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PROTECTIVE EFFECT OF *IONIDIUM SUFFRUTICOSUM* ON SPERMATOTOXICITY INDUCED BY ETHANOL IN EXPERIMENTAL RATS

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ABSTRACT

Chronic ethanol consumption associated with infertility in Wistar rats. To evaluate the protective effect of *Ionidium Suffruticosum* (*I.S*) on Sperm parameters in ethanol intoxicated experimental models. A total of 24 rats were equally divided into four groups. Group I control, Group II received ethanol in drinking water for 16 weeks. Group III received ethanol and simultaneous administration of *Ionidium Suffruticosum* extract (100 mg/kg body weight) for 16 weeks. Group IV received ethanol simultaneous administration of *Ionidium Suffruticosum* extract (300 mg/kg body weight) for 16 weeks. group II treated with ethanol shown significantly ($p<0.001$) reduced sperm count, motility, viability and increased abnormal morphology when compared to control. Treatment groups III & IV significantly increased ($p<0.05$) the sperm count, motility, viability, and retrieved the morphological abnormalities. *Ionidium Suffruticosum* plays major role in the reversal of the spermatogenesis in the ethanol intoxicated experimental model.

KEYWORDS: **Ethanol, Sperm motility, viability, morphology, *Ionidium Suffruticosum*.**

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INTRODUCTION

Infertility is an emerging major health problem among the couples and the male factor is accountable in 50% of the cases according to World Health Organization (1999), occurrences of male infertility rate is increased especially the last two decades varied between 8.97% and 14.63% in India [1]. Some chemicals can manifest the various disorders on male reproductive system. Ethanol is one of the endocrine disrupting chemicals and widely abused among the population in the world. Various studies have been demonstrated that the chronic ethanol consumption related with following disorders declined function of reproductive system in human [2] and delay in development of genital organs and behavior related sexual maturation in rats [3]. Also it decreases the pituitary Luteinizing Hormone and Follicle Stimulating Hormone release [4] and abnormalities in the seminiferous tubules that leading to defect in sperm production [5]. It has been disclosed that the ethanol consumption interrupts the function of pituitary gonadal axis in parent mice and their offspring [6]. Testicular oxidative stress is a result of the increased production of reactive oxygen species (ROS) and reduced level of antioxidants in seminal plasma which results in the sperm abnormalities and infertility. Chronic ethanol consumption induces the oxidative stress in testis which is a major factor of male infertility [7]. Earlier investigators explained the causative factor of oxidative stress (OS) and the role of various antioxidants such vitamin E, C and Coenzyme Q10, in preventing DNA damage and enrichment of the quality of spermatozoa [8]. The development of such an efficient antioxidant therapy may be important for the treatment of hypospermatogenesis [9].Plant derived bioactive compounds are interact with the proteins, DNA and other biological molecules to produce a desired outcome [10] especially in the treatment of male infertility. Plant derived bioactive compounds are collectively termed as phytonutrients or phytochemicals, are known for their antioxidant activity [11]. *Ionidum suffrutiosum (L.) Ging,* belongs to *violaceae* family. In India, it is a common weed of waste lands and open forests. It is small perennial, much branched, erect woody herbs. Leaves are simple, linear-lanceolate. Flowers are pink, axillary, solitary. Fruit are sub-globose, and yellowish. Whole plant has been used to cure the diseases like, urinary disorders, tuberculosis, asthma, fever, leprosy and eye disease [12]. It has been extensively used to improve the sexual function as a stimulant among folklore especially in south India. Plant has been used as diuretic, antigonorroetic, demulucent, and bowel complaints of children especially diarrhea [13] and plant steroidogenic activities has been demonstrated with various parameters on rat testis [14]. The plant morphological character and phytochemical values were identified by previous investigator, which contains steroids, triterpenes, sugars, flavenos, catechins, alkaloids, tanine, phenols, anthraquinones and aminoacids [15]. *Ionidium suffruticosum* well known for its antioxidant properties, but there is a lack of scientific support, its effect on infertility, caused by sperm parameter in ethanol intoxicated Wistar rats. The objective of this present study was to evaluate the protective effect of *Ionidium suffruticosum* on sperm characteristic and morphological changes of Wistar rats.

MATERIALS AND METHODS

(i) Chemicals

Ethanol solution (99.9% (product no:UN:1170) Hayman Limited,UK). Sucrose (Excela R (Part no:15925) Qualigens, India were purchased from ILE Company Chennai, Papinacloau stain, Heamatoxylin and eosin stain and other chemicals were of Analytical Grade.

