitable fixative, and prepared for histological, ultrastructural and molecular studies. The effective doses and the various concentrations of all treatments were determined by performing several initial experiments for each material used in this research.

Histology Study

This part of the research study of histological changes in the testis by light microscopy, which it were fixed in 10% Neutral buffered formaline Ph (2,7 to 4,7) for 48 hours (Hopwood , 2002), And then dewatering operations through a series of alcohol (70%, 90%, 100%) and imbedding in paraffin wax, it has also been cutting cross-sections thickness of 3-5 µm using Rotary microtome, and carried sectors on glass slides and then colored dye Himatoxlan- Eosin (Bancroft & Gamble, 2007).

Ultrastructure Study

To examine the transmission electron microscope was fixed small portions 1mm from the testes of rats in Jlauterolad head 4% in 0.2 mole of cacodylate buffer which coolant at a temperature of 4°C for 24 hours and post-fixed in the osmium tetraoxide dissolved in the same buffer at room temperature. Then samples were dehydrated through a concentration in ascending of ethanol and in propylene oxide, and then embedded in the ricin. The samples were cut 1µm thick semi-thin sections and loaded onto glass slides and stained with dye toluidin blue in 1% borax. Ultrathin sections were cut by diamond knife, and then loaded on copper grids where dye by lead citrate and uranyl acetate (Woods & Stirling, 2002).

Molecular Study

RNA Extraction

Frozen testicular tissues were thawed on ice, and total RNA was isolated using (QIAgene RNeasy mini kit, cat # 74104) according to the manufacturer's instructions.

Quantification and quality measurments and working RNA

The quality of the isolated RNA was assessed by electrophoresis on a 1% agarose gel based on the integrity of 28S and18S bands after ethidium bromide staining. The degree of purity of RNA, It was know through absorption wavelength ratios A 260 nm / A 280 nm RNA (Nano Drop Spectrophotometer).

Design of real-time quantitative polymerase chain reaction PCR primers

Table 1 Sequences of primers used for the RT-PCR analysis.

Isoforms	Primers sequence (5`-3`)
CYP1A1 F	GGG AGG TTA CTG GTT CTG G
CYP1A1 R	ATG AGG CTG TCT GTG ATG TC
CYP2C6 F	GAC CTC ATT CCT ACC AAC CT
CYP2C6 R	CCT CTC CTG CAC ACA TCC
CYP2E1 F	CCT TTC CCT CTT CCC ATC C
CYP2E1 R	AAC CTC CGC ACA TCC TTC C
CYP3A9 F	GGTGTTGTATCACATGGACCAGA
CYP3A9 R	CAGGAGTGAACAAAATTACTGCA
GAPDH F	GAT GGT GAA GGT CGG TGT G
GAPDH R	ATG AAG GGG TCG TTG ATG G

F - Forward, R - Reverse, GAPDH - glyceraldehydes phosphate 3-dehydrogenase

Primers for CYP3A9 were synthesized according to (Anakk et al., 2003). Primers for GAPDH, CYP1A1, CYP2C6 and CYP2E1 were synthesized according to (Mrozikiewicz et al., 2010). It was Request from a company: Invitrogen by Life Technologies (table 1).

Quantitative Real-time PCR

Quantitative polymerase chain reaction is used RNA as the basis interaction in the presence of a reverse polymerase to measure by using quantitative polymerase chain gene expression (qReal-time) detection QIAgen kit, according to the method recommended by the company distributed (Quantitect SYBR Green RT-PCR, cat # 204243)

Statistical analysis

Statistical analyses were performed using SPSS software version 21.00. One-way analysis of variance (ANOVA), post-hoc and least significant difference (LSD) were performed for inter-group comparison. $P > 0.05$, $P \leq 0.05$ and $P \leq 0.001$ were considered non-significant, significant and highly significant, respectively.

