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Histopathological Changes in Rat Testes Induced by Potassium Bromate and Potential Ameliorative Effects of Ruta chalepensis Oil Extract

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ABSTRACT

Potassium bromate (KBrO3) is used as a food additive mainly in the bread-making process. Ruta chalepensis (R. chalepensis) is an excellent and valuable bioactive plant that produces a range of complex flavonoids. The present study was aimed to investigate the possible protective and therapeutic effect of R. chalepensis against KBrO3 toxic effects on rat testis using histopathology investigation. Fifty adult male albino rats were used in the present study. The rats were divided into five groups each containing 10 rats. First group was kept as control, second group received oil extract of R. chalepensis, third group was treated with KBrO3, forth group (Protective group) and fifth group (Therapeutic group). All groups of animals were sacrificed at 2 and 4 weeks. The results of the present study showed that the administration of R. chalepensis caused disarrangement of spermatogonia throughout the lumen of seminiferous tubule. Additionally, abnormal widening of interstitial spaces with degeneration of interstitial cells. There was necrosis to the spermatocytes with inhibition of the spermatogenic process. KBrO3 group showed necrosis of seminiferous tubule, loss in some spermatogenic cells. There were fibrous stroma between the seminiferous tubules. Also revealed vacuolation, edematous, hyalinization and loss of interstitial connective tissue cell with haemorrhage between seminiferous tubules. Protective group showed seminiferous tubule atrophy, disrupted germ cell layers and disappearance of Leydig cells. The therapeutic group, showed interstitial hemorrhage, with necrosis in spermatogonia and vacuolation. It may be concluded that KBrO3 is toxic to testis and R. chalepensis at the tested dose is not beneficial as protective and curative agent.

التغيرات النسيجية المرضية في خصى الجرذان الناتجة عن برومات البوتاسيوم والتأثيرات التحسينية المحتملة لمستخلص الزيتي للفيجل

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الكلمات المفتاحية:
الدرسي
برومات البوتاسيوم
فيجل

الملخص

تستخدم برومات البوتاسيوم كمادة مضافة للغذاء بشكل رئيسي في عملية صنع الخبز. الفيجل هو نبات ينتج كمية من مركبات الفلافونويد المعقدة. هدفت الدراسة الحالية إلى معرفة التأثير الوقائي والعلاجي المحتمل للفيجل ضد التأثيرات النسيجية المرضية لبرومات البوتاسيوم على خصى الجرذان. استخدم في الدراسة الحالية

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خمسين من ذكور الجرذان. تم تقسيم الجرذان إلى خمس مجموعات تحتوي كل مجموعة على 10 جرذان. المجموعة الأولى مجموعة ضابطة، المجموعة الثانية جرعت بالمستخلص الزيتي لنبات الفيجل، المجموعة الثالثة استلمت برومات البوتاسيوم، المجموعة الرابعة (المجموعة الوقائية) والمجموعة الخامسة (المجموعة العلاجية). تم ذبح جميع الحيوانات بعد 2 و4 أسابيع. أظهرت نتائج هذه الدراسة أن المستخلص الزيتي للفيجل تسبب في اضطراب في عدد الحيوانات المنوية في تجويف الأنيبيبات المنوية. بالإضافة إلى ذلك، اتساع غير طبيعي للمساحات الخلالية مع تنكس الخلايا الخلالية. كما لوحظ نخر في الخلايا المنوية مع تثبيط عملية تكوين الحيوانات المنوية. أظهرت مجموعة برومات البوتاسيوم نخراً في الأنيبيبات المنوية، وفقدان في بعض الخلايا المولدة للحيوانات المنوية. كما شوهد سدى ليفي بين الأنيبيبات المنوية. كما وجدت فجوات، وذمة، تنكس زجاجي، وفقدان خلايا النسيج الضام الخلالي مع نزيف بين الأنيبيبات المنوية. في المجموعة الوقائية لوحظ ضمور في الأنيبيبات المنوية، وتمزق طبقات الخلايا الجرثومية واختفاء خلايا ليدج. في المجموعة العلاجية شوهد ظهور نزيف خلالي، مع نخر في الحيوانات المنوية وحدوث فجوات. نستنتج من دراستنا هذه وجود تأثيرات سامة لبرومات البوتاسيوم على خصى الجرذان والجرعة المختبرة للمستخلص الزيتي للفيجل ليست مفيدة كعامل وقائي وعلاجي.