(ii) Plant material

Ionidium suffruticosum (syn.Hybanthus enneaspermus (L.) belongs to family *Violaceae* commonly known as spade flower were collected from the farm house in the kanyakumari District, Tamilnadu. The plant has been identified by Dr.Manian Professor & Head of the Department of Botany, Bharathiyar University, Coimbatore, Tamilnadu.

(iii) Extraction and Drying

The plant materials (leaves) were air-dried at room temperature (26±2°C) for two weeks, after which it was ground to a uniform powder. The extracts of the samples were prepared by soaking 100g of dried powder in 300 ml of hydro-ethanol (70% ethanol and 30% distilled water) for 48 hrs. Procedure was repeated. The extracts were filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-ROTAVAP, India). This filtrate is used in this study.

(iv) Animals

The present study was performed on healthy 24 male Wistar rats, 6-7 week old, weight about 70-100 gram, were procured from the Tamilnadu Veterinary and Animal Sciences University, Chennai. The rats were maintained under standard conditions (12 h light and 12 h of dark cycle) and temperatures (22-26◦C), the all the animal were fed with standard rat pellet diet (Provimine Animal Nutrition India Pvt. Ltd., Bangalore, Karnataka State, South India) and water ad-libitum. The experimental procedures were approved and conducted as per the norms of the Institutional Animal Ethical Committee of SRM Medical College & Hospital, in accordance with the CPCSEA guideline, bearing the number is 44/IAEA/2011.

(v) Toxicity studies

Acute toxicity studies were carried out with Albino Rats. Each animal received a single dose of *Ionidium Suffruticosum* extract dissolved in 1ml of distilled water (3000 mg/kg body weight) administered per orally after overnight fasting. Animal were observed continuously for 2 hours for the gross behavioral changes and every 30 minutes than once in every 2 hours, finally at the end of 24 and 72 hours to note of any signs of toxicity including death [16].

(vi) Selection of dose of the extract

LD_{50} was done as per OCED guidelines for fixing the dose for biological evaluation. The estimated LD_{50} of *Ionidium suffruticosum* is above 3000mg/kg body weight when given orally. There by the therapeutic dose for the pharmacological evaluation by *Ionidium suffruticosum* was 1/10th of the maximum tolerated dose, which was fixed to be 100 and 300mg/kg of the body weight of the experimental animals. The selected dose in this study is similar to previous study [17].

(vii) Experimental design

After two weeks of acclimatization, the rats were randomly divided into five groups, each group of six animals. Group I rats served as control.

Group II rats were administered various percentage of ethanol 5%, 10%, 20% and 30% (v/v) as incentive of 2mg of sucrose in the feeding bottle.

The ethanol concentration was increased stepwise end of every 4th weeks over the course of 16 weeks, in the following manner: 5%, 10%, 20% and 30% (volume/volume).

Group III rats were administered various percentage of ethanol 5%, 10%, 20% and 30% (v/v) as incentive of 2mg of sucrose in the feeding bottle, simultaneously treated with extract of *Ionidium Suffruticosum* (100mg/kg of bwt) were given orally.

Group IV rats were administered various percentage of ethanol 5%, 10%, 20% and 30% (v/v) as incentive of 2mg of sucrose in the feeding bottle, simultaneously treated with extract of *Ionidium Suffruticosum* (300mg/kg bwt) were given orally.

(viii) Collection of sperm samples

At the end of 16 weeks of treatment rats were sacrificed with an overdose of ketamine (product no: K2753, Sigma-Aldrich, India). The testicles were removed through a lower abdominal incision. The epididymis were separated from testis carefully with the 11 sized surgical blades, and lacerated to collect the semen for the further investigation.

(ix) Sperm Analysis

The collected sperms from experimental rats were used to analysis the sperm count, motility, viability and morphology in according to the method [18].

(a) Total sperm count

Cauda epididymis were placed in a Petri dish containing 10ml of phosphate buffered saline

(PBS) pre-warmed to 35-37◦C and split with a surgical blade to open the caudal part of epididymis to release the contents. Epididymal fluid ratio of 1:20. The petri dish was then swirled to achieve a uniform sperm suspension from which a sperm count was carried out in the Neubauer haemocytometer.

$$\text{Sperm count per ml} = \frac{\text{Sperm count} \times 20}{4 \times 0.1} \times 1000 = \text{Sperm count} \times 50000$$

The number of spermatozoa counted was expressed in 10^6 /ml.

(b) Sperm motility

A small drop of liquefied semen placed on a microscopic glass slide, covered with cover slip and examined under the light microscope at a magnification of 1000x. Several fields have been scanned to get a total number of 200 sperm. Motility was classified as progressive, non-progressive and non-motile.

