https://doi.org/10.48047/AFJBS.6.5.2024.10607-10625

AfricanJournalofBiological Sciences

TheProtectiveRole of*Nigellasativa*AgainsttheTesticularDamage Induced by Aluminum Chloride in Adult Male Rabbits

SiouaniAmina[1,2],MoumenYasmina[1,2*],BoulahbelSouad[1],BenhamoudiKatia[1,2], Meziani Asma[1]

[1]Departmentof Life Sciences and Nature, Faculty of ExactSciences, Life SciencesandNature,University of Oum El Bouaghi, 04000, Algeria.
[2]LaboratoryofAnimalEcobiologyandPhysiology,UniversityofOumElBouagui,04000,Algeria.

Correspondingauthorcontactinformation
Dr.MOUMENYasmina
Email: moumen.yasmina@univ-oeb.comPhone number: (+213) 670212384

Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03Jun 2024
doi:*10.48047/AFJBS.6.5.2024.10607-10625*

Abstract

The objective of this study is to investigate the protective effect of *Negella Sativa (N.sativa)* seedsagainstthetoxicity ofreproductivemarkersinadult malerabbitstreated withaluminum chloride($AlCl_3$). Sixteenrabbitswererandomlyandequally divided into fourgroups,thefirst grouptaken as control (T),the second group(NS) wastreated with200 mg/kg/day of *Nigella Sativa* seeds extract; the third group (AL) was treated with 25 mg/kg/day of $AlCl_3$, and The last group (NS-AL) received the combined treatment of $AlCl_3$ and *N. sativa* extract at the same doses.The administration of $AlCl_3$ and seed extract was carried out orally for four weeks.

The results showthat the Al-treated group (AL) had reduced testicular weight, plasma levels of testosterone, LH, FSH, sperm speed, motility, and concentration compared to the control group (T). Furthermore, a microscopic examination revealed many histological alterations in the interstitial tissue and seminiferous tubules. The reproductive toxicity of $AlCl_3$ resulted in testicular degeneration in the majority of seminiferoustubules,accompanied byadecreasein the number of germinal cell layers in the seminiferous tubules, and the accumulation of multinucleated giant cells in the tubular lumen. In contrast, the groups treated with *N.sativa*alone (NS) or combined with aluminum (AL-NS) showed that the histopathological alterations were significantly attenuated by the administration of *N. sativa* extract. Based on theresults ofthis work,it seems thatthe supplementation of *N. sativa* extract may protectthe reproductive system from damage induced by $AlCl_3$.

Keywords:Aluminumchloride,toxicity,*Nigellasativa*,antioxidant,fertility,testis.

1. Introduction

Aluminum (Al) is one of the most common metals in the environment, it makes up about 8.8% of the earth's crust (Domingo, 1994), it can be found in a variety of forms, including oxide, hydroxide, chloride, and phosphate. Al is extensively available and used in the production oftoothpaste, foodadditives, cookware,tools, and cosmetics.In soil,rocks, clays, and jewels, Al can also be found in mixes with oxygen, silicon, fluorine, and other elements (Farina et al., 2002). Al is an extremely toxic trace element that can be harmful to humans as well asanimals (Yousef, 2004).It has several different waysthat Al can be absorbed, butthe three primary ones are ingestion, skin contact, and inhalation. In fact, adults canconsume between5and40 mgofaluminumorally eachday sincethemetal canbe found in food, drink water, air, various preparations, and medicinally in large quantities, as buffered aspirin and antacids (Gomesa et al., 2019). However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) notes that 2 mg/kg/week is the acceptable limit for Al consumption (Epstein, 1990). Al doesn't seemto play anyknown physiological role in the body. However, excessive exposure results in negative physiological effects (Ganrot, 1986). Several human pathologies, including neurological disorders, anemia, liver damage, and renal dysfunction, have been related to the accumulation of Al in the body (Baceria, 2002; Vittori et al., 2002; Martak etal.,2010 andRayan etal.,2011).Thereproductive functioncanalso beaffectedby aluminum toxicity, including ovarian damage, testicular dysfunction, and inhibition of ovulation (Ali mahrane et al., 2011 and Fu et al., 2014). Experimental studies on aluminum intoxication in animals support human studies, they reveal that exposure to Al causes low sperm quality, infertility, hormonal imbalance, and alterations in the tissue structure of reproductive organs (Lobet et al., 1995 and Pandey and Jain, 2017). Moreover, oxidative stress and the excessive production of free radicals (ROS) are the main causes ofaluminum's harmful effects (Yuan et al., 2012). According to clinical research, oxidative damage can affecttheblood-testesbarrier, changeor block enzymeactivity, disruptcell signaling, modify membrane function, and oxidize DNA (Sargazi et al., 2006 and Pandey, 2013).