جرذان
خصية
أمراض أنسجة

Introduction

Potassium bromate ($KBrO_3$) is a white crystalline substance that is readily soluble in water [1]. It is granules or powder and tasteless. It has no medicinal value but is added to flour as a maturing agent, to dough, to fish paste as a conditioner, and also to beer or cheese [2]. The chemical composition of $KBrO_3$is being exhibited as a bromate of potassium found in the form of white crystals or powder [3]. $KBrO_3$ retarded the growth in growing rats and consequently reduced the pubertal, testicular and epididymal weights and exposure to $KBrO_3$ alters the histology of the rat testis and impaired spermatogenesis [4]. $KBrO_3$ used as food additive in the manufacturing of bread is proven to be hazardous for the human health [3]. Reproductive and developmental toxicity related to bromate exposure has not been documented in humans. However, there is some concern in the literature over the lack of a study with respect to developmental toxicity of bromate [5]. Nevertheless, in another research showed no changes in the mean body weight and testis weight against $KBrO_3$ toxicity on testis of male rats [6]. *Ruta chalepensis* (*R. chalepensis*), commonly known as Fijel, belongs to the family of Rutaceae. It is a native herb of the Mediterranean region [7]. *R. chalepensis* affected spermotrophic activity and caused an increase in sperm count, motility, living percent, reduction in sperm abnormalities and a important increase in testosterone and FSH with no change in the LH and prolactin levels [8]. In the last years some research revealed marked hepatorenal protective effects of *R. chalepensis* extract against $KBrO_3$-induced liver and kidney damage and there was sign of recovery in hepatocytes in therapeutic effect against $KBrO_3$ [9], [10]. Thus, the aim of this study is to investigate the protective and therapeutic effect of *R. chalepensis* against $KBrO_3$ toxicity in rat testis using histopathology investigation.

Materials and methods

Fifty male albino rats (*Rattus norvegicus*), weighing between 275-300 g were used throughout the present study. They were obtained from the animal house of Zoology Department, Science Faculty, Omar Al-Mukhtar University. The animals were housed in groups of five in standardized cages and were located in the same room with constant environmental conditions such as temperature (22±3°C) and humidity (50-60 %). They were supplied with enough rat feed and drinking water *ad-libitum.* All animals were allowed to acclimatize in the environment for two weeks before the commencement of the study which lasted for four weeks.

Preparation of potassium bromate: Potassium bromate with the empirical formula $KBrO_3$ obtained from (BDH) company (England). $KBrO_3$ was orally administrated at a dose 100 mg/kg/b. w. dissolved in distilled water freshly prepared [12] daily for 2 and 4 weeks according to the group distribution.

Preparation of *R. chalepensis*: Leaves of *R. chalepensis* were collected from Al-Jabal Al-Akhdar region on the east coast of Libya. The extraction process for the *R. chalepensis* essential oil followed the methodology described by [11]. The collected leaves were weighed, washed with water, dried and then placed in acetone inside sealed jars for 48 hrs. Solvent was removed from samples by rotary evaporator and then oils were collected. *R. chalepensis* oil extract was orally administrated at dose of 0.5 g/Animal [8], daily for 2 and 4 weeks, which represents the overall experimental duration.

Both doses were orally given through a special stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury.

Experimental animals grouping: The animals were divided into 5 equal groups, each contains 10 male rats: **1) Control group:** Animals of this group received distilled water daily by oral gavage for four weeks. **2) *R. chalepensis* treated group:** Rats received *R. chalepensis* oil extract orally in a daily dose of 0.5 g/Animal, for four weeks. **3) $KBrO_3$ treated group:** This group include rats that were administrated $KBrO_3$ in a daily dose of 100 mg/kg b. w. for four weeks. **4) Protective group:** Animals of this group were first administrated *R. chalepensis* oil extract orally daily for two weeks and secondly administrated daily oral doses of *R. chalepensis* in association with $KBrO_3$ for an additional two weeks. **5) Therapeutic group:** Animals of this group were first provided with oral dose of $KBrO_3$ daily for two weeks, and then were treated orally with $KBrO_3$ in association with *R. chalepensis* oil extract for an additional two weeks.

