IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 5 Ser. VI (Sep. –Oct. 2020), PP 19-26
www.Iosrjournals.Org

Protective Effect of *Withania Somnifera* on Follicular Cells Against Endosulfan Exposed Mice

Poonam Kumari
P.G. Department of Zoology, College of Commerce, Arts & Science, Patna
Magadh University, Bodh- Gaya

Abstract
Indiscriminate use of pesticides has increased many folds in the recent times. Pesticides, fertilizers and improved seed varieties have contributed to substantial increases in crop yields. Despite of these benefits, pesticide uses are accompanied by potential risks to the environment and human health. Prolonged pesticide exposure may cause liver malfunction, immune malfunction, neurologic impairments and reproductive effects, Which has also led to hormonal imbalance in females leading to infertility. Medicinal plants have been used by people of all cultures for treatment of different diseases and toxicity since many decades. Thus, the present work is designed to study the effect of Withania somnifera against endosulfan induced toxicity on ovary of female mice.Treatment groups were administered endosulfan at the dose of 3.0mg/Kg body weight was administered by oral gavage method to female mice for1 week, 2 weeks & 4 weeks. Thereafter, aqueous extract of withania somnifera at the dose of 1000 mg/Kg body weight per day orally was administered for 4 weeks to observe the ameliorative effect of it on ovarian cells. The study reveals that after the exposure of endosulfan, showed the degenerated germinal epithelial cell and oocytes in graffian follicular cells in ovary of mice while Withania somnifera caused effective restoration in the different stages of the follicular cells in mice. that Withania somnifera not only possesses ameliorating and rejuvenating property but also maintains the histology of the ovarian cells leading to normal functioning of it. Thus, it proves to be one of the best antidote against endosulfan induced toxicity.

Keywords: *Corpus luteum; Endosulfan; Graffian follicles ; Light- Microscopy; Mice ; Oocytes; Withania somnifera.*

Date of Submission: 10-10-2020 Date of Acceptance: 26-10-2020

I. Introduction

In spite of harmful effects of poisons on environmentand other creatures, unfortunately at present the most effective and even the only applicable method for fighting with insecticides is chemicals. Chemical insecticides include different chemical compositions named as organochlorine in agriculture, industry, farming, animal husbandry and houses. The wide use of agrochemicals under conventional agriculture has caused severe health hazards for human beings. It also has caused numerous other side effects on the environment including destruction of the biodiversity. Endosulfan is an organochlorine insecticide effective against a wide range of pests like cereals, vegetables, fruits, coffee, cotton, oilseeds etc. It is easily absorbed in the gastro intestine by the stomach, lungs and skin and exposurethrough any route can be hazardous. The commercially produced endosulfan is composed of two isomers α- endosulfan and β- endosulfan and both these forms are genotoxic to human gonads [1].

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benza-dioxathiepin-3-oxide) is a polycyclic chlorinated hydrocarbon insecticide. According to World Health Organisation (WHO), endosulfan is currently classified as Class II – moderately hazardous to human health while the United States' Environmental Protection Agency (EPA) rates endosulfan as Category Ib – highly hazardous [2]. However, it still continues to be used in agriculture and public health. Endosulfan toxicity has been demonstrated in various organs such as the brain, kidney, liver, heart, and reproductive system [3].

Reproductive toxicity of endosulfan has been shown in some studies. Endosulfan reduces circulating follicle stimulating hormone (FSH) and luteinising hormone (LH). It has also been associated with decrease in daily sperm production (DSP), sperm count, and increase in the sperm abnormalities in males [4,5,6].

Withania somnifera, (WS) also known as ashwagandha, Indian ginseng, winter cherry, horse smell, Kaknaje Hindi, is a well-known medicinal plant in Solanaceae family used in traditional medicine in many countries such as Iran and India [7]. This plant is known to cure impotency and increase sex appeal and fertility when used solitarily or in combination with other medications [8,9]. This wild plant grows in dry and hot-semiarid climate such as southern Mediterranean region, Canary Islands, and northern Africa to northern India

(Iran, Jordan, Sudan, Palestine, Afghanistan, and Egypt) [10,11]. Different parts of this plant such as roots, leaves, flowers, seeds, stems, and fruits are used as remedy in traditional medicine of different countries [12-14]. Many phytochemicals have been extracted so far from this plant with possessing different pharmacologic and biological properties [15].