3. RESULTS

Morphological and body, testes weights changes

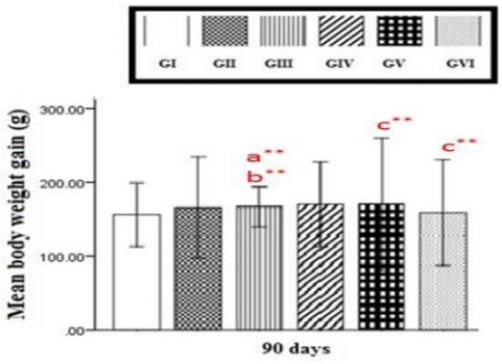

Figure 1 The body weight in rats treated orally with risperidone and *Costus speciosus* after 90 days (Values are mean±S.D of five animals) ** (P<0.01), (a=G1, b=G2, c=G3, d=G4, e=G5, f=G6).

No mortality and morphological changes were noticed during the experiment in general. Body and testes weight of the groups treated with Costus extract alone and with risperidone were more or less similar to the control group, Treatment with a low dose of risperidone induced a significant increase in body weight compared to control (figure1), while the rats treated with both doses of risperidone induced insignificant reduction in testes weight (figure 2).

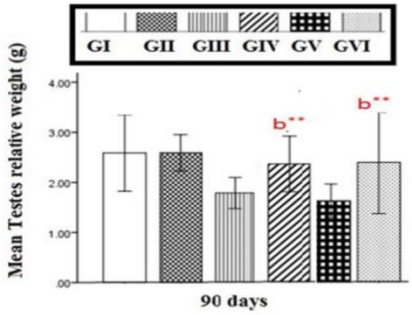

Figure 2 The testes weight in rats treated orally with risperidone and *Costus speciosus* after 90 days (Values are mean±S.D of five animals) ** (P<0.01), (a=G1, b=G2, c=G3, d=G4, e=G5, f=G6).

Changes of bioassay parameters

Treatment with risperidone only at low and high doses led to insignificant reduction in the level of testosterone after 90 days. Treatmeant with costus extract did not show significant differences throughout the study period, all values ranged within the control limit (figure 3).

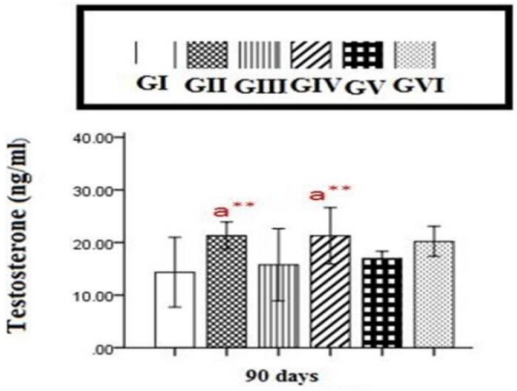

Figure 3 Plasma levels of testosterone in rats treated orally with risperidone and *Costus speciosus* after 90 days (Values are mean±S.D of five animals), ** (P<0.01), (a=G1, b=G2, c=G3, d=G4, e=G5, f=G6).

Histological and ultrastructural changes of testes

Histological observations of testes of rats treated with low and high doses of risperidone after 90 days, showed the following changes: Deformation and atrophy of several seminal tubules with a lack of germ cells. Cells separation and exfoliation of spermatogenic cells is towards the lumen of seminiferous tubules. Necrosis of spermatogenic cells with, pyknotic nuclei, were observed. Loss of sperms in most lumina of seminiferous tubules was noticed. An increase of intertubular connective tissue (mainly collagen fibers), cellular leakage and dilatation of blood vessels is with RBCs stasis. The severity of pathologic changes in the tissue of the testis was proportional to the increase of the cumulative dose of risperidone (figure 4C, E).

The ultrastructural changes of testis of treated rats with high- dose risperidone for 90 days showed: Disorganization of seminiferous tubules due to a disappearance of spermatogenic cells. Nuclei of spermatogenic cells appeared pyknotic, dark, with high electron density.Vacuolar degeneration cytoplasm was observed in spermatogenic cells type B. Aggregation of early spermatids in the lumen of seminiferous tubules were noticed. Atrophied Sertoli cells with degenerate cytoplasmic organelles were detected. Increased number of Leydig cells in the intertubular space was observed (figure 7). No histological (figure 4B) and ultrastructural (figure 6) alterations were observed in testes of rat treated with Costus extract only.