Collection of tissue samples: After 2 and 4 weeks the testes were removed from abdominal cavity, washed in saline and weighed then testis specimens were fixed in 10 % buffered neutral formalin solution [13], dehydrated in ascending grades of ethyl alcohol (70 %, 90 % and 100 %), cleared in xylene and impregnated and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotary microtome and stained.

RESULTS

Microscopically the testis of the control group showed the normal histological features of seminiferous tubules; the seminiferous tubules

was normal with bundles of normal spermatozoa. The seminiferous tubules were enclosed by basement membrane, lined by stratified spermatogenic and supporting sertoli cells (Figure 1). Animals administered *R. chalepensis* for 2 weeks showed the minimal changes in testis histological observations compared with the testis of control animals, which has illustrated slight degeneration in spermatogonia cells lining the seminiferous tubules and disarrangement of spermatogonia throughout the lumen of seminiferous tubule compared to control can be seen in figure (2). Additionally, in figure (3) after 4 weeks the animals administered with *R. chalepensis* showed abnormal widening of interstitial spaces with degeneration of interstitial cells. There is necrosis to the spermatocytes with inhibition of the spermatogenic process. No mature sperms were detected in the lumen of the seminiferous tubules. Administration of $KBrO_3$ for 2 weeks caused necrosis of seminiferous tubule, disorganization of germinal epithelium, and loss in some spermatogenic cells. There were fibrous stroma between the seminiferous tubules. Also revealed depressed spermatogenesis, loss of sperms, vacuolation and edematous, hyalinization with loss of interstitial connective tissue cell with haemorrhage between seminiferous tubules illustrated in figures (4). The testis specimens collected from animals administered with $KBrO_3$ for 4 weeks showed disturbed spermatogenesis, loss of sperms and detachment of germ cells from the basement membrane. Seminiferous tubule lumen is filled with amorphous material containing cellular debris (Figure 5). In addition nearly complete destruction of the seminiferous tubular contents-germ cells and necrosis and odema of the interstitial spaces. There is no spermatid or spermatozoid in the lumen illustrates in figure (6). In protective group the animals administered *R. chalepensis* for 2 weeks followed by double treatment with *R. chalepensis* and $KBrO_3$ for another 2 weeks and scarified after 4 weeks, the histological architecture of testis showed seminiferous tubule atrophy,disrupted germ cell layers and disappearance of Leydig cells (Figure 7). Finally, the testis in the protective group after 4 weeks exhibited damage close to destructive in testis of the $KBrO_3$ group. In the therapeutic group, no recovery was observed with *R. chalepensis* therapy at the end of the experimentation. The histological architecture of testis showed interstitial hemorrhage, with necrosis in spermatogonia and vacuolation. No improvement the histological structure of the testis. There was accumulation of degenerating germ cells, the sperm revealed more pronounced destructive changes (Figure 8).

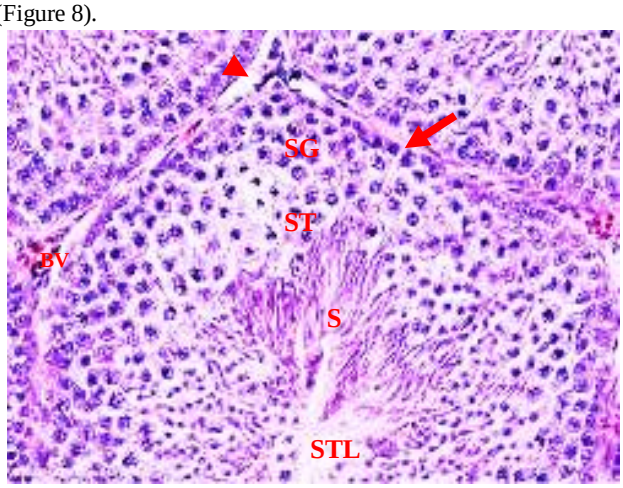

Fig. 1: Photomicrograph of a section in testis of rat from control group, demonstrating seminiferous tubule lumen (STL), interstitial tissue (Head arrow) and, spermatozoa (S), spermatogonia (SG), spermatid (ST), blood vessel (BV) and basement membrane (Arrow) (H & E X 400).