WS has been recommended for management of polyarthritis, lumbago, painful swellings, premature ejaculation, oligospermia, plague, asthma, vitiligo, general debility, impotency, ulcers, uterine infection, leucorrhoea, hemorrhoid, and orchitis in traditional Persian medicine [16,17]. All these therapeutic uses suggest its anti-inflammatory, aphrodisiac, semenogogue, and deobstruent features [18-21]. As far as there are no wide-spectrum and specific studies or systematic reviews about therapeutic effects of WS, on male and female reproductive system, the present study was trying to systemically review therapeutic effects of WS on reproductive system and fertility disorders.

Hence, the present work aims to study the ameliorative effect against Endosulfan induced toxicity in Swiss albino mice

II. Material & Methods

Animals: Adult Swiss albino mice were used in the experiment and their weight ranges from 30-35 g. The age of mice for the experiments was 12 weeks old. They were house in the different polypropylene cages containing sterile paddy husk as the bedding material. They were maintained under a well regulated light and dark (12h:12h) schedule at 24°± 3° and were allowed free access to laboratory food and tap water.

Test Chemical: Pesticide endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment.

Calculation of LD50 and Maximum Permissible Dose (MPD) of Withania Somnifera (Ashwagandha) aqueous root extract:

For calculating the LD50 value of Withania Somnifera for mice by standard method was reported by Balachandran and Govindrajan (2005), as 2500 mg/kg b.w as LD50. At 600 mg/Kg & 1100 mg/Kg b.w. although there were no death reports but the no side effects were seen at 1100 mg/kg b.w. So, 1000 mg/Kg b.w. was selected as Maximum Permissible Dose (MPD) for the experiment.

Study Protocol :

Mice were divided into five groups (n=10 per group) untreated control , endosulfan treated @ 3.0 mg/kg b .w. /day (one week, two week & four week) and endosulfan treated followed by withania somnifera (1000 mg/kg b.w./day). Endosulfan were administered orally because major available residue in the environment enter the non-target animal is by orally. The vehicle and test drug were administered to the respective group for 28 days. On 29th day the animal were sacrificed by cervical dislocation after the scheduled treatment. The ovary from all the animals were removed and cut into two pieces with the help of a sharp and sterlized blade. Accessory tissues were cleaned in normal saline and then fixed in formaline for light microscopy study.

III. Results:

Light Microscopic Study:

Microphotographs of ovary of control mice showed normal cortex and medullary region. Outer layer of germinal epithelial cells are in normal position. Primordial and primary follicles are in normal in shape (figure-1). Late primary and secondary follicle showing normal oocytes. In secondary follicle, formation of antrum has been developed. Stromal cells are also seen in normal in condition (figure -2). In graffian follicular cell, the cumulus oophorous cell surrounding the oocyte form a single layer of columnar cell and corona radiata. Stratum granulosa cells were observed around the oocyte with normal nuclei. All the follicular cells are well organized (figure-3), But after administration of endosulfan @ 3.0 mg/kg b.w /day orally treated for 1 week to ovary of swiss albino mice showed slightly degenerative appearances and hemorrhage in secondary follicle. It also showed irregularity in stromal cell shape. Fusion of most of follicular cells appeared (figure-4). Ovary of mice treated with Endosulfan for 3 weeks showed very degenerative appearances in outer germinal epithelial layer, primary and secondary follicular cells. The mature graffian follicular cell was ruptured and show tendency to decrease in size. Coagulated blood was dispersed. Ova were also degenerated wih some vacuolization (figure-5). Endosulfan 4 weeks treated ovary of female mice showed very highlyfragmented stromal cell. Very high degenerative appearances in germinal epithelium, primaty and secondary follicular cells. Mature graaffian follicles are showed completely ruptured and irregular. Corpus luteum are highly degenerated and fragmented cells are seen. Disrupted medullary region are seen. Ova were also degenerated. While after followed by withania somnifera @ 1000mg/ kg b.w. /day for four weeks showed the greatly impairment of germinal epithelium. Primary and secondary follicular cells have restored the outer layer. The disrupted grannulosa cell of graffian follicle are becomes arranged towards normalcy. The arrangement of follicular cells showed restoration

tendency, which indicate that withania somnifera play a vital role and to restore normal fertility against endosulfan toxicity on histology of ovary.