Sinceancienttimes,herbalremedieshavebeenanimportantsourceofsubstancesusedfor curing human diseases. According to estimates fromthe World Health Organization (WHO), upto80% of people continueto obtaintheir medical care from herbalremedies(Hadi Kamil, 2013). Black *Nigella Sativa* seedsare widely utilized since they have a variety of therapeutic uses (Tukruri and Dameh, 1998). They are annual herbaceous plants that belong to the Ranunculaceae family (Babayan et al., 1978). *Nigella Sativa* is primarily grown in North Africa,southern Europe,and SouthwestAsia,even though itis also grown in Algeria's arid

and desert regions. *Nigella sativa* seeds are rich in bioactive compounds, the most important of which have been reported by earlier studies.Indeed, the seeds contain 13.5-22 % protein, 38-40 % fat, 3,7 % moisture, 3,7 % ash, and 17-32 % carbohydrates (Al-Jassir, 1992; Abdelaal and Atia,1993; Sharma et al., 2009 and Ahmed et al., 2013).They consist of many antioxidant compounds, including 4-terpineol, carvacrol, t-anethole, and thymoquinone (TQ) (Sharieatzadeh et al., 2011). This last which is considered the major element of essential oil, has been involved in biological activities (Hanafy and Hatem, 1991) due to its powerful antioxidant quality, TQ inhibits lipid peroxidationand subsequently decreasesthe production of reactive oxygen species (ROS) (Burits and Bucar, 2000 and Al-Majed et al., 2006). In contrast, a range of pharmacological studies has been carried out on *N.sativa*seeds in recent years, which have revealed a large spectrum of activities, including anti-microbial activity, anti-oxidant, anti cancer, anti inflammatory, anti-hyperlipidemic, and anti-diabetic, hepato-protective, nephro-protective, neuro-protective,cardiovascular, immune-protective activity, ameliorative effect on the reproductive system (Kooti et al., 2016). Several previous studies have demonstrated the overall positive effect of *N. sativa* oil on the reproductive system in male rats, in particular the spermatogenic process.According to Cho Ping et al. (2014) *N. sativa* can affect the fertility potential by influencing sperm count, testis and epididymis weights, plasma testosteroneandLH levels, and other pituitary-testicular pathway hormones, as well as the fertility index. In addition, *N. sativa* seeds can enhance sperm motility and sperm count in the seminiferous tubules and epididymal duct, spermatogenesis in spermatocytesIandII,andthereproductiveorgans'weight.Infemalerats, *N.sativa*improves the fertility potential by increasing the number of pregnancies (Mukhallad et al., 2009; Al-Sa'aidi etal.,2009 and Kolahdoozetal.,2014).

Thisstudy investigatedthepotentialprotectiveimpactof *Nigellasativa*seedextractonmale adult rabbits *Oryctolagus cuniculus*, as well as the toxic effect of aluminum chloride on certain reproductive markers.

2. Material and methods

Ethanolicextractof*NigellaSativa*

N. Sativa seeds were purchased at a nearby market in Beskra, south Algeria, the seeds were powdered using an electric grinder, 20 g of the powder was maceratedwith aqueous ethanol 80 ml (v/v = 80/20) by using white filter paper, the mixture was filtered after the maceration process,whichlastedfor24hoursatroomtemperaturewithperiodicshaking.Thefiltratewas

evaporated byusingRotavaporatatemperature of60°C,and finallydriedina darkairyplace for 15 days.

Chemicals

Aluminum Chloride Anhydrous ($AlCl_3$), 98%, was purchased from (Sigma Aldrich. MO USA) by Oum El-Bouaghi University. the Al doses for each day were diluted in distilled water and administered orally.

Animals

Sixteen adult male rabbits (*Oryctolagus cuniculus*), ages 24 to 26 weeks, weighing 2.5–3.5 kg,wereobtainedfrom arearingcenter.Animalswereprovided acommercialdietand water*ad libitum.* Rabbits werekept in individual cages for adaptation for a period of15 days intheanimalhouseoftheLifeSciencesandNature Department,OumEl Bouaghi University.

Experiment design

After the adaptation period, animals were randomly divided into four groups. Group 1 (T): served as control. Group 2 (NS): received 200 mg/kg bw of *N.Sativa*extract. Group 3 (AL) received 25 mg/kg bw of $AlCl_3$, and group 4 (AL-NS) received 200 mg/kg bw of *N.Sativa*extractand25 mg/kgbwof$AlCl_3$.*N.Sativa*extract wasdissolvedin water and given orallyin a single daily dose, for 4 weeks.

Thebiologicalstudyofsemen

The sperm analysis was carried out according to the WHO (1990) method. The epididymis' seminal content was extracted by cutting off the head of the organ, and 0.1 ml of sperm was collected and diluted in 1 ml of physiological solution (NaCl 0.9%). diluted sperm was then used to estimatethe sperm speed, concentration, motility, and vitality.