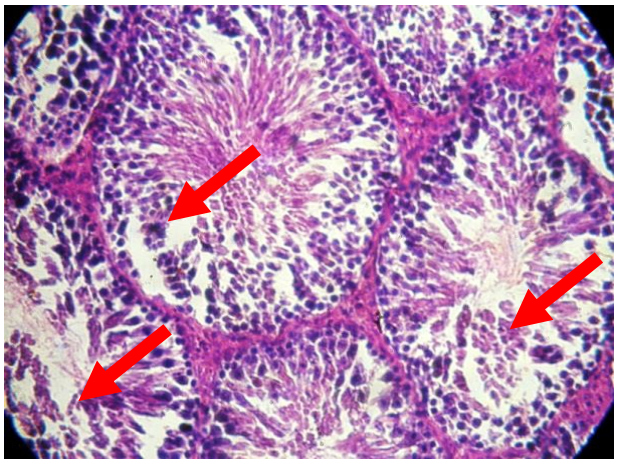

Fig. 2: Photomicrograph of a section in testis of rat treated daily with Rue (0.5 g/Animal) for 2 weeks, showing slight degeneration in spermatogonia cells lining the seminiferous tubules (arrows)(H & E X 400).

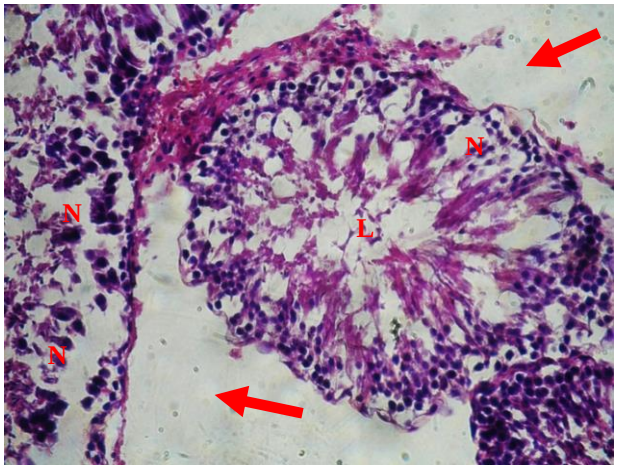

Fig. 3: Photomicrograph of a section in testis of rat treated daily with Rue (0.5 g/Animal) for 4 weeks, showing abnormal widening of interstitial spaces with degeneration of interstitial cells (Arrow). There is necrosis (N) the spermatocytes. No mature sperms were detected in the lumen of the seminiferous tubules (L)(H & E X 400).

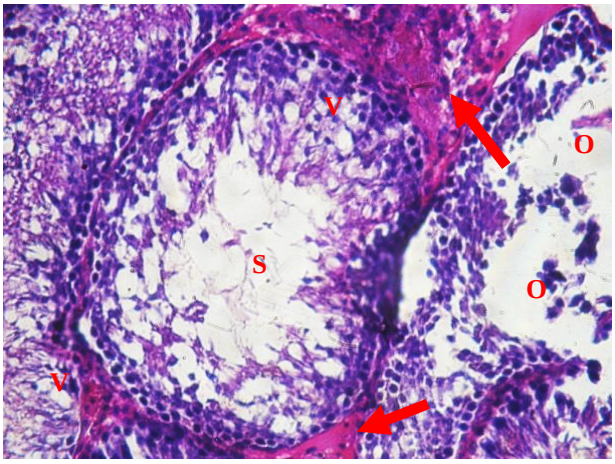

Fig. 4: Photomicrograph of a section in testis of rat treated daily with KBrO3 (100mg/kg) after 2 weeks showing depressed spermatogenesis, loss of sperms (S), vacuolation (V) and edematous in some seminiferous tubules (O) with haemorrhage between seminiferous tubules (Arrow) (H & E X 400).

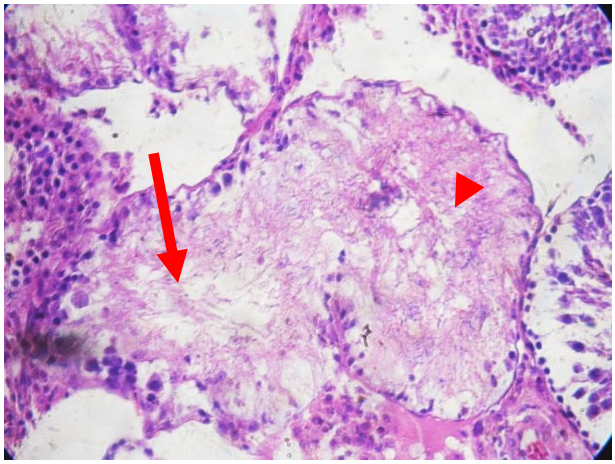

Fig. 5: Photomicrograph of a section in testis of rat treated daily with KBrO3 (100 mg/kg) after 4 weeks showing disturbed spermatogenesis, loss of sperms and detachment of germ cells from the basement membrane (Arrow). Seminiferous tubule lumen is filled with amorphous material containing cellular debris (Head Arrow) (H & E X 400).