Histological examination of testes of rat treated with both costus extract and risperidone at low and high doses after 90 days showed that: Seminiferous tubules had a regular architecture more or less similar to control. Spermatogenic cells in the testes were observed in regularly arranged rows with different stages of spermatogenesis (especially sperms in the lumen) (figure 4 D, F). Intertubular tissue appeared more or less similar to control (figure 5).

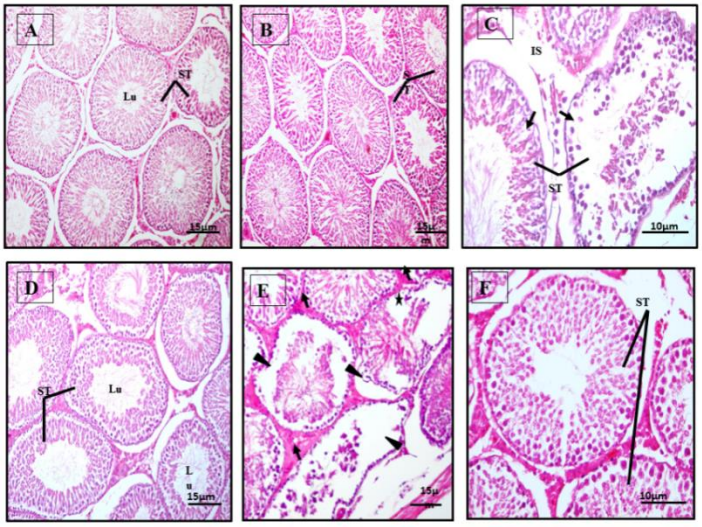

Figure 4 Light micrographs (LM). T.S. Testis of rat after 90 days. [Specimens fixed in 10% buffered formalin and stained with Haematoxylin and Eosin]. **A**. control rat Showing normal histological structure of the seminiferous tubules (ST) populated by spermatocytes and late spermatids (*) surrounded the tubular lumen (Lu). **B**. costus extract treatment. Showing regular and hyperspermatogenesis within seminiferous tubules (ST) more than control. **C**. low dose (LD) risperidone treatment. Showing disorganization of the normal appearance of the testis seminiferous tubules (ST) with atrophy, disorganization of germinal epithelium ,and loss of spermatogenic cells type (arrow) with massive and throughout germ cells destruction; interstitial space (IS); (compare with A). **D**. low dose (LD) risperidone and costus extract treatment. High magnification of the seminiferous tubules (ST) showing regular different successive stages of spermatogenesis surrounding a central lumen (Lu). **E**. high dose (HD) risperidone treatment. Showing degeneration of lining germinal epithelium (head arrows) and vacuolation (*) of seminiferous tubules (ST) and with intertubular oedema (arrows). **F**. high dose (HD) risperidone and costus extract treatment Showing evident restoration of spermatogenesis within regular circular seminiferous tubules (ST) ,compare with fig. (E). Notice, eosinophilic fibrillar interstitium.