Hormoneanalysis

after the sacrifice, the blood was removed and collected immediately in tubes containing heparin, it was separated by centrifugeat 4000 rpm/15min, the samples of plasma were used to estimate the testosterone, LH, and FSH levels.

Histologicalstudy

To obtain thin and clear histological sections that can be observed under a light microscope, the testes were sampled, and preserved ina dilute solution of formalin (10%). they were then subjected to a deshydration process, by using increasing concentrations of alcohol. after immersion of samples in paraffin, they were sectioned by a microtome into 4 µm sections, followed by staining with eosin and hematoxylin, the mounting and the drying were carried out, and the histological sections were finally ready for microscopic observation (Bancroft and Gamble, 2002).

Dataanalysis

Student t-tests were performed for statistical analysis to compare between two groups, and one-way analysis of variance (ANOVA) was used to analyze data for all groups. At the p-value was less than 0.05 ($p < 0.05$), the statistical data was defined as significant. a: test ANOVA. b, c, and d: testt-student. b:T vs NS. c:T vs AL. d:T vs AL-NS.

3. Results

Spermparameters

The obtained results show a highly significant increase in the speed of spermatozoa in the group treated by *N. sativa,* anda non significant reduction in the speed ofspermatozoa in the aluminum-treated group. Furthermore, a non significant improvement was recorded in the rabbits treated with both $AlCl_3$ and *N. Sativa* extract (Fig 1).

Ahighlysignificantdecreaseisobservedinthemotilityofspermatozoainrabbitstreatedwith $AlCl_3$alone,the*N.Sativa*extracttreatmentrevealsnonsignificant differencescomparedwith the control. However, an improvement in the motility of spermatozoa is recorded in the combined group AL-NS (Fig 2).

The concentration of spermatozoa reveals a very high significant decrease in the aluminum-treated group comparedto the control. However, a highly significant reduction was recorded in *N. Sativa* treated group and aluminum-treated group compared to the control (Fig 3).

Thevitality of spermatozoa reveals a veryhigh significant reduction in the aluminum-treated group compared to the control. However, treatment with *N. sativa* seeds extract improves slightly the vitality of spermatozoa. A very high significant improvement is recorded in the combined group AL-NS (Fig 4).