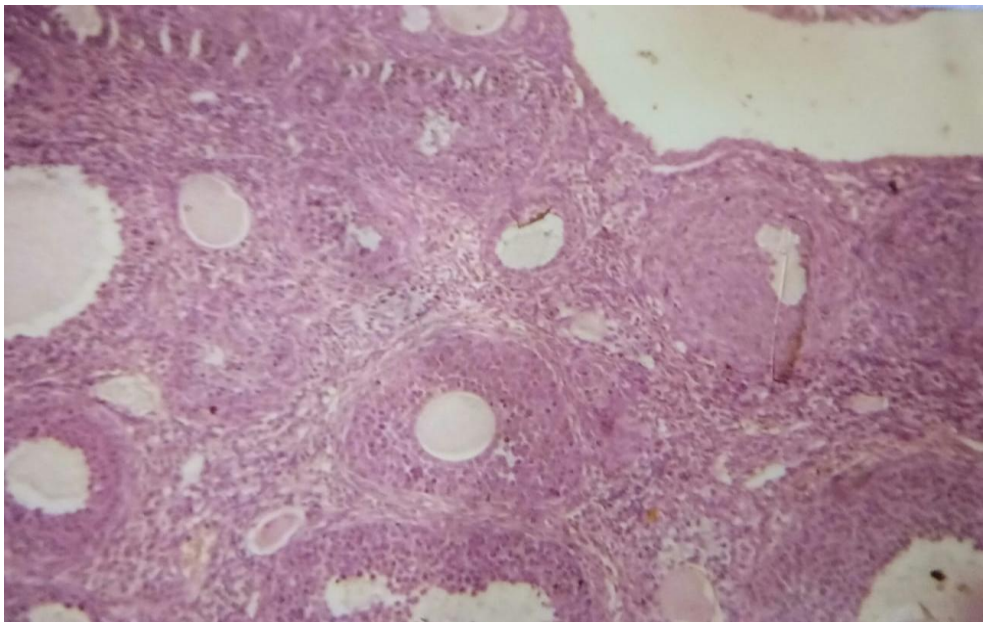

Figure-1: Microphotographs section of ovary of control (untreated) mice stained with haemotoxyline & Eosine showing normal cortex and medullary region. Primordial & primary follicles are normal in shape.

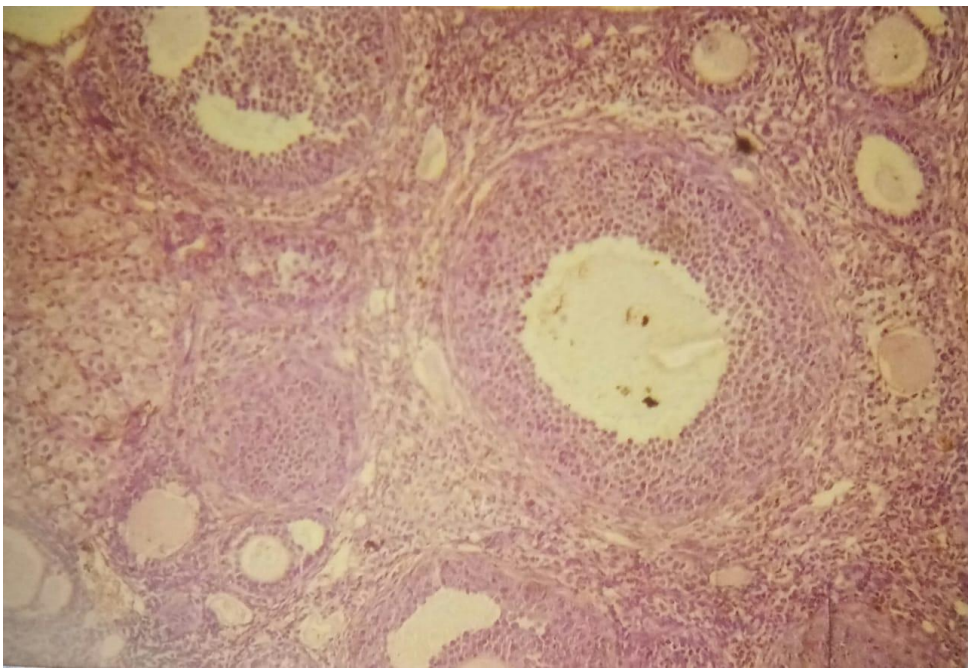

Figure-2: Microphotographs section of ovary of control (untreated) mice stained with haemotoxyline & Eosine showing primary and secondary follicle with normal primary oocytes.