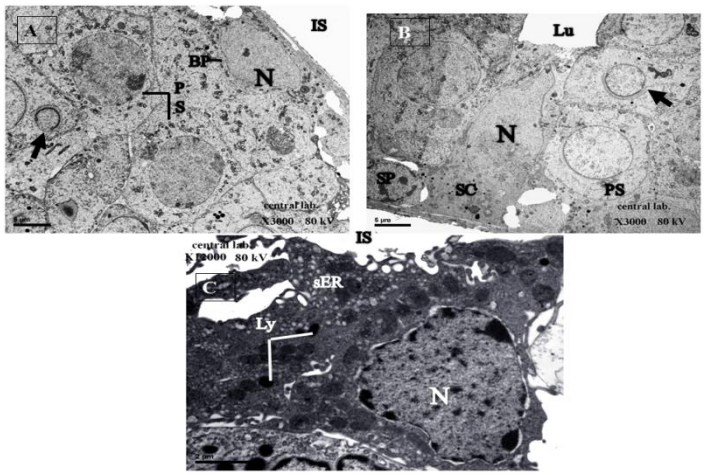

Figure 5 Transmission electron micrographs (TEM) . T.S. testis of Control rat after 90 days. [specimens fixed in 4%buffered glutaraldehyde fixed, post fixed in osmium tetroxide (OsO4), uranyl acetate and lead citrate stained preparations]. **A**. Showing different types of germinal epithelium , type B pale spermatogonia (BP),nucleus(N) of primary spermatocytes (PS), very early spermatid (arrow) , tunica propria (TP), testicular interstitial(IS). **B**. Showing Sertoli cell (SC) extend from the basal lamina to the luminal surface (Lu) of the seminiferous epithelium ,its nucleus (N) is large ,lightly stained and its long axis is oriented perpendicular to wall of the tubule ;spermatogonia; primary spermatocyte (PS); very early spermatid formation(arrow). **C**. showing portion of Leydig cell, the cytoplasm contains an abundance of sER, nucleus (N), lysosomes (Ly), interstitial space (Is).

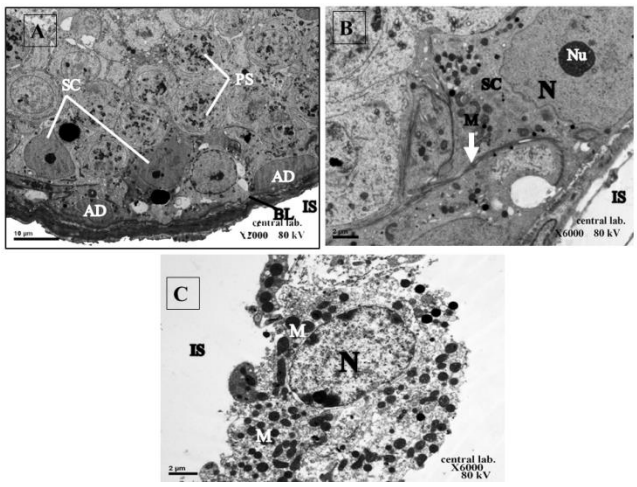

Figure 6 Transmission electron micrographs (TEM). T.S. testis of treated rat with costus extract after 90 days. **A.** Showing normal testicular architecture with regular course of spermatogenesis, spermatogonia (SG), type A dark (AD) primary spermatocytes (PS). Notice, active primary spermatocytes (PS); intertubular space (IS), basal lamina (BL); proliferation of sertoli cells (S), compare with control. **B.** A part of seminiferous tubule showing the Sertoli cell (SC) with nucleus (N) and prominent nucleolus (Nu) indicating highly activity. Notice, blood testis barrier, numerous distinct mitochondria (M). **C.** Showing Leydig cell with large number of mitochondria (M) compare with control.

