# Effect of *Junglans regia* and *Nigella sativa* on Ethanol Induced Infertility in Male Rats

**O. M. Kale[1], V. J. Chaware[2], V. K. Redasani[3]**

Department of Pharmacology, YSPM's Yashoda Technical Campus Satara, Maharashtra India.

**Abstract**

**Alcohol drinking is one of the major factor for male infertility. In undeveloped and some developing nations, medicinal herbs used to treat male infertility as an alternative to standard. The purpose of this work was to assess the effect of *Junglans regia* and *Nigella sativa* on male infertility in wistar rats caused by ethanol. During the course of 14 days, fourty two (42) male wistar rats being randomly assigned to seven groups of six rats each. Group I animals (Control group) were administered with distilled water, Group II animals (Negative or inducer group) were administered with 38% Ethanol at a dose of 10 ml/kg body weight; Group III animals (Positive group) received 38% Ethanol administered with Clomiphene citrate as the reference medication; Groups IV and V animals were administered with 38% Ethanol co-treated with *Junglans regia* oil at the dose of 5ml/kg and 10ml/kg respectively. Group VI and VII were administered with 38% ethanol co treated with *Nigella sativa* oil at the dose of 5ml/kg and 10 ml/kg respectively. Testicular histology, sperm parameters and testosterone hormone were assessed. Due to ethanol ingestion, the large fall ($p<0.05$) in testosterone levels was recorded, ethanol ingestion resulted in a significant rise in sperm abnormalities and a significant reduction ($p<0.05$) in sperm count, motility, and viability. These findings were consistent with changes seen in the testis during testicular histopathology. Nevertheless, the harmful effects of ethanol on the reproductive organs, sperm viability, motility, abnormality and testosterone level were reversed when animals treated with *Junglans regia* and *Nigella sativa* oil.**

## INTRODUCTION

A couple is considered infertile if they are unable to conceive after a year of consistent, unprotected sexual activity(1). Since having children is a top priority in families, the treatment and management of infertility has gained international attention. 15% of couples who are of reproductive age are said to be infertile, with males accounting for 50% of these cases(2). Many variables contribute to diminished spermatogenesis and the creation of defective sperm, making male infertility a complex condition. Numerous disorders, including genetic abnormalities and infections of the reproductive organs, can result in male infertility. In addition, cigarette smoking, anabolic steroids, medications, depression, physical inactivity, exposure to electromagnetic waves and ethanol abuse are closely related to our lives and are known as the main risk factors for male infertility(4) As well as oxidative stress brought on by an excess of free radicals. The sperm cells' mitochondrial activity is inhibited by the free radicals, which slows down their motility and causes teratozoospermia(5). This could provide insight into the potential causes of the rising number of male infertility cases.

According to Chia et al. 42% of males who experience infertility is due to use of alcohol(6). This suggests that alcohol use is a strong risk factor for male factor infertility(3). Additionally, studies have shown a link between drinking alcohol and lower testosterone levels. The Leydig cells in the testicular interstitium produce the hormone testosterone. Gonadotropin releasing hormone (GnRH) of the hypothalamus controls the release of luteinizing hormone, which in turn initiates its production when the anterior pituitary gland receives a negative feedback(7). For a germ cell to develop successfully, various hormones from the pituitary, hypothalamus, and testis must interact in a balanced manner(8). Reduced sperm density, which ultimately results in azoospermia, has been discovered linked to increased levels of LH, FSH, and either normal or low levels of testosterone (9).

The oldest known medical practice is herbal therapy. Throughout history, all societies have utilized herbs, and they continue to produce some of our most valuable treatments. An abundant variety of secondary metabolites, which are utilized as flavours, agrochemicals, medicines, and other products, can be found in plants. colours, scents, and biopesticides[10].Adelakun et al. (2019b) used a rat experiment to examine *Junglans regia's* ability to reproduce[11].The results of this study suggested that male rats demonstrated enhanced sperm count as a consequence of oil consumption, leading to higher reproductive potential. The results of Ikwuka et al. (2021) confirm that regular administration of *Junglans regia* increases male fertility in wistar rats[12]. Potentially antioxidant-rich alkaloids, glycosides, saponins, and PUFs may be the cause of this enhancement in sperm motility and count. Furthermore, as previously observed, saponins may also enhance sperm parameters. Likewise, Masterson et al. (2020) discovered that *Junglans regia* increased sperm motility and semen quality[13].The development and normal operation of the reproductive system depend on reproductive hormones. Testosterone and FSH are necessary for the completion of reproductive masculine abilities [14]. Research revealed a correlation between spermatogenesis and sex hormones such as levels of FSH, LH, and testosterone [15].