(c) Sperm morphology and viability

Pre-cleaned slide with 95% ethanol and a small drop of semen is placed on the slide. Then fraction was pulled out into a smear with a second slide. Smears were air-dried and fixed in 95% ethanol for 15 minutes. For scoring the normal and abnormal morphology of 200 spermatozoa per rat, sperm smeared microscope slide were stained with papanicolaou (PAP) and haematoxylin and eosin (H&E) [19]. Viability and non viability ratio was determined using 0.5g Eosin Y and 3g Nigrosin in 30ml of distilled water. Fresh semen sample were smeared with 2 drop of prepared solution and assessed by counting a minimum of 200 sperm. Slides were examined under oil immersion with magnification of 1000x [20].

STATISTICAL ANALYSIS

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 19. Homogeneity of variances and normality should be checked before using the ANOVA. Differences between the groups were calculated using One-way Analysis of Variance (ANOVA) followed by the post hoc (Tukey HSD) tests. Data were expressed in means ± SD (standard deviation) and the term 'statistically significant' was used for $p<0.05$.

RESULTS

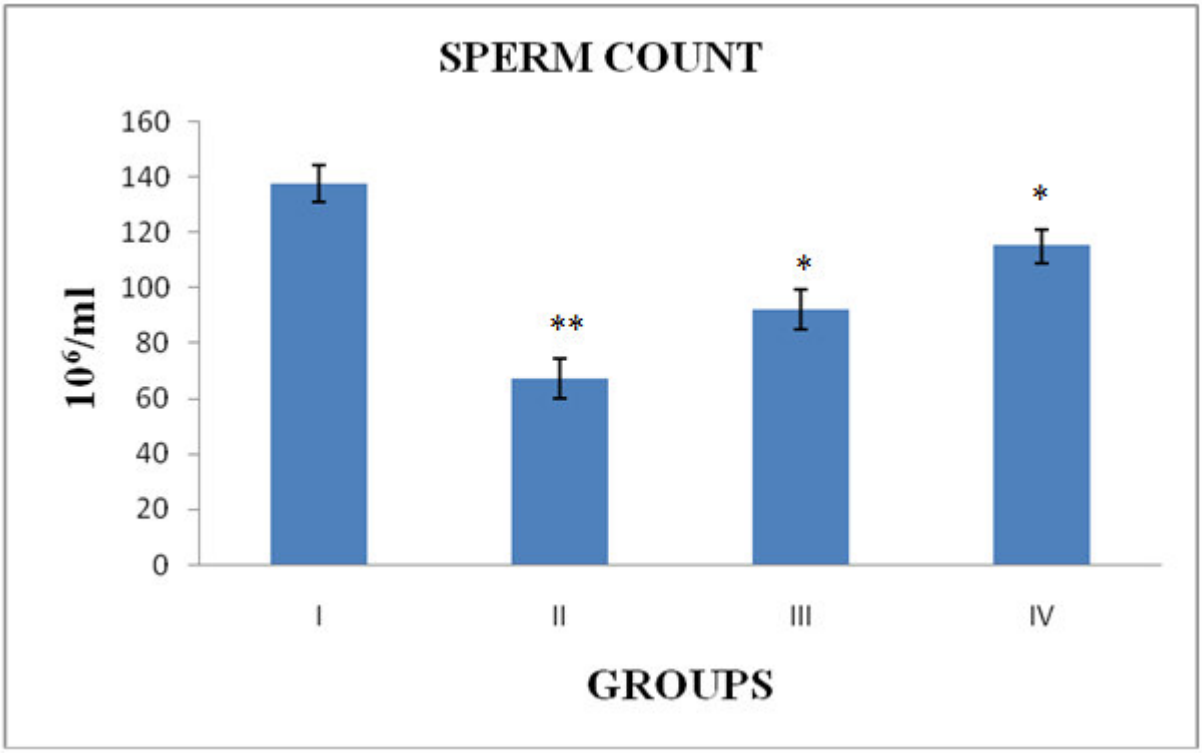
Result of toxicity studies and LD_{50} of the extract

No death was observed till the end of study. Hence, it could be concluded that the estimated LD_{50} of *Ionidium suffruticosum* is above 3000mg/kg body weight when given orally.

Effect of ethanol on sperm parameters.

The total number of sperm count in ethanol treated group was significantly ($p<0.001$) lower than control group (Figure.1). As shown in Table 1.The sperm motility in ethanol treated group was significantly ($p<0.05$) lower than the control group. As shown in Table 2.The sperm viability in ethanol treated group was significantly ($p<0.001$) lower than the control group and also the high percentage of morphological abnormalities were significantly ($p<0.001$) increased in ethanol group vs control.

Figure 1
Protective effect of Ionidium suffruticosum on sperm count of control and experimental groups.