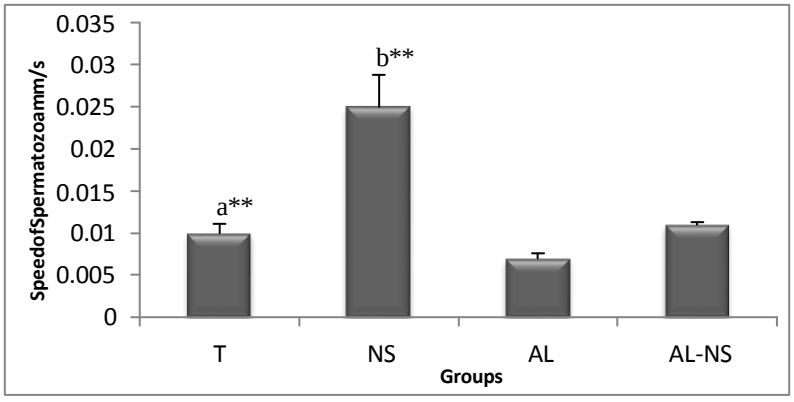

Figure1: Effectof*N.Sativa*extractand$AlCl_3$onspermspeedinmalerabbits

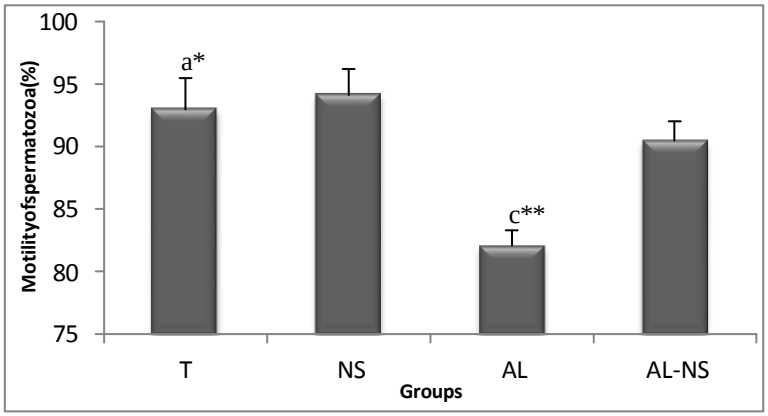

Figure2: Effectof*N.Sativa*extractandAlCl3 onspermmotilityinmalerabbits

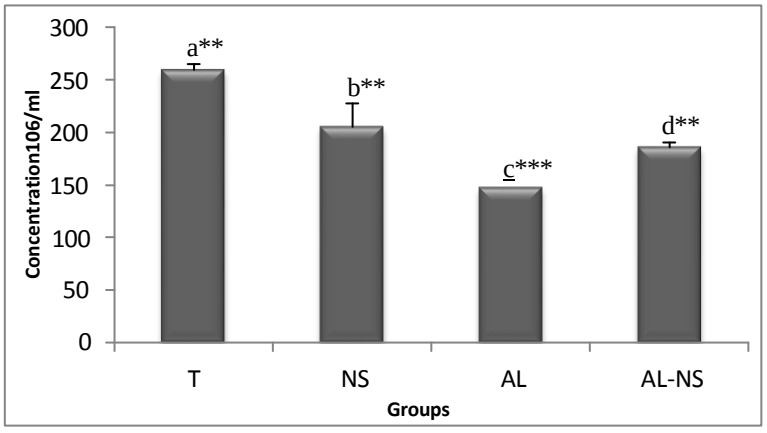

Figure3: Effectof*N.Sativa*extractand$AlCl_3$onspermconcentrationinmalerabbits

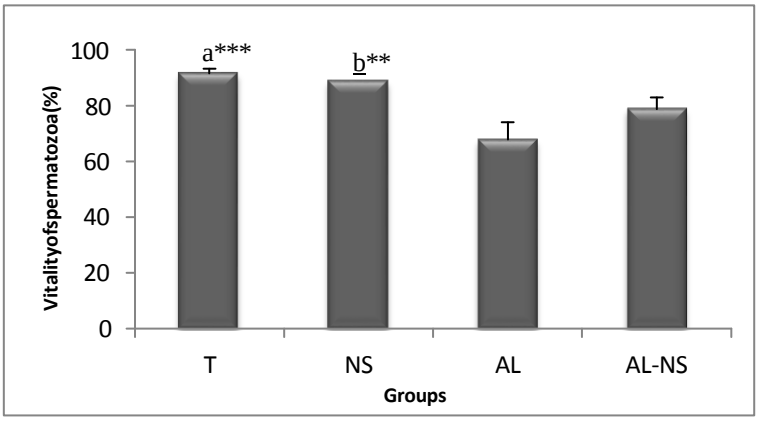

Figure4: Effectof*N.Sativa*extractand$AlCl_3$on spermvitalityinmalerabbits

Reproductivehormones

Resultsshowanonsignificant decreaseinplasmatestosteroneandFSH concentrations inthe aluminum-treated group compared to the control (T). while oral treatment with *N. Sativa* extract induces a non significant increase compared to the control. In the combined treated group AL-NS, testosterone and FSH levelsare also decreased, nevertheless, the effect is less important (Fig. 5 and 7).

The plasma LH level is significantly reduced in rabbits that received aluminum, the groups treated with *N.Sativa*extract alone or combined with aluminum show a non significant decrease compared to the control (Fig 6).

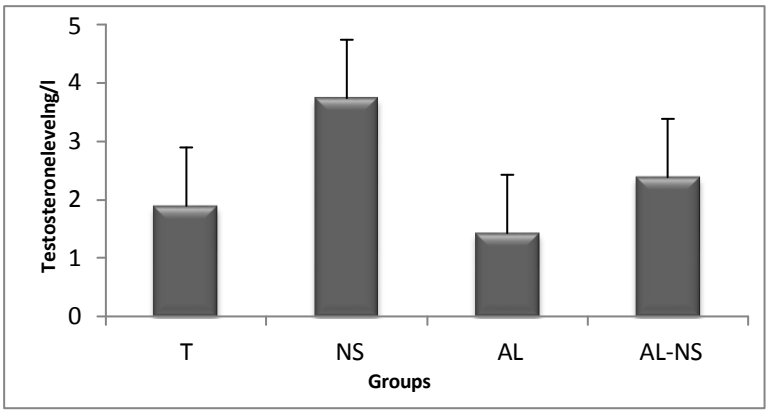

Figure5: Effectof*N.Sativa*extractand$AlCl_3$onplasmatestosteronelevelsinmalerabbits