Male infertility has been successfully treated with a variety of antioxidants, nutritional therapy and herbal medications [16]. Known by most as black seed, *Nigella sativa* is a medicinal plant that is a member of the Ranunculaceae family of plants [17]. People in Asia, the Middle East and Africa frequently utilize *Nigella sativa* seed as a spice, food preservation and medication [18]. One of the ingredients in traditional in Asian and Iranian treatments for infertility is *Nigella sativa*[21]. Studies on the effects of *Nigella sativa* oil on male rat spermatogenesis, fertility [19] and some reproductive features of male chickens have been reported [20].

## MATERIALS AND METHODS

### Plant material and extraction

Dried fruits of *Junglans regia* and *Nigella sativa* seeds purchased from local market of Satara, it was pressed in cold press machine and got oil. The oil was kept in dark cool place away from sunlight

### Study overview and design

Male wistar rats (n= 42) weighing 180-230gm were obtained from Animal House of Yashoda Technical Campus Satara. The animals were kept under temperature between 20-25°C in 12 hr light/dark cycle with pelleted food and water during 14 days of experimental study.

The experiment is conducted on 7 groups of animal containing six animals each. Group I animals were administered with distilled water. All groups of animals were ingested with 38% ethanol for 14 consecutive days except Group I animals. Group II animals were administered 38% Ethanol(10ml/kg,p.o.), Group III animals were administered Clomiphene(25mg/kg,p.o.) with 38% Ethanol(10ml/kg,p.o.), Group IV animals were administered *Junglans regia*(5ml/kg,p.o.) with 38% Ethanol(10ml/kg,p.o.), Group V animals were administered *Junglans regia*(10ml/kg,p.o.) and 38% Ethanol(10ml/kg,p.o.), Group VI animals were administered *Nigella sativa*(5ml/kg,p.o.) and 38%Ethanol(10ml/kg,p.o.),Group VII animals were administered *Nigella sativa*(10ml/kg,p.o.) and 38% Ethanol(10ml/kg,p.o.) for period of 14 days.

### Hormonal analysis

Testosterone, luteinizing hormone, and follicle-stimulating hormone were among the sex hormones assessed by enzyme-linked immunosorbent assay (ELISA) with ELISA assay kits .The process that was followed in the kit materials.

### pH of semen

Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The sperm smear on the pin was rubbed on a pH paper of range 4.0–10.0. The colour change corresponds to the pH and was read from the paper

### Sperm morphology

The sperm morphology procedure that was followed according to Dorado et al. (2011)[22].Ten milliliters of semen were put onto a slide by Briefly, and a thin warm plate at 37°C, a feathered-edge smear was produced and allowed to air dry completely for five minutes. .Each sample was processed into five air-dried smears. After applying a spectrally selective dye to the smears, they were studied by an oil immersion method. A hundred spermatozoa were counted, after which they were classified as either normal or abnormal and their percentages indicated which problems are more common.

**Sperm motility and count in the epididymis**

Cutting the epididymis's tail and carefully pressing it onto a clean slide yielded the epididymal contents, which included the sperm. Yokoi et al. (2003) approach was followed to determine progressive motility and cell count[106]. In a nutshell, the cauda epididymis was finely chopped using anatomical scissors in 2 ml of Earles buffer and then heated to 35°C for 10 minutes in a rocker. Following diluting, the quantity of spermatozoa resistant to homogenization was counted in a hemocytometer, and roughly 25 fields of view were inspected at 40× magnification using a light microscope.