I- Control; II- Ethanol+Sucrose; III- Ethanol+Sucrose+Ionidium Suffruticosum (100mg/bwt); IV- Ethanol+Sucrose+Ionidium Suffruticosum (300mg/bwt). Values were expressed as means ± S.D. of six rats in each group. **(p<0.001) vs control . *(p<0.05) vs Group II. NS indicates no significant vs control.

Effect of Ionidium Suffruticosum reverses the sperm count by its anti hypospermatogenesis properties.

Ionidium Suffruticosum (100mg/kg/bwt and 300mg/kg bwt) treatement significantly ($p<0.05$) increased the sperm count when compared with ethanol induced rats. Ethanol consumption was found to cause a significant decrease in the total antioxidant capacity in testis that leading to hypospermatogenesis as compare to control group (Figure.1).

Administration of Ionidium Suffruticosum enhances the sperm motility

Effect of *Ionidium Suffruticosum* on sperm motility in control and experimental groups. As shown in Table 1. *Ionidium Suffruticosum* treatment with dosage of 100mg and 300mg/kg bwt, were significantly increased the sperm motility when compared with ethanol treated group ($p<0.05$).

Table 1
Percentage of motile, progressive, and non-motile sperm cells.

Parameters	Group I	Group II	Group III	Group IV
Sperm motility (%)	94.00 ± 4.60	55.00 ± 2.16**	75.00 ± 4.37*	87.50 ± 6.67*
Progressive (%)	80.00 ± 5.06	21.50 ± 4.81**	54.00 ± 1.41*	71.00 ± 4.25*
Non-Progressive (%)	14.00 ± 4.98	33.50 ± 4.00**	21.00 ± 4.03*	16.50 ± 3.78*
Non-motile (%)	6.00 ± 4.60	44.50 ± 1.41**	25.00 ± 4.37*	12.50 ± 6.67*

I- Control; II- Ethanol+Sucrose; III- Ethanol+Sucrose+Ionidium Suffruticosum (100mg/bwt); IV- Ethanol+Sucrose+Ionidium Suffruticosum (300mg/bwt). Values were expressed as means ± S.D. of six rats in each group. **(p<0.001) vs control . *(p<0.05) vs Group II.

Impact of Ionidium Suffruticosum increases the sperm viability by anti-spermatolytic properties.

As shown in Table 2. *Ionidium Suffruticosum* treatment with dosage of 100mg and 300mg/kg bwt, were significantly increased the sperm viability when compared with ethanol treated group ($p<0.05$).

Impact of Ionidium Suffruticosum to prevent the formation of sperm abnormalities by its regulatory properties of ROS formation in the seminal plasma.

As shown in Table 2. *Ionidium Suffruticosum* treatment with dosage of 100mg and 300mg/kg bwt significantly reduced the sperm abnormalities when compare with ethanol treated group ($p<0.05$).

Table 2

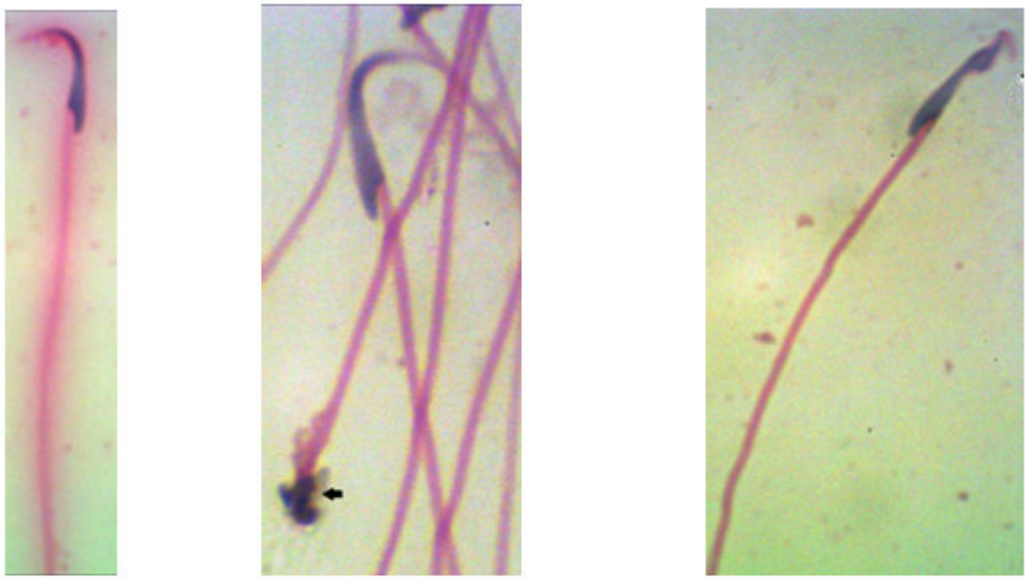
Percentage of viability, non-viability and morphology of sperm cells.