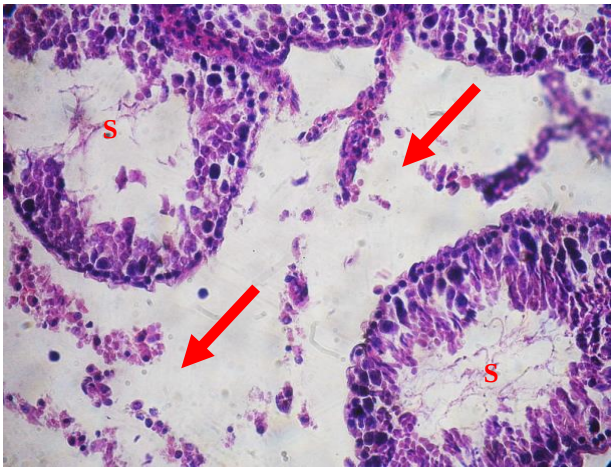

Fig. 6: Photomicrograph of a section in testis of rat treated daily with KBrO3 (100 mg/kg) after 4 weeks showing nearly complete destruction of the seminiferous tubular contents-germ cells and necrosis. Odema of the interstitial spaces (Arrow). There is no any spermatid or spermatozoid in the lumen(S) (H & E X 400).

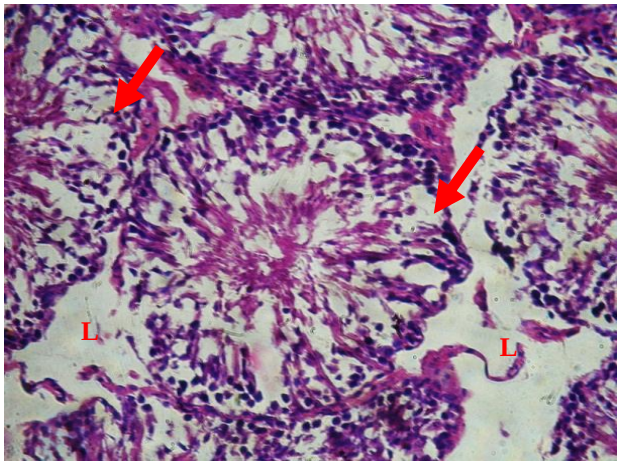

Fig. 7: Photomicrograph of a section in rat testis from protective group after 4 weeks, showing seminiferous tubule atrophy and disrupted germ cell layers (Arrow). Disappearance of Leydig cells (L) (H & E X 400).

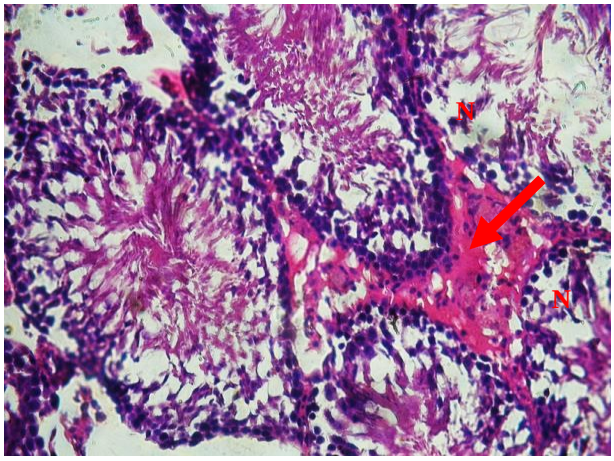

Figure (8): Photomicrograph of a section in rat testis from therapeutic group rat after 4 weeks showing interstitial hemorrhage (Arrow) with necrosis in spermatogonia(H & E X 400).