**Statistical analysis**

The results were expressed as means ± standard deviation (SD). The significance of differences among groups was analyzed statistically by One-way ANOVA (Analysis of Variance), followed by Student's unpaired t-test. The differences were considered statistically significant if $p < 0.05$

## RESULTS

**Body and reproductive organs weight**

The body weight gain was presented in Table1. The body weights of the rats significantly ($p$<0.05) increased with the group ingested with ethanol alone gaining more body weight when compared with the control and extract-treated groups (Table1). The weights and relative weights of testis and accessory glands are presented in Table2. In the group administered with ethanol alone, there was significant decrease in both absolute and relative weights of the testis and other reproductive organs as compared with the control animals ($p$<0.05;Table2). Due to recovery effects, co-administration of ethanol with either extract or standard drug significantly increased ($p$<0.05) the weight of testis ,epididymis, seminal vesicle and prostate gland when compared with the rat treated with ethanol alone, although the increment was not up to control level (Table 2). Co-administration of ethanol with either extract or standard drug significantly prevented the decrease in the weights of the testis (g) and epididymis (g), but did not affect the relative weights of the organs (g/100 g body weight) and affected the relative weights of the seminal vesicles and prostate glands. However, co-treatment of ethanol with the highest concentration of the extract showed more pronounced effect on all the reproductive Organ weights than the drug (Table2).

**Table 1: Average Body Weight (G/Rat) Of Control And Ethanol Induced Male Albino Rats Treated With *Junglans Regia* And *Nigella Sativa***

| Groups | Weight(g/rat) | | Weight gain /loss | Weight gain/ Loss (%) |
|---|---|---|---|---|
| | 1st day | 14th day | | |
| I | 205±3.71 | 217 ± 6.85 | 12 | 5.8$^a$ |
| II | 196 ± 16.20 | 224 ± 9.24 | 28 | 14.2$^b$ |
| III | 208±8.46 | 223 ± 3.27 | 15 | 7.2$^c$ |
| IV | 184±8.57 | 203 ± 12.69 | 19 | 10.3$^b$ |
| V | 202 ± 12.10 | 211 ± 6.54 | 9 | 4.4$^c$ |
| VI | 212 ± 7.81 | 224 ± 9.48 | 12 | 5.6$^b$ |
| VII | 208 ± 6.52 | 228 ± 14.07 | 20 | 9.6$^c$ |

Values represent mean ± *SD* (*n*=6). Values with different superscript letters on the same column are significantly different ($p<0.05$).

**Table 2: Reproductive Organ Weights Of Control And Ethanol Induced Male Albino Rats Treated With *Junglans Regia And Nigella Sativa***

| Groups | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameters** | **Control group** | **Ethanol /Negative group** | **Positive group** | **Test 1 low (5ml/kg)** | **Test 1 high (10ml/kg)** | **Test 2 low (5ml/kg)** | **Test 2 high (10ml/kg)** |
| **Right testis** | | | | | | | |
| **Absolute weight(g)** | 1.93±0.9 | 0.86± $0.02^{*}$ | 1.68± $0.09^{\#}$ | 1.08± 0.07 | 1.38±0.10 | 1.10± 0.9 | 1.22± $0.5^{\#}$ |
| **Weight(g/100gBW)** | 0.88±0.03 | 0.40± $0.01^{*}$ | 0.75± 0.02 | 0.53± 0.02 | 0.65± 0.03 | 0.49±0.02 | 0.53± 0.01 |
| **Left testis** | | | | | | | |
| **Absolute weight(g)** | 1.85±0.12 | 0.74± $0.04^{*}$ | 1.54± $0.11^{\#}$ | 1.02± $0.9^{\#}$ | 1.29± $0.12^{\#}$ | 1.11± 0.10 | 1.31± 0.2 |
| **Weight(g/100gBW)** | 0.85±0.05 | 0.34± $0.01^{*}$ | 0.69± 0.04 | 0.50± 0.02 | 0.61± 0.03 | 0.49± 0.01 | 0.57± 0.00 |
| **Epididymis** | | | | | | | |
| **Absolute weight(g)** | 1.42±0.11 | 0.57± $0.01^{*}$ | 1.21± 0.08 | 0.75± 0.00 | 1.03± $0.07^{\#}$ | 0.89± $0.04^{\#}$ | 0.96± 0.06 |
| **Weight(g/100gBW)** | 0.65±0.05 | 0.26± $0.01^{*}$ | 0.54± 0.02 | 0.36± 0.01 | 0.48± 0.02 | 0.39± 0.03 | 0.42± 0.02 |
| **Seminal vesicle** | | | | | | | |
| **Absolute weight(g)** | 1.08±0.09 | 0.28± $0.01^{*}$ | 0.89± 0.02 | 0.41± 0.02 | 0.69± 0.05 | 0.49± 0.01 | 0.57± 0.03 |
| **Weight(g/100gBW)** | 0.49±0.02 | 0.13± $0.00^{*}$ | 0.39± 0.02 | 0.20± 0.01 | 0.32± 0.03 | 0.21± 0.01 | 0.25± 0.00 |
| **Prostate gland** | | | | | | | |
| **Absolute weight(g)** | 1.22±0.11 | 0.43± $0.05^{*}$ | 1.08± 0.02 | 0.64± 0.01 | 0.81± $0.05^{*}$ | 0.54± 0.02 | 0.89± 0.06 |
| **Weight(g/100gBW)** | 0.56±0.01 | 0.20± $0.03^{*}$ | 0.48± 0.02 | 0.31± 0.00 | 0.38± 0.02 | 0.24± 0.01 | 0.39± 0.01 |