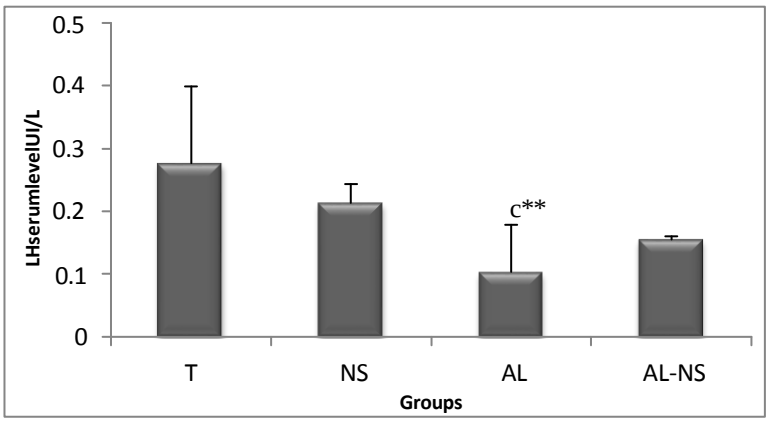

Figure6: Effectof*N.Sativa*extractand$AlCl_3$onplasmaLHlevelsinmalerabbits

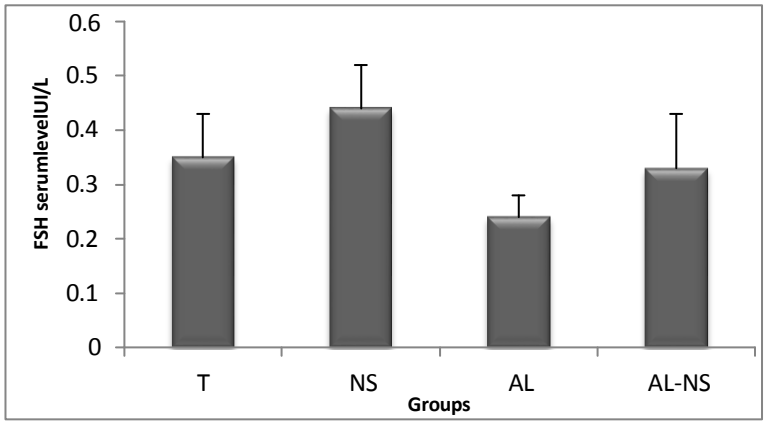

Figure7: Effectof*N.Sativa*extractand$AlCl_3$onplasmaFSHlevelsinmalerabbits

Weightoftestes

Theresultsshownon significant decreaseintheweightsoftestes inthe Algroupcomparedto the control. The treatment with *N.Sativa*extract reveals a non significant decrease in the testicular weight. Whereas, the combined treatment (AL-NS) improves slightly the testes' weights (Fig. 8).

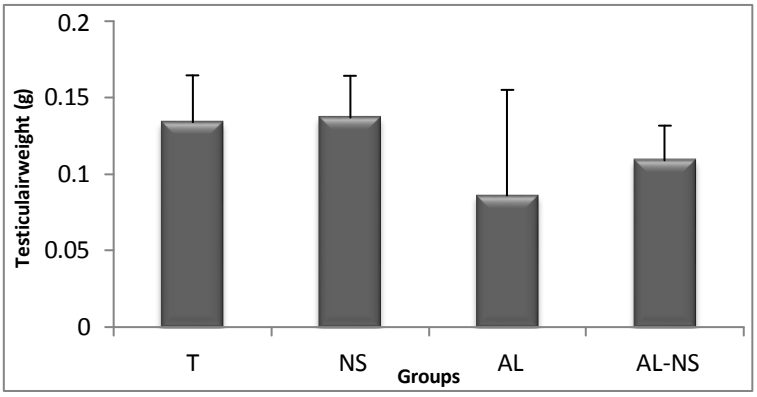

Figure8: Effectof*N.Sativa*extractand$AlCl_3$ontestesweightsofmalerabbits

Histologicalexaminationoftestis

The microscopic study ofthe testesin the controland *N. sativa*-treated groups shows that the seminiferous tubule walls are thicker and contain all the layers of cell differentiations from spermatogonia to mature spermatozoa. This indicates a normal arrangement of the seminiferous tubules, spermatogenic cells in tubules, Leydig cells, and interstitial connective tissue (Fig 9 A, B, C, and D).

Themorphology ofthetestisinthe Al-treatedgrouprevealssevere damage,areductioninthe seminiferous tubule thickness from 16.65 µm in the control group to 14.20 µm in the Al-treated group, andan increase in the lumen of the seminiferous tubule dueto thereduction of spermatogenic cells number and the absence or presence of mature spermatogenic cells (Fig 9. E and F). Although the germinal cells layer is significantly reduced in the histological sectionsofrabbit testistreated withaluminumand *N.sativa* seedsextract(AL-NS) thetubule walls are thicker and include all the layers of cell differentiation. It was noted that Leydig cells have a normal histological structure. However, the tubular structure appears to be affected in certain parts ofthe histological section (Fig. 9. G and H).