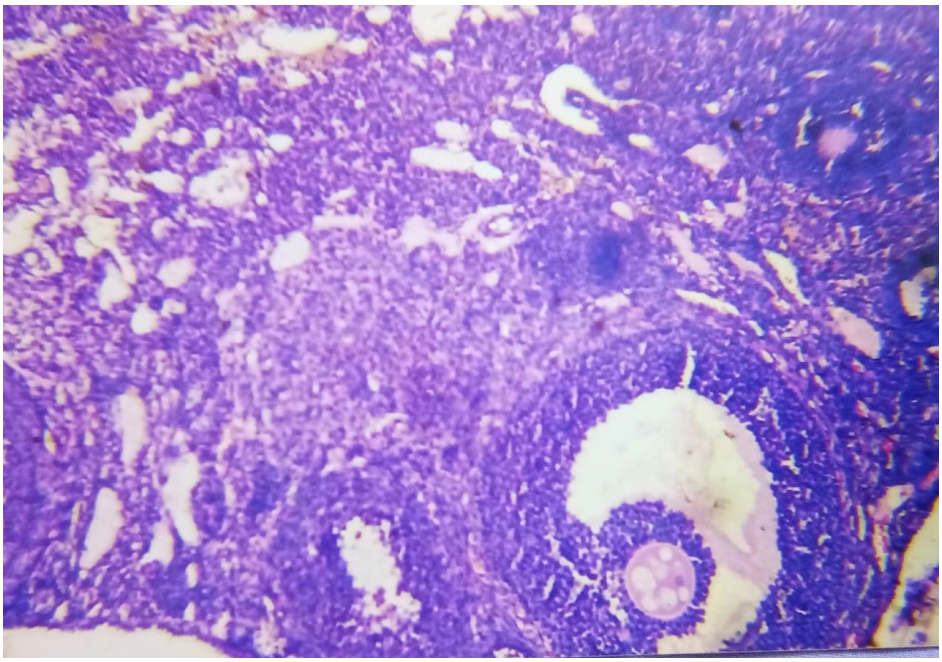

Figure-3: Microphotographs section of ovary of control mice stained with haematoxylin & Eosine showing normal or mature graffian follicles containing prominent secondary oocyte.

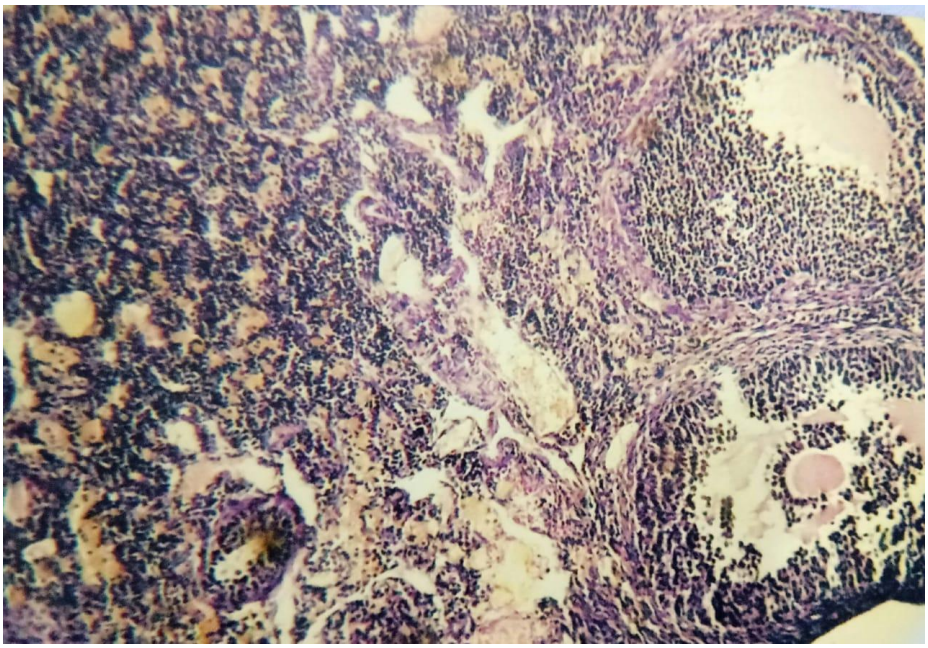

Figure-4: Microphotographs section of ovary of one-week endosulfan administered mice show slightly degenerated in follicular cells.