Data are expressed as mean ± SD, (n= 6).*Significantly different (*p*<0.05) compared with the control group. #Compared with the ethanol group.

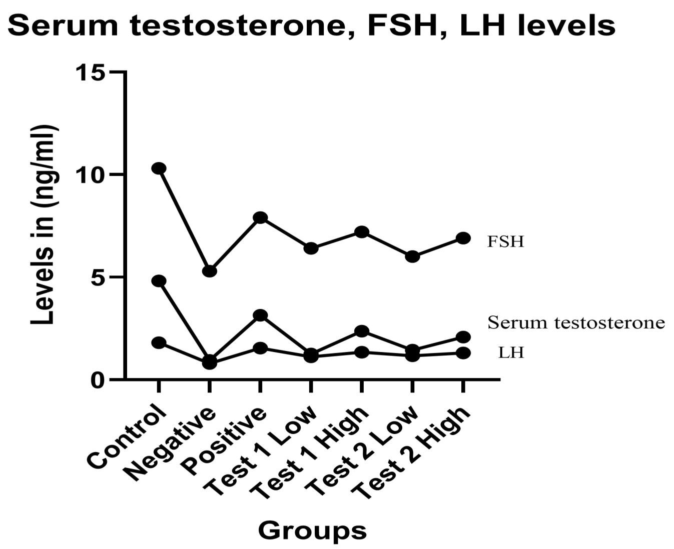
**Biochemical parameters**

Results of biochemical parameters in rat, showed that the level of Serum Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were significantly decreased in the ethanol administered rats ascompared with ethanol and control group

**Table 3: Levels Of Fsh, Lh, Serum Testosterone In Control And Ethanol Treated With *Junglans Regia* And *Nigella Sativa***

| Groups | Control | Negative | Positive | Test 1 low (5ml/kg) | Test1 high (10ml/kg) | Test 2 low (5ml/kg) | Test 2 high (10ml/kg) |
|---|---|---|---|---|---|---|---|
| **Serum testosterone (ng/ml)** | 4.82 ± 0.32 | 0.94 ± 0.07$^{*}$ | 3.15 ± 0.24$^{\#}$ | 1.26 ± 0.11 | 2.37 ± 0.19 | 1.44 ± 0.13 | 2.08 ± 0.10 |
| **FSH(ng/ml)** | 10.3 ± 1.7 | 5.3 ± 0.21$^{*}$ | 7.9 ± 0.34 | 6.4 ± 0.28$^{\#}$ | 7.2 ± 0.46 | 6.0 ± 0.08$^{*}$ | 6.9 ± 0.19 |
| **LH(ng/ml)** | 1.80 ± 0.03 | 0.79 ± 0.02$^{*}$ | 1.54 ± 0.06 | 1.12 ± 0.09 | 1.34 ± 0.02 | 1.17 ± 0.03 | 1.31 ± 0.01$^{\#}$ |

Data are expressed as mean ± SD, (n= 6). *Significantly different (*p*<0.05) compared with the control group. #Compared with the ethanol group.