Discussion

Examination of the rats testis administrated of *R. chalepensis* showed slight degeneration in spermatogonia cells lining the seminiferous tubules. In addition, marked the necrosis of the spermatogonia and spermatocytes in the lumen with inhibition of the spermatogenic process, the broadened interstitial testicular tissue and no mature sperms were detected in the lumen of the seminiferous tubules. Our results obtained in treated rat testis with *R. chalepensis*, agree with those obtained by other researches, they reported that testis of animals examined after treatment with cyclophosphamide exhibited marked degeneration of the spermatogenic cells [14]. As well as, Al-Taie [15] reported that, the testis of the rat treated with 300 and 600 mg/kg b. w. of *R. chalepensis* revealed degeneration of seminiferous tubules with absence of sperm in tubular lumen. Also, produced a significant reduction in the sperm count and motility. Therefore, any agent that damages the viability and function of Sertoli cells may have profound effects on spermatogenesis [16]. In addition, any defective cytoskeleton proteins level could be related to seminiferous tubules damage, including defective spermatogenesis and tight junction between the Sertoli cell and germ cell [17]. So, disturbs genes of cell cycle control may result in histopathological abnormalities of the reproductive organs [18]. Although several studies have demonstrated the noxious effects of various plants and/or their products on sperm motility and morphology, Plants may induce deterioration of sperm

functions either due to the direct action of the active ingredients of plants on sperm cells and/or by targeting Leydig cells or Sertoli cells and the associated functions [16].

The rats administrated with $KBrO_3$, demonstrated necrosis of seminiferous tubule, disorganization of germinal epithelium, and loss in some spermatogenic cells. There were fibrous stroma between the seminiferous tubules. Also, revealed depressed spermatogenesis, loss of sperms, vacuolation and edematous with loss of interstitial connective tissue cell. Moreover, there were no spermatids or spermatozoids in the lumen. Furthermore, thinning of the tubule wall due to reduction in germ cells that make up tubular wall. In addition, seminiferous tubule lumen is filled with amorphous material containing cellular debris. The results of this study are in agreement with the work of Libet *et al.*, [19] they recorded that the spermatocytes and spermatids are necrosis. In accordance with the findings of the present study, Rao and Chinoy [20] suggested that the accumulation of protein occurred in testis and epididymus due to androgen deprivation to target organs. This deprivation effect led to a reduction in testicular and cauda epididymus sperm population, loss of motility in the latter and an increase in number of abnormal spermatozoa, thereby manifesting 100 % failure in treated animals. The maturation arrest observed in the present study was explained by El-Wessemy [21]; they correlated this arrest to the testosterone inhibition which caused stopping of spermatogenesis. The histopathological sections of the testes revealed impairment of spermatogenesis process. These changes are due to an iodine deficiency induced by $KBrO_3$ in water. Moreover, iodine helps to induce eliminate the oxidative stress, which lead to germ cell apoptosis, because it neutralizes the hydroxyl ions [22]. The observation such as hypospermatogenesis (decrease in germ cells) appeared in $KBrO_3$ treated rats is similar to a study by [23]. In conditions with severe depletion or absence of germ cells within the seminiferous tubule, resulted from atrophy of tubules [23]. Additionally, there was an increased fibrosis of peritubular tissue causes a reduction in the size of seminiferous tubules. This is a normal feature of aging. However, increased fibrosis in tunica propria in earlier stages is usually associated with infertility as a result of decrease in rate of sperm production [23], [24]. In our study, the sloughing of early spermatids and vacuolar degeneration in some damaged seminiferous tubule cells proved the presence of signs of deterioration. The vacuolation and exfoliation might be a sign of testicular toxicity and cell degeneration [25]. According to this study, sever oedematous fluid infiltration in the interstitial spaces of the seminiferous tubules caused atrophic changes, impairment of the spermatogenic process. There was deformity of the histological appeared in the seminiferous tubules and necrosis of the primary spermatocytes which showed a karyolysis of their nuclei. These results are in agreement with Eldurssi and Gheth [26] when rats treated with Monosodium glutamate. Moreover, our result agreed with Yucel *et al.* [27] they reported that changes after treatment with Cisplatin, were maturational loss in germinal cells and arrest of spermatogenesis at the primary spermatocyte stage. Mild perivascular fibrosis, disorganization, and hyalinization of intertubular connective tissue were also observed. Furthermore, agreement with Eldurssi *et al*. [28], they stated that the testicular sections which were examined revealed congestion, exudation and necrosis of their tissues with treated by Chlorpyrifos. The discuss of causes by $KBrO_3$ in the current study maybe within following reasons: Toxicant could be attributed to direct effect on testicular tissue which leads to reproductive dysfunction such as reduced sperm count, motility and morphology [29]. In addition, Sperm membrane contains a characteristic high level of unsaturated fatty acids which makes spermatozoa particularly susceptible to oxidative damage [30]. Likewise, the decrease in sperm count and quality is correlated with decrease in testosterone levels and oxidative damage as evident from suppressed antioxidant enzyme activities [31]. The pathological changes of seminiferous epithelium may cause the disruption of Sertoli and germ cells, which results in impaired spermatogenesis and may also lead to germ cell loss [32]. $KBrO_3$ treatment decreased the serum level of testosterone, FSH and LH. Secretion of testosterone is probably impaired due to excessive oxidative stress and the degeneration of Leydig cells [33]. Toxic effects of $KBrO_3$ may result in the failure of pituitary to secrete FSH and LH and that will result in testicular dysfunction leading to infertility [34]. The highly specific toxicity for the endothelial cells of the testicular capillaries in the post pubertal rat and result in a rapid and massive increase in permeability. The resultant edema markedly reduces blood flow and results in ischemic necrosis of the testis. The presence of an inflammatory infiltrate that penetrates the necrotic tubules and proceeds to fibrosis [35].