Parameters	Group I	Group II	Group III	Group IV
Viability (%)	94.00 ± 4.60	55.00 ± 2.16**	75.00 ± 4.37*	87.50 ± 6.67*
Non viability (%)	00 ± 00	18.16 ±4.08**	4.58 ± 1.53*	1.33 ± 0.68*
Normal Sperm morphology (%)	80.00 ± 5.06	21.50 ± 4.81**	54.00 ± 1.41*	71.00 ± 4.25*
Morphological Abnormalities in Sperm (%)	19.83 ± 5.31	78.00 ± 4.32**	46.00 ± 1.41*	29.00 ± 4.25*
Head defect (%)	00 ± 00	20.00 ± 5.23**	5.50 ± 2.89*	1.50 ± 0.89*
Tail defect (%)	19.83 ± 5.31	58.50 ± 8.57**	40.50 ± 3.09*	27.50 ± 4.15*

I- Control; II- Ethanol+Sucrose; III- Ethanol+Sucrose+Ionidium Suffruticosum (100mg/b wt); IV- Ethanol+Sucrose+Ionidium Suffruticosum (300mg/b wt). Values were expressed as means ± S.D. of six rats in each group. ** ($p<0.001$) vs control. * ($p<0.05$) vs Group-II.

Figure 2

Histological evaluation of sperm in control and experimental groups

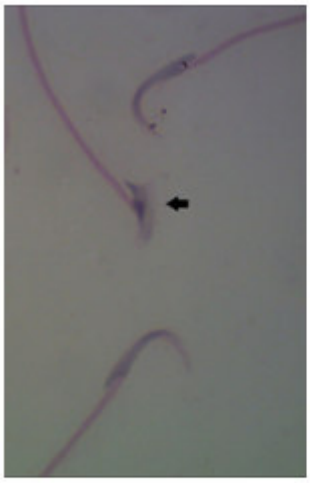


2A. Normal sperm -H &E

2B. Amorphous Head (Arrow) –H&E

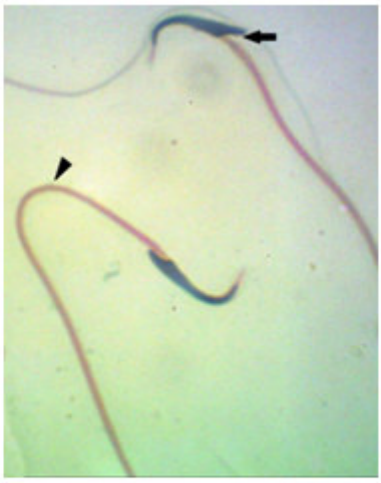
2C. Banana Head -PAP

Group: I



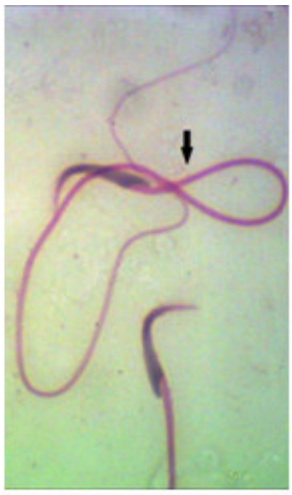
2D. Triangular Head (Arrow) H&E

Group: II



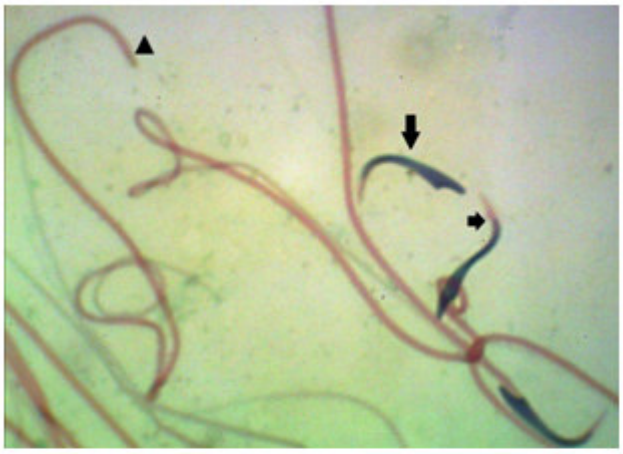
2E. Neck defect (Arrow) & Mid-piece bent (Arrow head) PAP