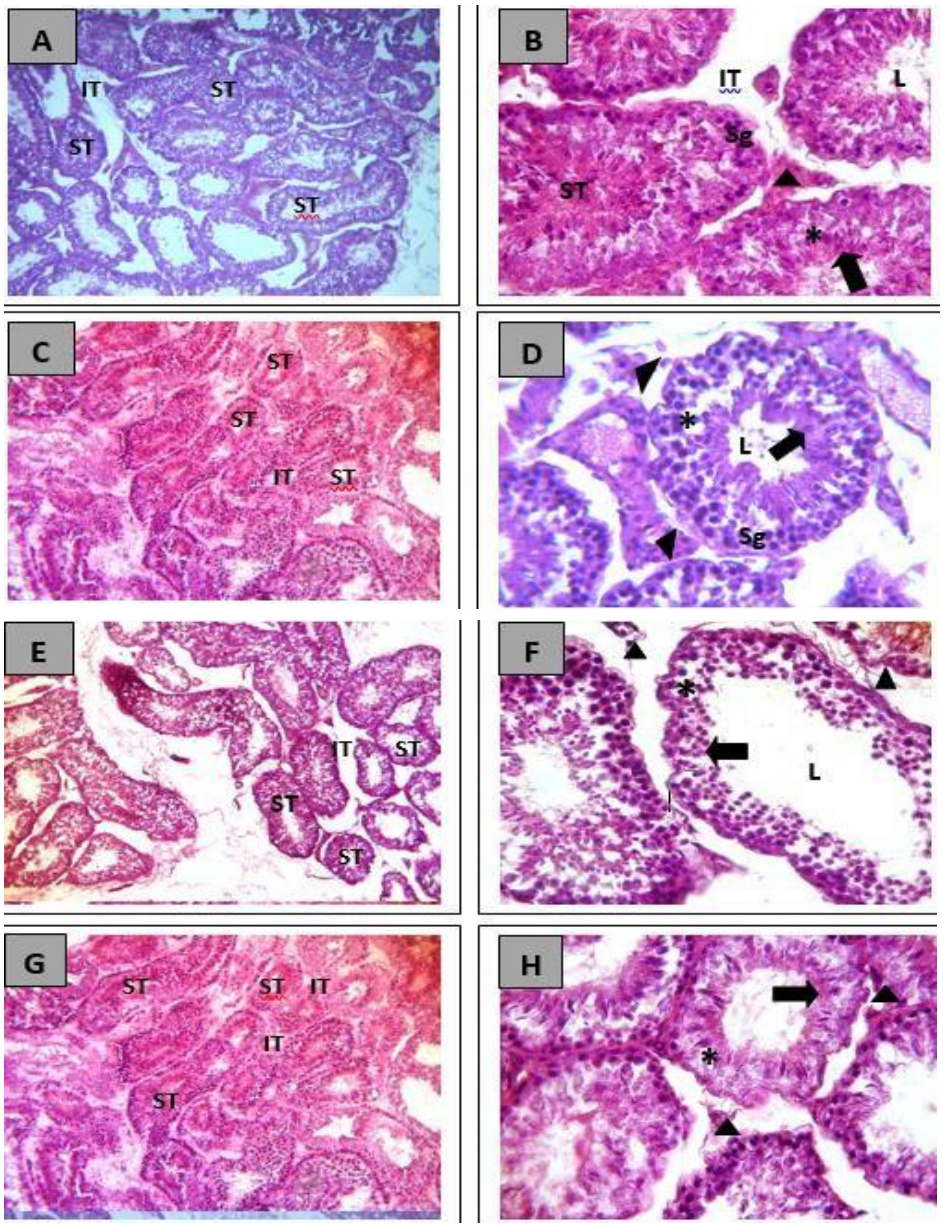

Figure 9: Histologicalsectionsofthetestes**.**(AandB): the sectionsofthe control(T).(Cand D): sections of the *N.sativa*treated group (NS). (E and F): sections of the Al-treated group (AL). (G and H): sections of aluminum and *N.Sativa*treated group (AL-NS). A, C, E, and G were magnified x100. B, D, F and H were magnified x400. Lumen of seminiferous tubules (L), seminiferoustubule(ST), spermatogonialstem cell (Sg), intertubular spaces(IT),Lydig cells (arrowheads), mature spermatozoa (arrows), germinal epithelium (asterisk).

4. Discussion

The reproductive system is exposed to severe damage caused by oxidative stress, since it is oneoftheleastresistantsystemsto oxidativereactions(Aitken,1995),andalsocontainslarge quantitiesofpolyunsaturatedfattyacids,whicharesusceptibletooxidation.Naturalproducts, due to their antioxidant components, can improve the reproductive system function, and its response to oxidative damage, by strengthening the endogenousantioxidant system.

On this basis, the results obtained in rabbits treated with *N. sativa* extract for four weeks showed an increase in certain reproductive parameters, an increase in sperm speed, and motility, plasma levels of FSH and testosterone, the weight oftestis, was observed in rabbits treated with the plant's extract, compared to the control. Although these elevations are not all significant, they demonstrated the positiveeffect of the plant's components on sperm quality. Theseresultsareinagreement with several previous studies whichhave demonstratedthat *N. sativa* extract has a positive effect on reproductive organs, Leydig cells and spermatozoa (Reza et al., 2005), it contributed to increased testicular weight and size (Al-Sa'aidi et al., 2009),andincreased organ weightand sexual hormones FSH, LH (El Khasmi etal.,2011).