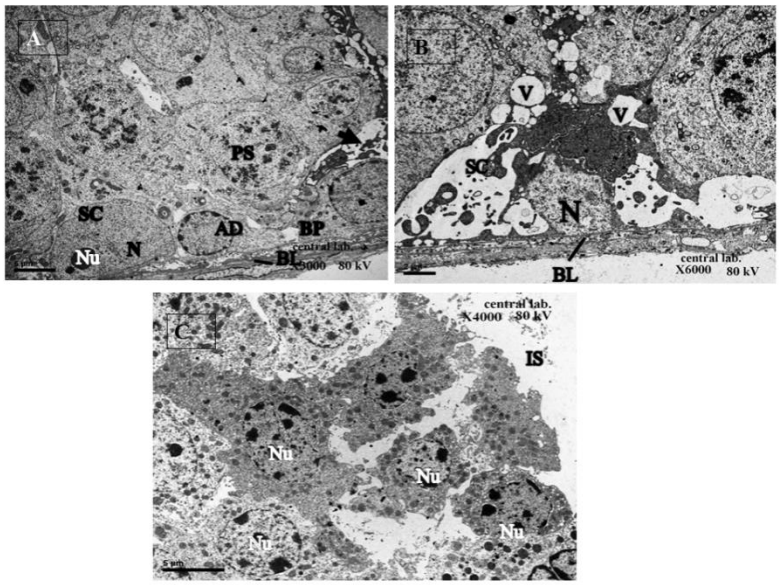

Figure 7 Transmission electron micrographs (TEM) . T.S. testis of treated rat with high dose (HD) of risperidone after 90 days. **A.** Showing dense chromatin of primary spermatocytes (PS); phagolysosome (arrow); type B pale spermatogonia (BP); nucleus (N) &nucleolus of Sertoli cell (SC). **B.** Showing karyorrhexis of Sertoli cell (SC) with highly vacuolated cytoplasm (V). **C.** Showing Leydig cells with marginated multinucleoli (Nu); interstitial space (IS).

The ultrastructural changes of testis of treated rats with high dose risperidone and Costus for 90 days showed that: Recognizable developmental stages of spermatogenic epithelium including sp, ss, and ps were detected. Sertoli cells appeared as elongated cells with its giant nuclei, prominent nucleoli, ER and mitochondria. The intertubular space was full of moderate number of Leydig cells and regul bloodvessels. In general, treatment with Costus inhibited the histopathological alterations induced by risperidone within the testis (figure 8).

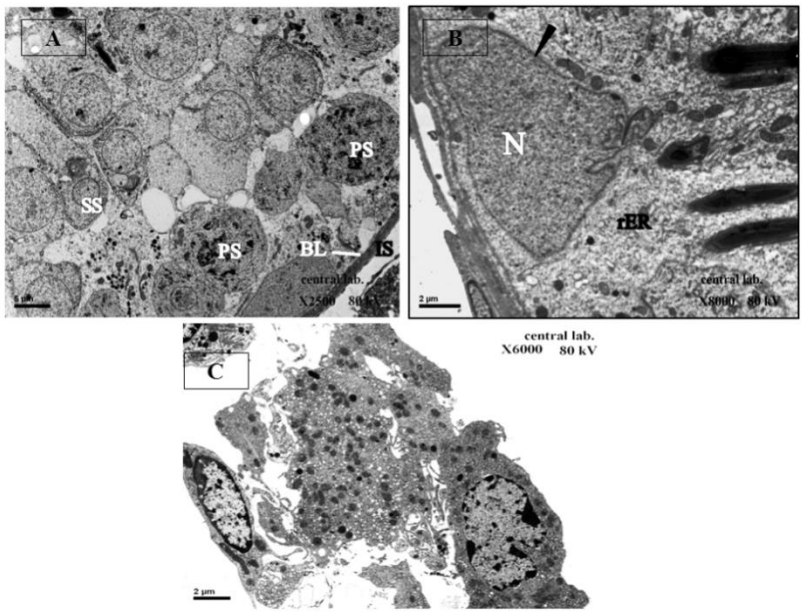

Figure 8 Transmission electron micrographs (TEM) . T.S. testis of treated rat with high dose (HD) of risperidone and costus extract for 90 days. **A.** Showing normal testicular architecture with the regular course of spermatogenesis, primary spermatocyte (PS), secondary spermatocyte (SS). Notice, basal lamina (BL), interstitial space (IS). **B.** Showing sertoli cell (SC) with regular nuclear envelope (head arrow), as well as developing spermatozoa embedded in sertoli cell cytoplasm. C. Showing leydig cells (LC) with regular nuclear envelope of nuclei (N1, N2), almost disappearance of folded invaginated cleft and multiple nucleoli, interstitial space (IS).

Molecular changes

Oral administration of high dose of risperidone for 90 days induced some variations in some genes of cytochrome P450: In testis, there was an increase of *CYP*3A, *CYP*2E1, *CYP*2C6 level, and a decrease of *CYP*1A1 level. Oral administration with costus induced a decrease of *CYP*2E1, *CYP*1A1 level; there is an increase of *CYP*2C6 and *CYP*3A9 levels in testis. Administration of high -dose risperidone with Costus induced a decrease of all gene levels of cytochrome P450 (*CYP*3A9, *CYP*2E1, *CYP*2C6, *CYP*1A1) in testis were observed (figure 9A, B).