Group: II



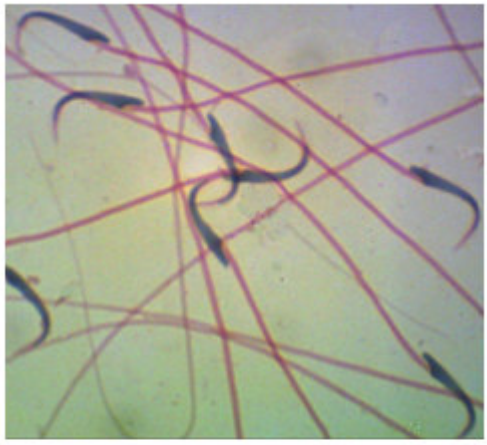
2F. Highly coiled with multiple abnormalities in tail (Arrow) H&E

Group: II



2G. Tailless Head (Long Arrow), Less Angulations (Short Arrow) & Headless with coiled tail (Triangle) PAP

Group: II



2H. *Ionidium Suffruticosum* 300mg (Group IV) treatment showed normal morphology. H&E

Group: IV

DISCUSSION

In the present study the total sperm count in ethanol treated group was significantly decreased ($p<0.001$) when compare to control group. The obtained result is similar to that previous investigators [21]. Declined sperm count is associated with prolonged exposure of seminiferous epithelium to high levels of ROS and high level of ROS facilitates the process of apoptosis and DNA damage, leading to testicular atrophy and declined spermatogenesis [22] that leading to reduced the sperm count in azoospermia condition [23].

The percentage of sperm motility in the ethanol treated group was significantly lower ($p<0.001$) than in control group. The sperm motility is based on their functional ability of the mitochondria, which is constantly providing the energy to their motility. It has been reported that the significant elevation of ROS production in the seminal plasma, in turn affect the mitochondrial function [24]. In our results sperm motility was agreement with previous study who observed that the adverse effect of ethanol on secretory products in relation with sperm maturation in epididymis [25]. The percentage of viability of sperm in ethanol treated group was significantly lower ($p<0.001$) than in control group and our result is similar to previous study which demonstrated that impaired fertility in Wistar rats caused by intraperitoneal injection of ethanol [26]. Previous study suggested that the increased sperm cell death due to alcohol induced oxidative stress and ROS is formed when the acetaldehyde interact with protein and lipids. Acetaldehyde was a by-product of alcohol metabolism, which is capable to damaging the mitochondria result in decreased ATP (adenosine triphosphate) production leading to sperm cell death [27]. Also reported that ROS-induce the lipid peroxidation of sperm membrane eventual rupture leading to discharge of cells and its organelle [28] and oxidation of DNA bases results impaired sperm function and loss their viability [29]. Percentage of motility, viability and total sperm count is drastically reduced in ethanol treated group compared to control, which indicates diminished spermatogenesis in testis and spermatozoa maturation in epididymis. The motility, viability and total sperm count were significantly ($p<0.05$) increased in rat treated with dose of 300mg than 100mg of *ionidium suffruticosum.* As shown in Fig.2 (B,C,D,E,F & G), the high percentage of morphological abnormalities are observed as head, neck, midpiece, principle piece and tail in ethanol administered group compare to treatment (III & IV) groups (Fig.2H). The head without hook, head without tail or detached head, tail with coiled, tail with broken, tail without head were similar to that previous study [30]. Banana head, triangular head, similar findings were observed by previous experimental study [31] and hooks at wrong angled and bent hook observed by us were similar to previous study [32]. As expressed in earlier studies that detached heads and abnormal tails associated with sperm viability also evidence of cell death occur after releasing from the sertoli cells, as blunt hooked head may caused by acrosomal degeneration and banana head, abnormal angulations of head, and less hooked due to DNA damage may due to increased level of ROS in seminal plasma [33]. As a mid piece defects are bent, curved, looped, as principle piece defects are bent, curved, coiled and looped, rudimentary tail or short tail, headless tail observed abnormalities were similar in previous study [34] may caused by underdevelopment of flagellum or energy depletion or excessive residual cytoplasm. Normally spermatozoa expel their cytoplasm from mid piece. Investigator explained that association between the potential OS and spermatozoa with excessive residual cytoplasm and infertility [35]. We observed defect in the neck region, few highly coiled, and spermatozoa multiple anomalous more tailless head and headless tail in ethanol treated group, and our results were similar to earlier studies and showed that the total sperm count, motility and viability significantly decreased in ethanol treated groups when compare to control and showed that the ethanol increase the sperm chromatin and DNA damage and recommended that administration of proper antioxidant could neutralize the harmful effects [36] were generated by ROS in alcohol abusers. Our results are positively correlated with ethanol-induced reproductive toxicity were ameliorated by administration of Kolaviron [37]. A study showed that the potential benefits of *Telfairia occidentalis* seed oil on alcohol induced testicular damage and sperm abnormalities [38]. An another study demonstrated that the effect of ethanol on sexual performance, hormonal level and sperm parameters and normalize an ethanol inducing effect by administration of the poly herbal drugs [39]. It has been shown that the chronic ethanol consumption can reduced the total sperm count, total motility, viability and increased the sperm abnormalities. Administrations *of Ionidium suffrutticosum* which modulates the antioxidant level and enhance the quality of sperm by the way of neutralize the OS due to ethanol consumption.