**Graph 1: Levels of FSH, serum testosterone, LH of ethanol induced male albino rats treated with *Junglans regia* and *Nigella sativa* oil**

**Result of sperm analysis**

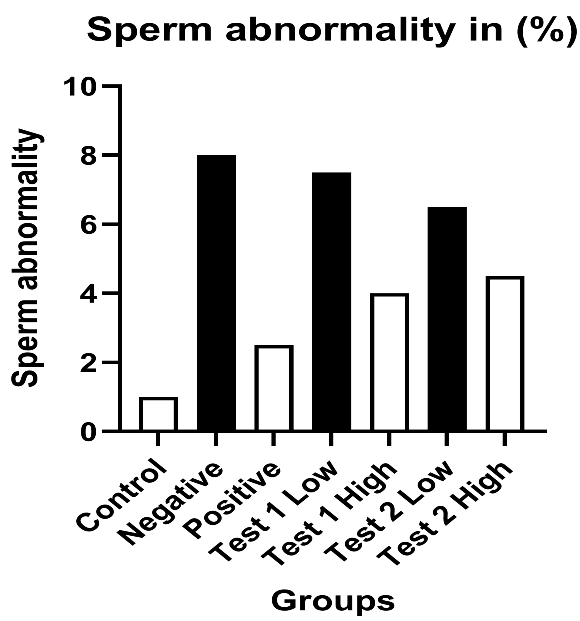
Results of sperm characteristics showed that the treatment of rat with ethanol reduced sperm count and viability and increased sperm abnormalities in experimental group when compared with the control and extract/drug-treated groups Statistical analysis showed that reduction in sperm count, viability and abnormalities in experimental group is significant (*p*<0.05) when compared with control and extract-treated groups. Also, there is significant difference in sperm characteristics between ethanol-and extract treated groups .

Motility analysis of spermatozoa indicated that the total motility significantly ($p<0.05$) decreased in ethanol administered group when compared with the control, while the extract significantly increased the sperm motility but not up to the control level. The rate of sperm movement was also decreased by ethanol. The pH of the semen was not affected in all the treatment groups as the value falls with in the neutral value which is favorable to the semen survival (Table4).

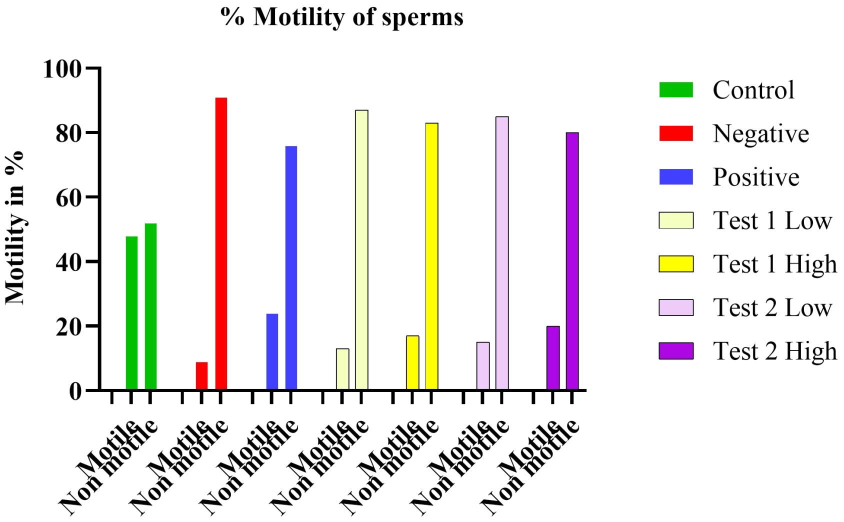
**Table 4: Characteristics Of Abnormal Spermatozoa In Rats Treated Under Ethanol-Induced Condition With *Junglans Regia And Nigella Sativa*(%)**