In protective group showed seminiferous tubule atrophy, disrupted germ cell layers and disappearance of Leydig cells. Our results accepted with many researchers such as Ibrahim *et al.* [36] they showed seminiferous tubules were completed depletion of both germinal and sertoli cells leaving only a basement membrane when treated with CaCl. Also, there were atrophied seminiferous tubules characterized by complete hyalinized scarring of the tubule with varying degrees of tubular collapse. Moreover, atrophy of the testicular tissues was showed [37]. Our result disagree with Al-Roujeaie *et al*. [38], who stated that histopathological evaluation revealed damaged testicular tissues in diabetic rats, which was protected following *R. graveolens* treatment. Thus, it was suggested that *R. graveolens* treatment improves sexual functionality and also protects against diabetic-induced testicular damage in rats. Prasad and Prasad [39] showed that *R. graveolens* prevented lower sperm counts, sperm motility, daily sperm production, and higher abnormal sperm numbers induced by cyclophosphamide. Most of the plants impair steroidogenesis by targeting the enzymes involved in the process at the level of Leydig cells and/or at the level of the hypothalamo-pituitary-gonadal loop. Additional studies are warranted to understand intensely the molecular mechanisms by which plants or their active ingredients hamper steroidogenesis in various species [16]. In the therapeutic group, showed interstitial hemorrhage, necrosis in spermatogonia with vacuolation and accumulation of degenerating germ cells. Our results similar to findings according to Soliman *et al.* [40] who illustrated that rats treated with fluoxetine lead to distortion of seminiferous tubules, germ cell degeneration with sloughing, vacuolation and the interstitium appeared wide containing degenerated Leydig cells and germ cell degeneration or loss. The reason of affect germ cells resulted from the coordinated function of all other cell type and processes within the testis, and any disturbance of their environment generally results in their death or exfoliation into the tubular lumen. Sertoli cell and Leydig cell were very sensitive to chemical damage responded to biochemical disturbances rather than cell death [41]. Also, agreed with Vidal and Whitney [42], they showed degeneration of spermatocytes during meiotic division lead to specific lesion with subsequent depletion. Therefore, our result agreement with Hasanin *et al.* [43] they showed complete degeneration of the spermatogenic, basement membrane appear markedly disrupted and thickened, distorted seminiferous tubule with degenerated spermatogenic epithelium, seminiferous tubules degenerative changes of the whole of the cell lineage of the seminiferous epithelium, Wide spaces between the cells, Spermatids were distorted, degenerated and vacuolation of the cytoplasm after exposed to acrylamide for 6 weeks. Furthermore, De Souza *et al.* [44] indicated that after treatment with anabolic steroid, alcohol and nicotine, observed interstitial hemorrhage and seminiferous tubules with vascular congestion.

Conclusion

In conclusion, the administration of *R. chalepensis* oil extract against $KBrO_3$ toxicity following the protocol presented in this investigation did not have beneficial as protective and curative effects in adult male rats. With regard of previous studies. Therefore, we guess administration of other dose and increasing of experimental period may result in significant differences of mention parameters and more studies are needed to clarify the properties of this herb.

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