The positive effect of *N. sativa* on the reproductive system and sperm quality is attributed to its wealth of effective bioactive components, such as antioxidants; vitamins A, B and C, minerals such as zinc, copper, and magnesium (Ahlobom et al., 2001; Kanter and et al., 2005),anethol,carvacrol,thymol,thymoquinone,4-terpineol,andthymoquinone(TQ).These components can influence spermatogenesis, sex hormone production and the hypothalamus-hypohyse-testicular pathway. Thymoquinone (TQ) is one of the most important components of*N.sativa*,itisa powerfulantioxidantthat scavengesandneutralizes free radicalsgenerated by oxidative stress, reducing their damage in the body. TQ may limit the generation of free radicals by transferring electrons to oxidizing agents (Burits and Bucar, 2000 and Adedara et al., 2014). Previous research has demonstrated that the antioxidant effect of *N. sativa* may reduce the production of free radicals caused by oxidative stress (Chen etal.,2006;Elbetieha et al., 2011 and Parveen and Shadabig, 2011). Other studies have shown that *N. sativa* oil contributes totheinhibition of membrane lipid peroxidation(Kanter, 2011),the promotion of antioxidant defensesystems suchasGSHandantioxidant enzymes(Mohamadinet al.,2010), reproductive parameters and the immune system (Mosbah et al., 2016), leading to the enhancement of fertility parameters (Reza et al., 2015). The plant can also increase the activity of 17-KSR (17-ketosteroid reductase), accelerate the steroidogenesis process and increase testosterone production, leading to improvement in sperm proliferation (Mosbah et al.,2016).Theenhancement ofcertainfertilityparameterscanbeexplainedbytheeffectof*N.*

*sativa*components onthehypothalamus-pituitary-testicularpathway, whichpositivelyaffects themechanism of steroidogenesisand spermatogenesis(El Khasmi et al., 2011).Histological sections performed on the testes of rabbits treated with *N. sativa* extract demonstrated a beneficial effect of the plant components on reproduction. This is clearly observed in the morphologyoftesticulartissue,whichappearshealthyandsimilartothehistologicalstructure of the control. The observations are in agreement with those obtained by Al-Sa'aidi et al. (2009) who revealed that administration of *N. sativa* extract led to an increase in Leydig cell clusters, which are responsible for testosterone synthesis, an increase in spermatogenesis, in the thickness of the seminiferous tubule wall, and the number of mature spermatozoa in the lumen of the seminiferous tubule and epididymis, demonstrating the role of *N. sativa* in improving reproductive performance.

Exposure of rabbits to $AlCl_3$ showed a decrease in reproductive parameters; sperm speed, concentration, motility, and vitality, in rabbits treated with 25 mg/kg bw for 4 weeks, compared to thecontrol. Theseresultsarein agreement with several previousstudies, both *in vitro* and *in vivo*, which suggested that exposure to aluminum resulted in a significant decrease in sperm motility and vitality, a reduction in the number of live sperm compared to dead sperm, a reduction in sperm count in the ejaculate, and ejaculate volume, changes in sperm morphology, as well as an increase in the abnormal sperm percentage (Khattab, 2007; Yousef etal.,2007; Abdul-Rasoul etal.,2009 and Martinez etal.,2017). The disturbances in reproductive parameters are due to the accumulation of aluminum in the reproductive organ tissues, leading to oxidative damage, inflammation, and abnormal development of the reproductiveorgans.Asaresult,thespermatogenesisprocess,spermcount,andspermquality decreased (Miska-Schramm et al., 2017). Dawson et al. (1998) demonstrated that high concentrations of aluminum in reproductive organs and testicular fluids, such as semen, are closely associated witha decreasein sperm efficiencyand performance, such as motilityand viability. Furthermore, Yousef et al. (2005) evaluated the impact of $AlCl_3$ on the concentrationoffructoseinsemenandthemotilityofspermatozoa.Theyfoundareductionin seminal fructose and an elevation in pH, those findings may be related to the sperm's lack of energetic metabolism. The reduction in the motility of sperm could therefore be associated partly with the reduction in semen fructose. In addition, aluminum may affect sperm mitochondrial enzymes, disrupting mitochondrial function and the mechanism of energy production used by sperm for motility (Yousef et al., 2007). Aluminum can also affect the midpiece of spermatozoa, by stimulating the production and secretion ofTRAP, a compound

that can cause deformations of the sperm midpiece, resulting in reduced sperm motility and sperm fusion with the egg (Kim and Parthasarathy, 1998).