| Parameters | Tailless head | Headless tail | Bent tail | Coiled tail |
|---|---|---|---|---|
| Control group | 2.50 ±0.23$^{a}$ | 2.50 ± 0.13$^{a}$ | 3.50 ± 0.10$^{a}$ | 1.00 ± 0.02$^{a}$ |
| Negative group | 5.50± 0.30$^{b}$ | 8.00± 0.65$^{b}$ | 10.50 ± 1.04$^{b}$ | 8.00± 0.62$^{b}$ |
| Positive group | 2.00± 0.11$^{b}$ | 2.00± 0.05$^{b}$ | 5.50 ± 0.34$^{b}$ | 2.50± 0.15$^{b}$ |
| Test 1 Low (5ml/kg) | 5.50 ± 0.26$^{a}$ | 7.00± 0.64$^{a}$ | 5.50 ± 0.24$^{a}$ | 7.50± 0.36$^{a}$ |
| Test 1 High (10ml/kg) | 2.50 ± 0.04$^{b}$ | 4.00 ± 0.07$^{c}$ | 5.00 ± 0.45$^{a}$ | 4.00± 0.12$^{a}$ |
| Test 2 Low (5ml/kg) | 4.50 ± 0.12$^{a}$ | 7.50 ± 0.34$^{a}$ | 6.50 ± 0.33$^{c}$ | 6.50 ± 0.35$^{b}$ |
| Test 2 High (10ml/kg) | 3.00± 0.41$^{a}$ | 4.50± 0.08$^{b}$ | 6.00± 0.52$^{b}$ | 4.50± 0.10$^{b}$ |

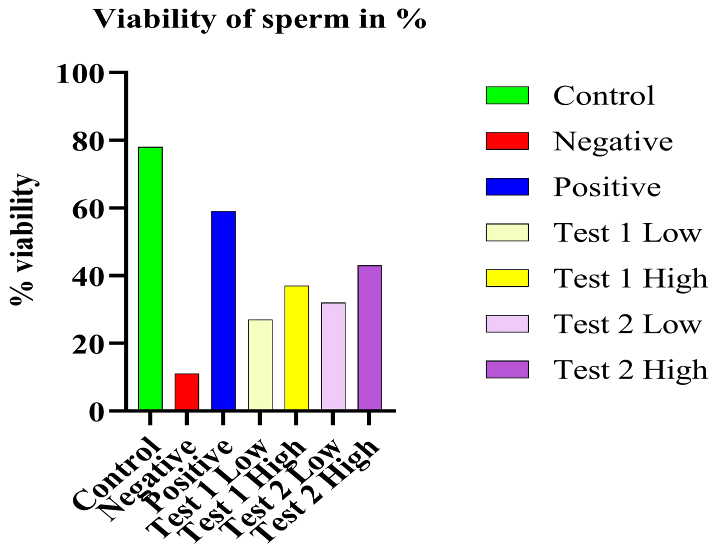
Data are expressed as mean ± SD, (n= 6). *Significantly different ($p<0.05$) compared with the control group. #Compared with the ethanol group.




**Graph 2: Total sperm abnormality of ethanol induced male albino rats treated with Junglans regia and Nigella sativa oil**




**Graph 3: Total sperm motility of ethanol induced male albino rats treated with *Junglans regia* and *Nigella sativa* oil.**

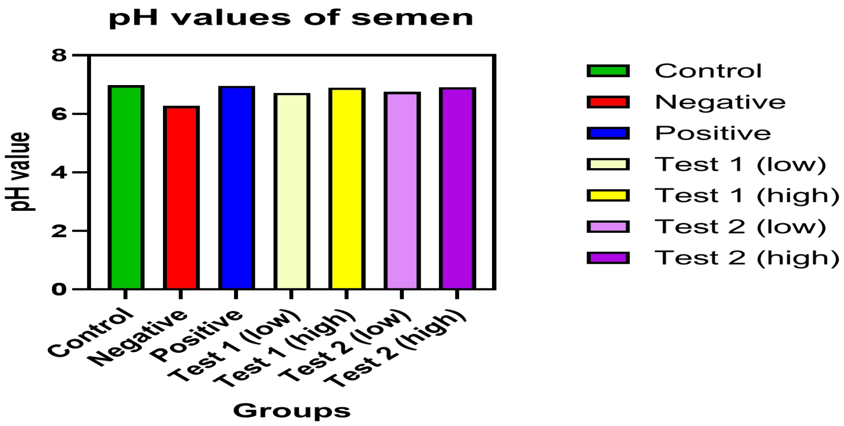



**Graph 4: Total sperm viability of ethanol induced male albino rats treated with *Junglans regia* and *Nigella sativa* oil**