CONCLUSION

The data of our study suggest that the presence of antioxidant properties in the herb, reduced or nullified the effect of the oxidative stress due to chronic ethanol consumption, and rat treated with 300mg showed less morphology than 100mg. we conclude the 300mg/Kg of drug have more antioxidant activities than 100mg/Kg, this drug could be used not only to improve the sexual function also used for reproductive toxicity due to chronic alcoholism. Moreover, for the first time, we demonstrated that the protective effect of *Ionidium suffruticosum* on spermatotoxicity induced by ethanol in male rats.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Samal S., Dhadwe SK., Gupta U., Gupta NK. Epidemiological study of male infertility. Indian Medical Gazette, 174-180, (2012).
2. Muthusami KR., Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. Fertil Steril, 84 (4): 919-924, (2005).
3. Marisela Hernández-González., Koral Elizabeth Rivers Sánchez., Martha Verónica Oropeza Blando., Sandra Orozco-Suarez., Marcela Arteaga Silva., Miguel Angel Guevara. Effects of alcohol on behavioral and morphologic indices of sexual maturation in male rats. Alcohol, 33: 117-126, (2004).
4. Iiris Slonen., Ilpo Huhtaniemi. Effects of chronoic ethanol diet on pituitary - testicular function of the rats. Biology of Reproduction, 42: 55-62, (1990).
5. Joanne Weinberg., Wayne Vogl A. Effects of ethanol consumption on the morphology of the rat seminiferous epithelium. Journal of Andrology, 9 (4): 261-269, (1988).
6. Albadri CT., Al Ani IM., Hiba HMA. Alcohol consumption and its effect on testicular structure and on sperm count and motility in parent mice and their offspring. The International Medical Journal Malaysia, 12 (1): 43-48, (2013).
7. Ashok Agarwal., Gurpriya Virk., Chole Ong., Stefan S du Plesis. Effect of oxidative stress on male reproduction. World J Mens Health, 32 (1): 1-17, (2014).
8. Kartikeya Makker., Ashok Agarwal., Rakesh Sharma. Oxidative stress & male infertility. Indian J Med Res, 129: 357-367, (2009).
9. Elshaari FA., Fatum AE., Sheriff DS. Spermatozoa- A unique representation of oxygen antioxidant paradox. Acta Medica Mediana, 49 (1): 48-53, (2010).
10. Joana Gil-Chávez G., José A Villa., Fernando Ayala-Zavala J., Basilio Heredia., David Sepulveda., Elhadi M Yahia., Gustavo A González-Aguilar. Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: An Overview. Comprehensive Reviews in Food Science and Food Safety, 12: 5-23, (2013).
11. Mark Percival., Antioxidants. Clinical Nutrition Insights, 1-4, (1996).
12. Pullaich T. Encyclopedia of World Medicinal Plant, Vol 3, Regency Publication: New Delhi, 1109, (2006).
13. Khare CP, Indian Medicinal Plants: An Illustrate Dictionary, Springer-Verlag Berlin/Heidelberg, 329, (2007).
14. Senthil Kumar B., Vijaya Kumar J. Effect of *Ionidium Suffruticosum* on testes of young albino rat. IJPBS, 2 (3): 106-112, (2012).
15. Arunkumr B., Sonappanavar., Jayaraj M., Ankalikar AA. Pharmacognostical

