Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 8
ISSN: 2321–3647(online)

Restorative effect of *Withania somnifera* on histology of chlorpyrifos induced ovary of Swiss albino mice

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ABSTRACT

Pesticides are widely used in agriculture for protection of crop from pests. Chlorpyrifos is a pesticide of organophosphate group which is used for both agricultural and non-agricultural purpose. Chlorpyrifos elicits a number of adverse effects including hepatic and reproductive dysfunctions. *Withania somnifera*, commonly known as Ashwagandha, possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. It also appears to benefit the endocrine, cardiopulmonary, and central nervous systems. The study is designed to evaluate the bioremedial effect of *Withania somnifera* on ovary of chlorpyrifos exposed mice. The control group received distilled water as drinking water. The 'treatment' groups received Chlorpyrifos 6 mg/kg b.w daily by Gavage method for four and eight weeks followed by two and four weeks administration of alcoholic extract of root of *Withania somnifera* (50 mg/kg/b.w/day). Animals were sacrificed after the scheduled treatment. Chlorpyrifos administered group showed degenerated corpus leuteum. Ova were also degenerated with degeneration of granulosa cells of mature Graffian follicle. These changes finally may lead to infertility in female mice. *Withania somnifera* causes remarked restoration in ovarian follicle with restored germinal epithelium. Mature Graffian follicles were also observed. Thus the study indicates that *Withania somnifera* plays a vital role against chlorpyrifos toxicity on ovary and may restores normal fertility in female mice.

Keywords: Chlorpyrifos; Corpus leuteum; Graffian follicle; *Withania somnifera*; Gavage.

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Received 26 June 2014, Accepted 10 July 2014

Please cite this article in press as: Anshupriya *et al*., Restorative effect of *Withania somnifera* on histology of chlorpyrifos induced ovary of Swiss albino mice. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Pesticides are considered a vital component of modern farming. They are used to control organisms that are considered to be harmful. Pesticides also prevent the transmission of diseases such as malaria and prevent sickness in humans that could be caused by moldy food. However, concerns about human health and environmental effects of pesticides have increased over the past decade.[1] Pesticide exposure can cause a variety of adverse health effects. These effects can range from simple irritation of the skin and eyes to more severe effects, such as affecting the nervous system, mimicking hormones causing reproductive problems, and also causing cancer. Strong evidence also exists for other negative outcomes from pesticide exposure including neurological changes, birth defects, fetal death,[2] and neurodevelopmental disorder.[3]

Chlorpyrifos, an organophosphate insecticide, is used increasingly with increasing concern about being a neurotoxicant. It has been shown to inhibit DNA synthesis, mitosis, neurite outgrowth and neural cell replication and differentiation.[4] It also interferes with signaling cascades including serotonergic, cholinergic and catecholinergic pathways.[5] It causes synoptosomal AChE activity in different parts of brain.[6] Organophosphate pesticides are known to alter the activity of Na^+/K^+-ATPase,[7,8] Mg^{+2}-ATPase, Ca^{+2}-ATPase, besides being potent anti-choline esterase compound.[9] Chlorpyrifos causes many hepatic and renal dysfunctions. As they are lipophilic in nature, their main target is bilayer lipoidal membrane of the cell.

Since last two decades, the phyto-remediation for treatment of various pesticide borne diseases has been done extensively. *Withania somnifera*, commonly known as Ashwagandha, possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. The herb is termed a rasayana in Ayurvedic practice, which means it acts as a tonic for vitality and longevity. It is also classified as an adaptogen. Roots of *Withania somnifera* have shown inhibitory effect on fore stomach and skin carcinogenesis in mice.[10] *Withania somnifera* administration causes restoration in glomerulus, bowmen's capsule, PCT and DCT as well as biochemical parameters of kidney of stress induced mice.[11] Thus the reported study was designed to evaluate the bioremedial effect of *Withania somnifera* on ovary of chlorpyrifos exposed Swiss albino mice.

MATERIALS AND METHODS

Pesticide

Chlorpyrifos (T_N –Dursban) was used at an effective concentration, EC = 20% (w/v).

Herbal Plant

Roots of *Withania somnifera* were selected as the plant material for herbal treatment against pesticide induced toxicity. The roots were purchased from herbal store of Haridwar with certification receipt.

Experimental model

Female Swiss albino mice (*Mus musculus*) weighing 30±5gm were selected as an experimental model in the present study. Animals were housed at controlled environmental conditions 22±2ºC, relative humidity 50±10%, and 12h dark-light cycle. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Methodology

Chronic Toxicity Studies:

Selected pathogen-free mice were sorted and chlorpyrifos was administered at 6 mg/kg b.wt dose level for 8 weeks by Gavage method. Sacrifice was done on 4^{th} week and 8^{th} week of chlorpyrifos administration in each group.

Herbal Administration:

Chlorpyrifos administration at 6 mg/kg b.wt for 8 weeks was followed by the administration of alcoholic extract (5% Ethanol) of roots of *Withania somnifera* for 4 weeks at 50 mg/kg b.wt. Animals were sacrificed on 2^{nd} week and 4^{th} week of herbal treatment. The dose of chloropyrifos were selected after calculating Maximum permissible dose (MPD) and Lethal Dose 50% (LD_{50}).