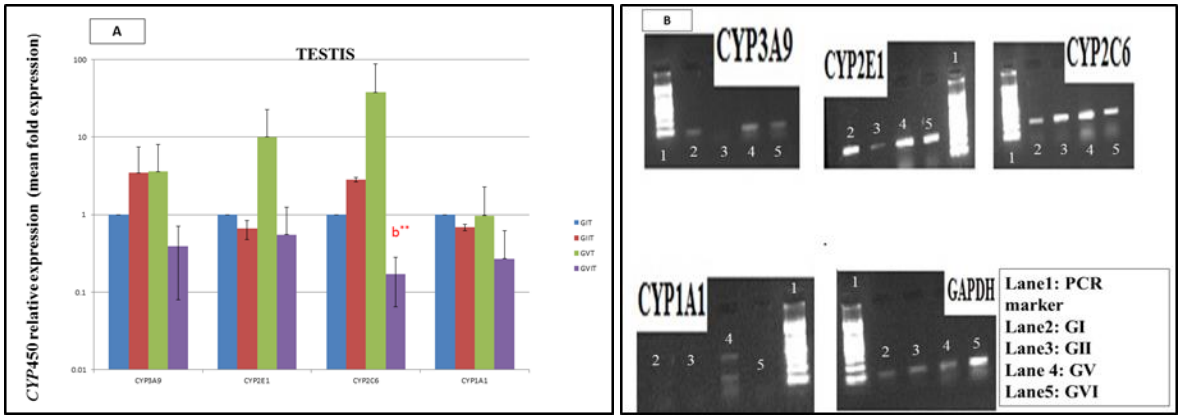

Figure 9 The influence of risperidone and *Costus speciosus* on the activity of cytochrome p450 in the testis rats after 90 days of treatment (Values are mean±S.D of five animals), ** (P<0.01), (a=G1, b=G2, e=G5, f=G6).

4. DISCUSSION

Results of the current study showed that male rats treated with risperidone low and high doses have recorded a decrease in testicular weight in addition to low testosterone level after 90 days compared to the control group, due to the low level of testosterone in the blood plasma of rats treated with the drug, but the level of the hormone group was treated with a drug risperidone less than the minimum has been attributed to the deterioration of cells Lydge (Konarzewska *et al*., 2009; Soliman et al., 2014). The current study also showed that both groups treated with risperidone a sharp decrease in the number of sperm cells, seminiferous tubules diameters, and increase the number of Sertoli cells which caused a decrease spermatogenesis. These

findings caused changes in the testes tissue, increase prolactin levels, As an increase in prolactin levels can hypogonadism occurs because of inhibition of secretion of gonadal hormones, including: testosterone, leading to a delay in the formation of sperm and changes in testes form or because of the low level of Inhibitor B (IB) in the blood plasma for less than the normal limit after treatment with of atypical antipsychotic. Dysfunction cells Sertoli the existence of a very strong positive relationship between the level of B inhibitor in plasma and between testicular volume and number of sperm that have appeared in patients who suffer from infertility with a high level of FSH. In general, there is a relationship between the damage in sperm levels and manufacturing damper B (Meachem et al., 2001; De Rosa et al., 2003; Chen et al., 2009; Peuskens, 2014). But it turned that the dopamine receptors in the germ cells lining the seminiferous tubules associated with antipsychotic thus these drugs directly affect the sperm. And this explains the effect of risperidone on spermatogenesis process in one of two mechanisms: either a low testosterone level or a direct impact on germ cell receptors (Hyun *et al*., 2002; Otth *et al*., 2007).