Investigation of *Ionidium Suffruticosum* Ging. A Seasonal multipotent medicinal herb. Decan J. Natural Products, 1(2): 43-50, (2010).
16. Gosh MN. Fundamentals of Experimental Pharmacology, 2nd Edn, Scientific Book Agency: Calcutta, 153-158, (1984).
17. Narayanaswamy VB., Manjunatha Setty M., Malini S., Annie Shirwaikar. Preliminary aphrodisiac activity of *Hybanthus enneaspermus* in rats. Pharmacologyonline, 1: 152-161 (2007).
18. Terry R Reynolds and B.S. Narang. Semen Analysis. Medical Laboratory Technology A Procedure Manual for Routine Diagnostic Tests. In: Kanai L Mukherjee (ed.),Vol 2, Tata McGraw-Hill, New Delhi, India, 1988, pp.871-876.
19. Marilyn Gamble. The Hematoxylins and Eosin. Theory And Practice of Histological techniques. In: John D Bancroft (ed.), Churchill Livingstone, 2008, pp121-128.
20. William M. Buckett. Predictive value of hypo-osmotic swelling test to identify viable non-motile sperm. Asian J Androl, 5: 209-212, (2003).
21. Oremosu AA., Akang EN. Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague-Dawley rats. Middle East Fertility Society Journal, 1-5, (2014), In press.
22. Gil-Guzman E., Ollero M., Lopez MC., Sharma RK., Alvarez JG., Thomas AJ Jr., Agarwal A. Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. Human Reproduction, 16 (9): 1922-30, (2001).
23. Raghuveer Choudhary., Chawala VK., Soni ND., Jayant Kumar., Vyas RK. Oxidative stress and role of antioxidants in male infertility. Pak J Physiol, 6 (2): 54-59, (2010).
24. John Aitken R., Keith T Jones., Sarah A Robertson. Reactive oxygen species and sperm function- in sickness and in health. Journal of Andrology, 33 (6):1096-1106, (2012).
25. Srikanth V., Malini T., Arunakaran J., Govindarajulu P., Balasubramanian K. Effects of ethanol treatment on epididymal secretory products and sperm maturation in albino rats. The Journal of Pharmacology And Experimental Therapeutics, 288 (2): 509-515, (1999).
26. Anthony E Ojieh. Changes in reproductive hormones and sperm viability induced by ethanol and associated morphological alternations in gonads of wister rats. Journal of Medical and Applied Biosciences, 3: 37-44, (2011).
27. Ashok Agarwal., Sushil A Prabhakaran. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Indian Journal of Experimental Biology, 43: 963-974, (2005).
28. Tamilselvan P., Bharathiraja K., Vijayaprakash S., Balasubramanian MP. Protective role of Lycopene on Bisphenol a induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Int J Pham Bio Sci, 4 (4): 131-143 (2013). www.ijpbs.net.
29. Suresh C Sikka. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Frontiers in Bioscience, 1: 78-86, (1996).
30. Smara U Oliva., Alessandra G Messias., Daniela AF Silva., Oduvaldo CM Pereira., Daniela CC Gerardin., Wilma G Kempinas. Impairment of adult male reproductive function in rats exposed to ethanol since puberty, Reproductive Toxicology. 22: 599-605, (2006).
31. Himanshu Gupta., Kamal Kishore Maheswari., Nitin Kumar. Reversible germ cell toxicity of *Sulphasalazine* and *Ampicillin* combination in male rats. J Reprod Infertil, 14 (3): 126-132 (2013).
32. Sudha M., Kavimani S. The productive role of spirulina on Doxorubicin induced genotoxicity in germ cells of rats. Int J Pharma Bio Sci, 2 (3): 214-222, (2011). www.ijpbs.net.
33. Sally D Perreault., Aida M Cancel. Significance of incorporating measures of sperm production and function into rat toxicology studies. Reproduction, 121: 207-216, (2001).
34. Adebowale Bernard Saba., Olayinka Ayotunde Oridupa., Matthew Olugbenga

Oyeyemi., Oluwaseun Dapo Osanyigbe. Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of *Lagenaria breviflora Roberts*. African Journal of Biotechnology, 8 (7): 1170-1175, (2009).

35. Anil K Rengan., Ashok Agarwal., Michelle van der Linde., Stefan S du Plessis. An investigation of excess residual cytoplasm in human spermatozoa and its distinction from the cytoplasmic droplet. Reproductive Biology and Endocrinology, 10 (92): 1-8, (2012).
36. Marzieh Rahimipour, Ali Reza Talebi., Morteza Anvari., Abolghasem Abbasi Sarcheshmeh., Marjan Omidi. Effects of different doses of ethanol on sperm parameters, chromatin structure and apoptosis in adult mice. European Journal of Obstetrics & Gynecology and Reproductive Biology, 170: 423-428, (2013).
37. Oluwatosin A. Adaramoye., Muritala Arisekola. *Kolaviron*, a biflavonoid complex from *Garcinia kola seeds*, ameliorates ethanol-induced reproductive toxicity in male wistar rats. Niger. J. Physiol. Sci., 28: 009-015, (2013).
38. Edidiong Akang. Ademola Oremosu., Olufunke Dosumu., Adedayo Ejiwunmi. *Telfairia occidentalis*, a prophylactic medicine for alcohol's damaging effect on the testis. Macedonian Journal of Medical Sciences, 4 (4): 380-387, (2011).
39. Mitra SK., Muralidhar TS., Rao DRB. Experimental assessment of relative efficacy of drugs herbal origin on sexual performance and hormone levels in alcohol exposed and normal rats. Phytotherapy Research, 10: 296-299, (1996).