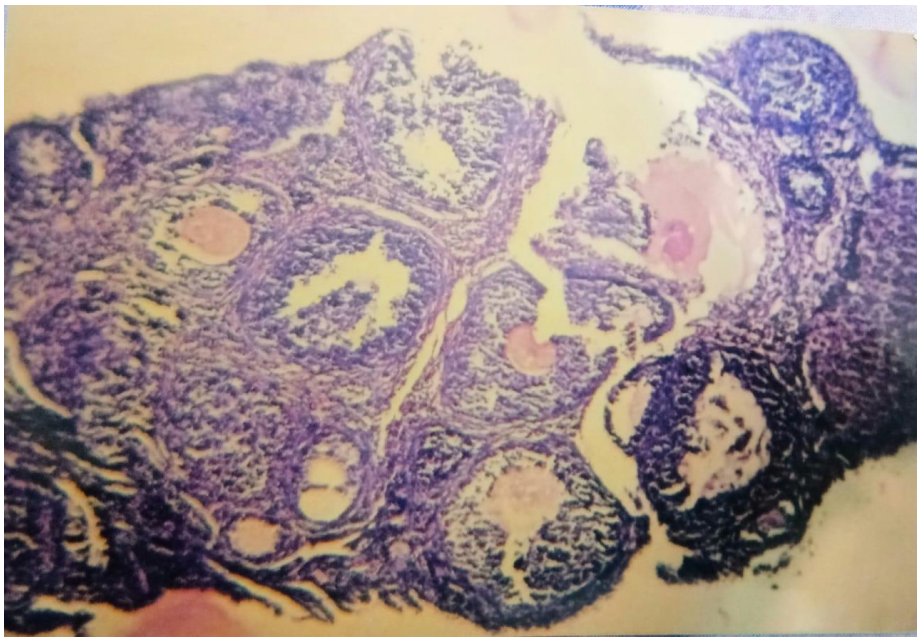

Figure-5: Microphotographs section of ovary of three-week endosulfan administered mice show very high degenerative appears in outer germinal epithelium and disrupted graffian follicles

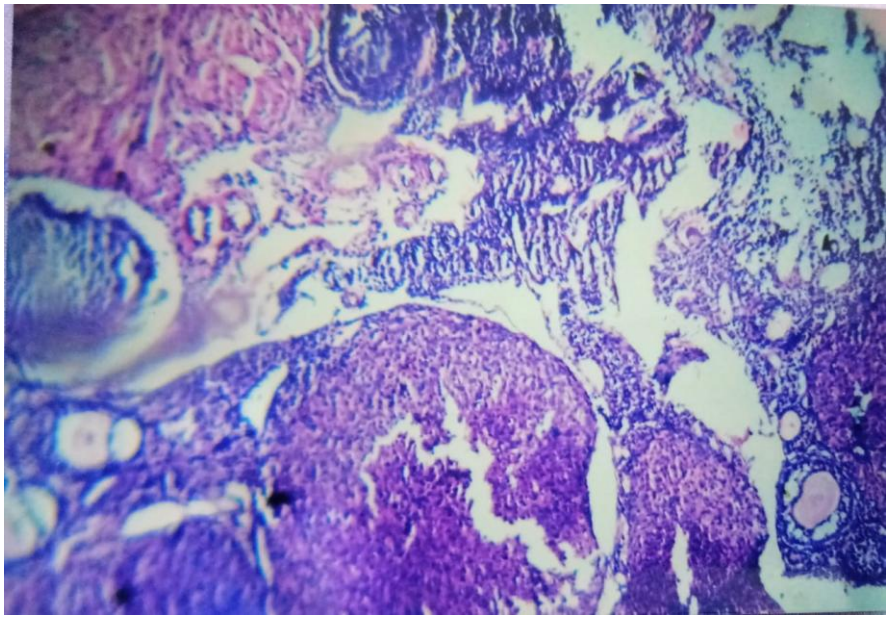

Figure-6: Microphotographs section of ovary of four -week endosulfan administered mice show degenerated graffian follicle and corpus luteum, ova were also degenerated.