The presence vaculoes in Sertoli cells usually due to the decomposition of the germ cells abnormal, premature exfoliation of germ cells, which disintegrated and swallowed by Sertoli cells, which did not digest whole, but piled up like fatty droplets in the cytoplasm of Sertoli cells (Soliman et al., 2014). The low number of germ epithelial that appeared in the current study may have been partly due to the lack of cell division (Nejad et al., 2012). The study appeared in the current Pre-apoptotic spermatogonia such as shrinking and separation from each other and from the basal lamina and mitochondrial vacuolization. The results of this research indicated that increasing the number of cells Lydge in the interstitial tissue after treatment risperidone is believed that the atrophy of seminiferous tubules stimulates the fast-spreading factors, which in turn affect the multiplication of cells Lydge (Nagata et al., 1999; Gomaa, 2000; Cotton et al., 2011). The results of the molecular study showed for this study increase in the level of gene expression, CYP2C6, CYP3A9 and CYP2E1 in the testes of rats treated with high-dose of risperidone for 90 days, while the level of CYP1A1 gene expression of the testes of rats decreased, there are a number of evidence of structural and functional heterogeneity between CYP2 and dopamine which supports that there may be similarities between therapeutic methods for these drugs and substrates CYP2, and there are similar aspects to the above links between the receptor CYP3 and P (P-glycoprotein) (Zhang et al., 1998; fischer et al., 1998).

The results of this research indicates that the treatment with Costus extract only did not show any change in the shape or structure of the testis, while treatment by Costus extract and resperidon together either low or high dose showed effective use of Costus extract in relieve the damage caused by the drug and improve the pathological and anatomical changes. Considering the first study of Costus extract on the histological, ultrastructural and molecular level it was consistent with a number of different studies that have examined the impact of Costus extract on several organs independently with the difference in the type of pathological changes of the organs depending on the different drug in each study. It was found that the flavonoids that are found the roots of Costus extract: Quercetin, Rurin, Apigenin known active neurological characteristics, through beneficial effects in certain diseases such as cancer and cardiovascular, neurological disorders (Williams et al., 2004). Sperm has response for Quercetin, quality improved in terms of (the movement, vitality and focus), in addition to treatment with Quercetin did not cause increased body male weight of rats compared to controls, consistent with the results obtained from the current study (Taepongsorat et al., 2008).

Studies revealed the presence of flavonoids in the roots of Costus extract, which is very strong rid of waste products from type of reactive oxygen ROS factors which effectively prevent the oxidation of red blood cells factors (El Sawi et al., 2010). Oxidation occurs when there is an imbalance between the production of reactive oxygen species ROS and defense system anti-oxidants and thus overcomes side oxidation factors; damage from the physiological due to an increase ROS damage cellular molecules such as fats and proteins as well as DNA (Finkel & Holbrook, 2000). The results also showed that the roots of Costus extract contain some phenolic acids plant such as 2,4-Dihydroxybenzoic which is characterized by multiple characteristics as anti-oxidant and anti-microbial. The results of previous studies that the roots of Costus extract contains many minerals and phenolic compounds (flavonoids and phenolic acids) and it is because the effectiveness of Costus extract as an anti-microbial (bacteria and yeasts) as an antioxidant and as a therapeutic agent for many diseases and disorders.

Costus extract is considered a rich source of antioxidants that work to reverse the free radicals and other reactive chemicals effect, the root of Costus extract also contain zinc and copper (Ocakoglu et al., 2008; El Sawi et al., 2010) which plays an important role in the activity of certain enzymes which are essential for many biological functions in all stages of life, such as the repair of cells and protect them from damage caused by certain antibiotics (Bugel et al., 2005). It must be noted that no results of previous studies on the histological, ultrastructure and molecular level on testes treated with risperidone and Costus extract separate or together to make a comparison.

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5. CONCLUSION

From the present study we conclude the following: Concerning the histological and ultrastructural alterations of testis the oral administration of risperidone has an adverse impact on testis. This effect might be the result of direct toxic impacts on the respective components and/or the indirect effects mediated by bioassay parameters dysregulation. More preclinical investigations are needed for better understanding of the mechanisms. The oral use of costus has a beneficial protective effect against the histopathological and ultrastructural alterations in testis induced by risperidone. The oral administration of risperidon has an adverse impact on the investigated haematological parameters and some detoxification genes.

Ethical approval

This study was approved by the research ethical committee of Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia (27/2019).

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Conflict of Interest

The author declares that they have no conflict of interest.

Data and materials availability

All data associated with this study are present in the paper.

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