The resultsrelated toreproductive hormones showed a decrease in FSH, LH and testosterone concentrations in the aluminum-treated group. The results are in agreement with those obtained by El-Ashmawy et al. (2007); Khattab (2007); Nuhair (2015) and Yakubu et al. (2017). Exposure to aluminum induces to disruption of reproductive hormone levels in the body. It can directly affect Leydig cells, the most important site of testicular androgen production (Reza and Palan, 2006), or indirectly, through an increase in nitric oxide (NO) levels, whichinturnactivatesnitricoxidesynthase.Thelatterinhibitstestosteroneproduction by inhibiting Leydig cell function (Dobashi et al., 2001 and Guo et al., 2005a). indeed, aluminum ingestion reduces the activity of coenzyme A, involved in the conversion of androstenedione to testosterone, and decreases 3',5 cyclic monophosphate (cAMP) in the testis(Guo et al., 2005a),leadingto a reduction in plasma testosterone concentration (Yousef et al., 2005). Aluminium also disrupts the function of the hypothalamus-pituitary-testicular pathway by changing the function of calcium ion channels. Shahraki et al. (2004) suggested that aluminum blocks calcium channels in the hypothalamus, particularly as calcium is involved in gonadotrophin-releasing hormone (GnRH) secretion. The inhibition of calcium channels leads to a depletion of GnRH secretion and, therefore, a reduction in LH and FSH production (Shahraki et al., 2004). Aluminum can affect testicular androgen receptors by decreasing orinterferingwithLHreceptors((RezaandPalan,2006),thedirectactionofAlon LH receptors causesa reductioninreproductive hormone synthesis(Sun et al.,2011),leading to oligospermia and exfoliation oftheseminiferoustubules (Al-Eisaand Al-Nahari,2017).In addition,aluminum accumulatesinreproductive organs, causing disruptionsin their structure and function.Our study showed that $AlCl_3$ causedtheenlargement oftheseminiferoustubule lumen and the absence of mature spermatozoa in it, as well as a decrease in the tubule wall thickness, which represents the different stages of spermatogenesis. Aluminum also caused the development of giant multinuclear cells in the lumen of some seminiferous tubules, and the degeneration oftubular cells. These observations are similar to those found by Guo et al. (2005b) who observed histopathological modifications in the morphological structure of the testis, resulting in a clear spermatogenetic failure, and necrosis, which occurs mainly in the final stages of the spermatogenesis (spermatidsand spermatozoa). Other studies have shown that aluminum causes hypertrophy of certain germ cells, and degeneration of others (Chinoy et al., 2005),absence of germ cells and necrosis of seminiferous tubules, edema ofinterstitial tissue(Halaetal.,2010),severedegenerationindifferentstagesofspermatogenesis,

congestion of blood vessels, abnormal structure of Leydig cells, and the complete absence of maturespermatozoain theepididymalduct(Abdul-Rasouletal.,2009).Thedegenerativeand necrotic action of aluminum on the testes led to a decrease in testicular weight in the aluminum-treated group compared to the control. These results are in agreement with the findingsofBatainehetal.(1998);El-Ashmawy etal.(2007)and Khattab(2007)whoreported thataluminum exposure caused a decrease intherelativeand absolute weight ofreproductive organs, suchastestesand seminalvesicles.Pandeyetal.(2014)demonstratedthattheadverse effect of aluminum on reproductive organs was attributed to its close relation to oxidative stress, which leads to the generation of large quantities of free radicals, resulting in oxidative damage in testicular tissues and cells. A similar correlation has been described by previous studies in animals exposed to metals as mercury (Moumen et al., 2011 and Kalender et al., 2013), lead (Dorostghoal et al., 2013), cadmium (Predes et al., 2011 and Moumen et al., 2020),arsenic (Morakinyo et al.,2010),and molybdenum(Pandey and Jain,2015).Whereas, Ige and Akhigbe (2012) demonstrated that the accumulation of Al in testicular tissue following its passage across the blood testis barrier leads to membrane damage of spermatogenic cells. In addition, aluminum affects Sertoli cells directly, by breaking the intercellular bridges, which can lead to germ cell exfoliation. Kim et al. (2001) reported that down-regulation of a cell adhesion protein, such as Sertoli cell cadherin, enhanced the sloughing of seminiferous epithelial cells, resulting in atrophy of the tubules. While Moselhy et al. (2012) demonstrated that $AlCl_3$ causes the cytoplasmic membrane to lose its barrier function, leadingto the release of substancesand enzymes stored in the cells.

Conclusion

This study indicates that exposure to $AlCl_3$ can result in adverse effects on reproductive and sperm parameters (speed, concentration, motility, and vitality of spermatozoa, plasma LH, FSH, and testosterone levels), as well as, on the histological architecture of the testis. However, the administration of *N. sativa* seed extract has revealed an improvement in all reproductiveparameters,indicatingthattheantioxidantcomponentspresentin *N.sativa*seeds could have protective effectsagainst $AlCl_3$ toxicity. Whereas the combined treatment of AL- NS showed an attenuated role of *N. sativa* against aluminum-induced toxicity.

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