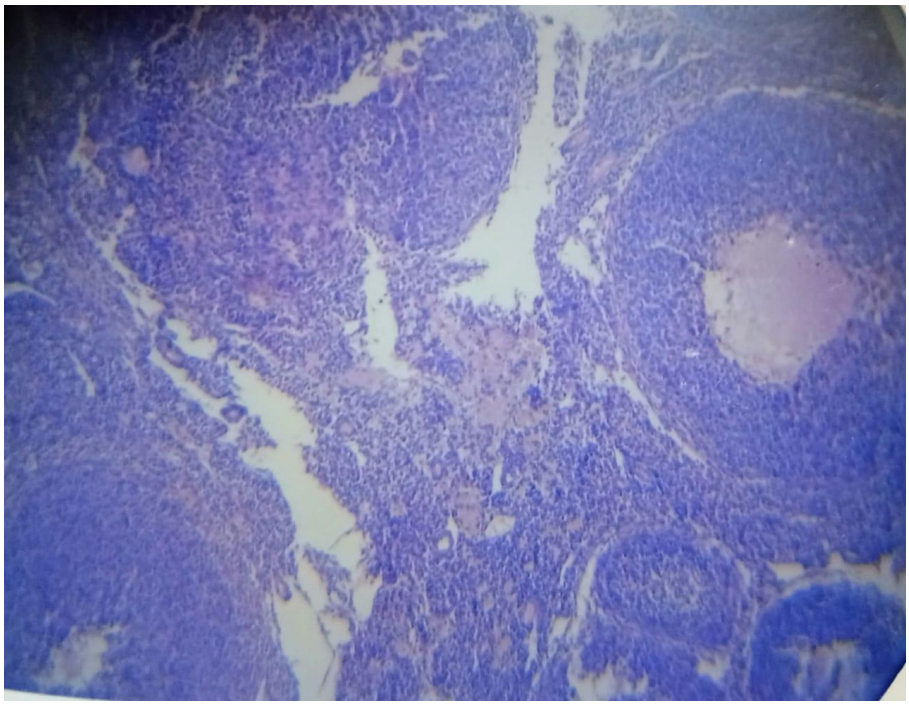

Figure-7: Microphotographs section of ovary of four -week withania somnifera administered mice show restoration in follicular cell and corpus luteum.

IV. Discussion

Traditional and complementary medicines have been more popular now a days to cure health related conditions [22]. This proposes a new strong potential in traditional and complementary medicine to come up with new medical combinations with fewer side effects [23-25]. Traditional Persian medicine is one of the most well-known categories of traditional medicine using herbal medicine as one of the main therapeutic modalities [26].

Withania somnifera is one of the herbal medicines widely used for the treatment of infertility and sexual dysfunction. This plant has been known to contain more than 80 types of phytochemicals such as steroidal and nonsteroidal alkaloids, steroidal lactones and saponins like isopelletierine, anaferin, anahygrine, hygrine, cuscohygrine, tropine, pseudotropine, withananine, ashwagandha, withaferins, withananinine, pseudowithanine, somnine, somniferine, somniferinine, 3-tropyltigloate, withanine, withasomine, visamine, mesoanaferine, sitoindoside (27–30), hentriacontane, amino acids such as aspartic acid, glycine, tryptophan, proline, alanine, tyrosine, hydroxyproline valine, cystine, glutamic acid, and cysteine, calcium, phosphorus, iron, flavonoids, starch, reducing sugars, proteolytic enzyme "chamase," glycosides, dulcitol, and volatile oil. Of all these components, withaferin A and sitoindosides had the key role in WS therapeutic effects [31].

Based on the present study, it was shown that extracts of WS fruits, leaves, stems, and especially roots enhance sperm quality indices such as motility and count in men and also decrease the effects of chemical toxins on gonads in both men and women. WS can increase gonadal weight in both sexes, enhance folliculogenesis and spermatogenesis, and improve LH, FSH, and testosterone balances [32]. Sexual behavior indices such as female sexual function index and female sexual distress index improve statistically significant after WS extract administration [33].

The mechanism of WS effect on the reproductive system is not known entirely yet, but this mechanism is proposed to be linked to the antioxidative features and ability to improve the hormonal balance of LH, FSH, and testosterone and improve detoxification process. Also, the GABA mimetic feature of WS extract is thought to play the main role in inducing gonadotropin releasing hormone secretion and improving hormonal balance [34]. In the male reproductive system, it is assumed that WS by providing metal ions facilitates enzyme activities, modifies oxidative stress, and prevents cell apoptosis. The root extract of WS has been shown to induce alanine transaminase activity which increases alanine in seminal fluid leading to a less oxidative stress index and improved semen quality. Normalizing lactate, phenylalanine, glutamine, citrate, and histidine in seminal fluid are another feature of WS extract which improves enzymatic processes in tricarboxylic acid cycle (TCA) and fatty acid metabolism [35].

V. Conclusion

Based on the results, thus it is concluded from entire study that endosulfan causes degeneration in primary, secondary, graffian follicles and corpus luteum. Germinal epithelium was also degenerated with frequent vacuolization, while *Withania somnifera* has a positive effect in the treatment of infertility in female. Due to the growing interest in using herbal medicine especially those which possess the antioxidative and reproductive system supporting properties, further studies are needed to be designed with higher population and more-structured methodology so a more precise and decisive conclusion can be made.