**Table 5: Ph Values Of Semen Of Control And Ethanol-Induced Male Albino Rats Treated With *Junglans Regia* And *Nigella Sativa***

| Groups | Control group | Negative group | Positive group | Test 1 low (5ml/kg) | Test 1 high (10ml/kg) | Test 2 low (5ml/kg) | Test 2 high (10ml/kg) |
|---|---|---|---|---|---|---|---|
| **pH values** | 6.98 ± 0.20 | 6.27 ± 0.10 | 6.95 ± 6.15 | 6.72 ± 0.30 | 6.89 ± 0.27 | 6.75 ± 0.21 | 6.91 ± 0.11 |

Data are expressed as mean ± SD, (n= 6)




**Graph 5: pH values of semen in ethanol induced male rats treated with *Junglans regia* and *Nigella sativa* oil**

## Discussion

The findings of this investigation suggested that ethanol may affect the pituitary-gonadal axis, steroidogenesis, oxidative stress, and inflammation, all of which might affect reproductive function[24]. Through mitochondrial damage, the synthesis of homocysteine and acetaldehyde a disturbance in the equilibrium of critical components shows. Increase in microsomal proliferation, alcohol consumption can cause oxidative stress and inflammation[25]. Additionally, alcohol can cause structural abnormalities in sperm cells by causing genotoxicity and seminiferous tubules as a result of lipid buildup in Sertoli cells and spermatogenic cells in rats given alcohol[26]. Our findings demonstrated that ethanol dramatically decreased the concentration of testosterone in the serum, which is consistent with earlier research[27]. Furthermore, oxidative damage and inflammation brought on by ethanol may be the primary causes of decreased testosterone levels and impaired reproductive function[29].The testes had two purposes in mammals: spermatogenesis and steroidogenesis. Nevertheless, a number of factors, including medication, chemotherapy, toxins, polluted air, deficiency in nutrients and vitamins, and toxins can disrupt spermatogenesis and shows negative impact on sperm production[28].Alcohol abuse is a widespread problem in many civilizations and research indicates that it has detrimental effects on human health. It has been shown in numerous studies that consuming ethanol leads to reduced testosterone production, low sperm quality, and changed sexual behavior in males[30].The purpose of this study was to investigate action of *Junglans regia* and *Nigella sativa* on ethanol induced infertility in male rats. Our findings demonstrated that, in comparison to the control group, the reproductive parameters of rats exposed to 38% v/v Ethanol for 14 days was dramatically reduced. Our outcome is consistent with earlier data.