Acknowledgement

The authors are grateful to P.G.Department of Zoology, College Of Commerce, Arts and Science, Patna for his kind support and co-operation

References

[1]. **Agency** of Toxic Substances and Disease Registry, Toxicological Profile for Endosulfan, 2000
[2]. WHO. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification. Geneva: World Health Organization; 2000. p. 2. [Google Scholar]
[3]. Anonymous. Toxicological profile for endosulfan. U. S. Department of health and human services. Public health service. Agency for toxic substances anddisease registry. 2000;323pages.
[4]. Anonymous. Final report of the investigation of unusual illnesses allegedly produced 38. Nag SK, Raikwar MK. Organochlorine pesticide residues in bovine milk. Bulletin of Environmental Contamination and Toxicology, 2008; 80:5–9.byCampoy C, Jimenez M, Olea-Serrano MF, Moreno-Frias M, Canabate F, Olea N, Bayes R, Molina-Font JA. Analysis of organochlorine pesticides in human milk: preliminary results. Early Human Development, 2001; 65: 183–190. Endosulfan exposure in padre village of Kasargod district (N.Kerala). National Institute of occupational Health (Indian Council of Medical Research). 2002; 98 pages.
[5]. Cerrillo I, Granada A, Lopez-Espinosa MJ, Olmos B, Jimenez M, Cano A, Olea N, Olea-Serrano MF. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk. Environmental Research, 2005; 98: 233–239.
[6]. Campoy C, Jimenez M, Olea-Serrano MF, Moreno-Frias M, Canabate F, Olea N, Bayes R, Molina-Font JA. Analysis of organochlorine pesticides in human milk: preliminary results. Early Human Development, 2001; 65: 183–190.
[7]. L. Mishra, B. B. Singh, and S. Dagenais, "Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review," *Alternative Medicine Review*, vol. 5, no. 4, pp. 334–346, 2000.
[8]. A. Mahdi, K. K. Shukla, and M. K. Ahmad, "*Withania somnifera* improves semen quality in stress-related male fertility," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 576962, 9 pages, 2011.
[9]. V. Sharma, S. Sharma, Pracheta, and R. Paliwal, "Withania somnifera: A rejuvenating ayurvedic medicinal herb for the treatment of various human ailments," *International Journal of PharmTech Research*, vol. 3, no. 1, pp. 187–192, 2011.
[10]. T. K. Biswas and S. Pandit, "In Search of Spermatogenetic and Virility Potential Drugs of Ayurvedic Leads: A Review," *Andrology-Open Access*, vol. 04, no. 02, 2015.
[11]. A. Kumar, R. Kumar, M. S. Rahman, M. A. Iqubal, G. Anand, P. K. Niraj et al., "Phytoremedial effect of Withaniasomnifera against arsenic-induced testicular toxicity in Charles Foster rats," *Avicenna Journal of Phytomedicine*, vol. 5, no. 4, p. 355, 2015.
[12]. N. J. Dar, A. Hamid, and M. Ahmad, "Pharmacologic overview of Withania somnifera, the Indian Ginseng," *Cellular and Molecular Life Sciences*, vol. 72, no. 23, pp. 4445–4460, 2015.
[13]. G. Singh, P. Sharma, R. Dudhe, and S. Singh, "Biological activities of Withania somnifera," *Annals of Biological Research*, vol. 1, no. 3, pp. 56–63, 2010.
[14]. R. Jain, S. Kachhwaha, and S. Kothari, "Phytochemistry, pharmacology, and biotechnology of Withania somnifera and Withania coagulans: A review,"*Journal of Medicinal Plants Research*, vol. 6, no. 41, pp. 5388–5399, 2012.
[15]. A. Saiyed, N. Jahan, S. F. Majeedi, and M. Roqaiya, "Medicinal properties, phytochemistry and pharmacology of Withania somnifera: an important drug of Unani Medicine," *Journal of Scientific & Innovative Research*, vol. 5, no. 4, pp. 156–60, 2016.
[16]. Q. Uddin, L. Samiulla, V. K. Singh, and S. S. Jamil, "Phytochemical and pharmacological profile of Withania somnifera dunal: A review," *Journal of Applied Pharmaceutical Science*, vol. 2, no. 1, pp. 170–175, 2012.
[17]. S. Imtiyaz, S. J. Ali, M. Aslam, M. Tariq, and S. S. Chaudhary, "Withania somnifera: a potent unani aphrodisiac drug," *International Research Journal of Pharmaceutical And Applied Sciences*, vol. 3, pp. 59–63, 2013.
[18]. I. S. Avicenna, *Kitāb al-Qānūnfī al-Tibb (Canon of medicine)*, Senior Press Superintendent, Jamia Hamdard Printing Press, New Delhi, India, 1998.
[19]. A. M. Khan, *Eksir-e-Azam (The great elixir)*, Institute of medicine historical studies, Islamic and complementary medicine, Tehran, Iran, 2007.
[20]. M. A. Khorasani, *Makhzan al Advieh (The storehouse of medicaments). Research institute for Islamic and Complementary Medicine*, vol. 351, Bavardaran Press, Tehran, Iran, 2001.
[21]. D. T. Tuhfat al-mu'minin, *The Present for the Faithful*, Research Center of Traditional Medicine: NashreShahr Press, Tehran, Iran, 2007.
[22]. P. Fisher and A. Ward, "Complementary medicine in Europe," *British Medical Journal*, vol. 309, no. 6947, pp. 107–111, 1994.
[23]. V. C. Karande, A. Korn, R. Morris et al., "Prospective randomized trial comparing the outcome and cost of in vitro fertilization with that of a traditional treatment algorithm as first-line therapy for couples with infertility," *Fertility and Sterility*, vol. 71, no. 3, pp. 468–475, 1999.
[24]. M. C. Inhorn, *Quest for Conception: Gender, Infertility And Egyptian Medical Traditions*, University of Pennsylvania Press, 1994.
[25]. M. B. Gewali and S. Awale, *Aspects of traditional medicine in Nepal*, Institute of Natural Medicine University of Toyama, Japan, 2008.
[26]. M. J. Abbasi-Shavazi, M. C. Inhorn, H. B. Razeghi-Nasrabad, and G. Toloo, "The 'Iranian ART Revolution': infertility, assisted reproductive technology, and third-party donation in the Islamic Republic of Iran," *Journal of Middle East Women's Studies*, vol. 4, no. 2, pp. 1–28, 2008.
[27]. D. R. Smith, E. A. Tanagho, and J. W. McAninch, *Smith's general urology*, Lange Medical Books/McGraw-Hill, 2008.
[28]. C. Krausz, "Male infertility: Pathogenesis and clinical diagnosis," *Best Practice & Research Clinical Endocrinology & Metabolism*, vol. 25, no. 2, pp. 271–285, 2011.

[29]. A. Direkvand-Moghadam, Z. Suhrabi, M. Akbari, and A. Direkvand-Moghadam, "Prevalence and predictive factors of sexual dysfunction in Iranian women: Univariate and multivariate logistic regression analyses," *Korean Journal of Family Medicine*, vol. 37, no. 5, pp. 293–298, 2016.
[30]. M. Rafieian-Kopaei, "Medicinal plants and the human needs," *Journal of HerbMed Pharmacology*, vol. 1, no. 1, pp. 1-2, 2011.
[31]. L. Mishra, B. B. Singh, and S. Dagenais, "Scientific basis for the therapeutic use of *Withaniasomnifera* (ashwagandha): a review," *Alternative Medicine Review*, vol. 5, no. 4, pp. 334–346, 2000.
[32]. V. Sharma, S. Sharma, Pracheta, and R. Paliwal, "Withaniasomnifera: A rejuvenating ayurvedic medicinal herb for the treatment of various human ailments," *International Journal of PharmTech Research*, vol. 3, no. 1, pp. 187–192, 2011.View at: Google Scholar
[33]. A. A. Mahdi, K. K. Shukla, and M. K. Ahmad, "*Withania somnifera* improves semen quality in stress-related male fertility," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 576962, 9 pages, 2011.
[34]. V. R. Ambiye, D. Langade, S. Dongre, P. Aptikar, M. Kulkarni, and A. Dongre, "Clinical evaluation of the spermatogenic activity of the root extract of Ashwagandha (*Withania somnifera*) in oligospermic males: a pilot study,"*Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 571420, 6 pages, 2013.
[35]. N. H. Shaikh, V. M. Deshmukh, and M. V. Walvekar, "Alteration in testicular morphology and sperm count due to Glycowithanolides treatment during aging," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 8, no. 3, pp. 72–77, 2015.View at: Google Scholar

Poonam Kumari. "Protective Effect of Withania Somnifera on Follicular Cells Against Endosulfan Exposed Mice." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(5), (2020): pp. 19-26.