The medicinal advantages of *Junglans regia* have been known since prehistoric times. Walnuts were utilized in traditional medicine to cure a variety of conditions, including diabetes, cancer, inflammation, diarrhoea, problems with the prostate, and cardiovascular issues [31]. Polyphenols, fatty acids, cardiac glycosides, carbohydrates, steroids, minerals, tannins, proteins, dietary fiber, melatonin, α-tocopherol, plant sterols, folate, vitamin E, vitamin C, and vitamin A are present in walnut fruit [32]. *Junglans regia* oil served as a representative of the flavonoids, saponins, and phenols found in the current investigation[33].*Junglans regia* has a rich source of polyunsaturated fatty acids. In comparison to the control group, Robbin and colleagues found that a daily intake of 75 g of walnuts improved the morphology, vitality, and motility of sperm in a group of young men in good health. They also found that increased level of omega-6 and omega-3 PUFs in blood serum were linked to improvement of semen quality and sperm motility [35].Significant amounts of polyunsaturated fatty acids, including linoleic acid, oleic acid, and linolenic acid are present in *Junglans regia*[36]. By inhibiting 5-α reductase walnut oil stops testosterone from being converted to dihydrotestosterone, restoring plasma testosterone levels in men and oestrogeninin postmenopausal women [37]. But *Junglans regia* oil appears to have increased testosterone levels because of its impact on leydig cells and its interference with testosterone production was most likely caused by stimulus of prostaglandin production. The development and normal operation of the reproductive system depend on reproductive hormones. Male reproductive potential is fulfilled by FSH and testosterone [38]. Research revealed that there is a connection between spermatogenesis and sex hormones such as testosterone, LH, and FSH [39]. The levels of FSH, LH, testosterone, and estradiol were measured in animals that were male and female. *Junglans regia* oil at low and high dosages significantly increased the levels of FSH and LH in female rats. Similarly, it was discovered that level of testosterone considerably higher in male rats, suggesting that *Junglans* regia oil may have fertility-boosting properties. These findings support earlier research that discovered how *Junglans regia* considerably raises sex hormones, mostly LH, FSH that enhance testicular growth. Higher dosages of *Junglans regia* oil considerably raised FSH levels which leads to enhance fertility; this was likely because the pituitary was more sensitive to gonadotropin-releasing hormone [40]. According to Alahmar (2019), oxidative stress is essential to the pathophysiology of several inflammatory disorders as well as reproductive processes. While reactive oxygen species play a crucial role in physiological processes as important signaling molecules, its high concentrations can potentially influence pathological processes related to reproduction[41].

A number of beneficial results have seen of *Nigella sativa* on male infertility. Sperm are protected by *Nigella sativa's* protective action, which have been demonstrated for its ability to scavenge superoxide anion, direct cytoprotective effects, indirect antioxidant, androgen activities[42]. Thymoquine's antioxidant properties allow it to act on mitochondrial deterioration and enhance spermatogenesis [43]. Antioxidants such as *Nigella sativa*, which can boost sperm factors or neutralize free radicals in the semen fluid, can promote steroidogenesis and spermatogenesis. Among which, several compounds, such as flavonoids, anthocyanin, carotene, isothiocyanate, and carotenoids that are also present in *Nigella sativa*. Because *Nigella sativa* oil contains UFAs, such as oleic acid (about 20%) and linoleic acid (about 60%), it can increase sperm parameters like motility and count while also removing the sperm with anomalies. Phenolic and alkaloid substances raise the amounts of FSH and testosterone in testicular tissues. Because *Nigella sativa* contains zinc, copper, magnesium, and vitamins, the testes and epididymis may grow[44]. Testicular tissue and testosterone levels. According to study a rise in LH levels can raised the amounts of testosterone hormone generated by leydig cells, which is beneficial in growth, development, function, and weight of the male reproductive organs including the prostate and testes and epididymis

Clomiphene is a ovulatory stimulant class of drug it act by stimulating GnRH, LH and FSH it leads to stimulate or increase the level of testosterone in the animal

Our study shows that the both *Junglans regia* and *Nigella sativa* use to increase reproductive potential. The data shows that the ethanol have the negative mechanism on fertility it can damage the reproductive organs and hampers the fertility. Control group with no alcohol/ ethanol treatment has the highest results of fertilation parameters. Alcoholic group shows the worst results. The positive group which is consist of standard drug Clomiphene co received with ethanol shows better results than ethanolic group because standard drug Clomiphene had prevent the testicular damage cause due to ethanol. Our testing drugs *Junglans regia* and *Nigella sativa* are given in low and high dose with ethanol both are preventing the testicular damage cause by ethanol, the high dose group shows good results than low dose group but not as promising as positive group. The testicular injury cause by ethanol is overcome by both the test drugs

## CONCLUSION

This study has revealed that *Junglans regia* and *Nigella sativa* effectively treat the infertility caused by ethanol in male rats. The findings of this research provide a safer natural remedy for infertility problems. Due to side effects and economic considerations, plant-based remedies for infertility problems will be more widely accepted than chemical ones.

## ACKNOWLEDGEMENT

The Authors are thankful to the Research guide, Principal, Department of Pharmacology, Faculty of Pharmacy of YSPM’s Yashoda Technical Campus Satara, for providing essential facilities for research work.

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