Sub-cellular Studies:

Mice were sacrificed from each group for histological analysis. The ovary was dissected out and washed three times in isotonic saline (0.85 w/v %) and then fixed in 10% neutral formalin solution and the tissue was processed. Slides were stained with Hematoxylene-Eosin (H & E) and examined morphometrical under Light Microscope.

RESULTS AND DISCUSSION

Ovary of control mice showed normal corpus luteum. Mature Graffian follicle was also observed with distinct ova. Different stages of follicles were visible (Figure. 1).

Ovary of chlorpyrifos four weeks administered mice showed degenerated mature Graffian follicle. Ova were not distinct. Many vacuolated spaces were observed in medulla of ovary. Degenerated germinal epithelium was observed with degenerated cytoplasm (Figure. 2). Ovary of chlorpyrifos four weeks administered mice showed degenerated ova in mature Graffian follicle. Degenerated germinal epithelium was observed (Figure. 3). Ovary of chlorpyrifos eight

weeks administered mice showed many vacuolated spaces with degenerated cytoplasm of ovarian cortex as well as medulla. Degenerated ova were also observed. Corpus luteum was also degenerated (Figure. 4). Ovary of chlorpyrifos eight weeks administered mice showed degenerated ova with degeneration of granulosa cells of mature Graffian follicle. Many vacuolated spaces were also observed (Figure. 5). Ovary of chlorpyrifos eight weeks administered mice followed by two weeks administration of *Withania somnifera* showed restoration in cytoplasm of ovarian medulla with few vacuolated spaces (Figure. 6). Ovary of chlorpyrifos eight weeks administered mice followed by two weeks administration of *Withania somnifera* showed restoration in germinal epithelium of ovary. Ovarian cortex was also restored to some extent (Figure. 7). Ovary of chlorpyrifos eight weeks administered mice followed by four weeks administration of *Withania somnifera* showed restoration in germinal epithelium. Different stages of follicles were restored. Both ovarian cortex and medulla were restored in structure. Cytoplasmic material was well distributed in ovarian cortex (Figure. 8). Ovary of chlorpyrifos eight weeks administered mice followed by four weeks administration of *Withania somnifera* showed restoration in ovarian follicle with restored germinal epithelium. Mature Graffian follicles were also observed. Least vacuolization were observed (Figure. 9).

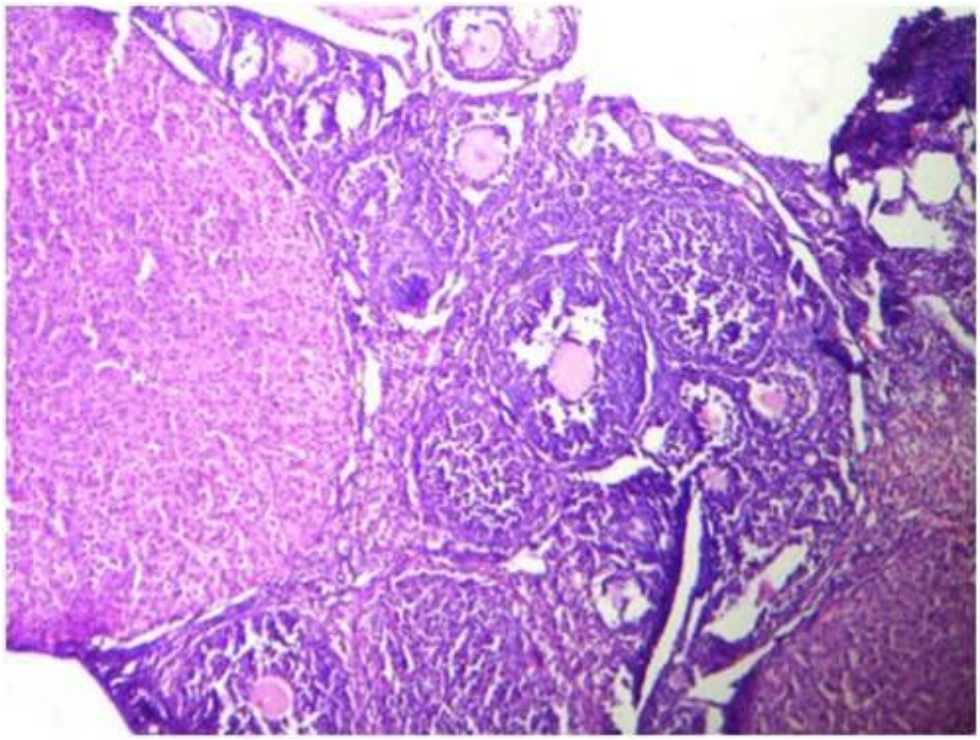

Figure .1: Ovary of control mice shows normal corpus luteum. Mature Graffian follicle were also observed with distinct ova. Different stages of follicles are visible. X200

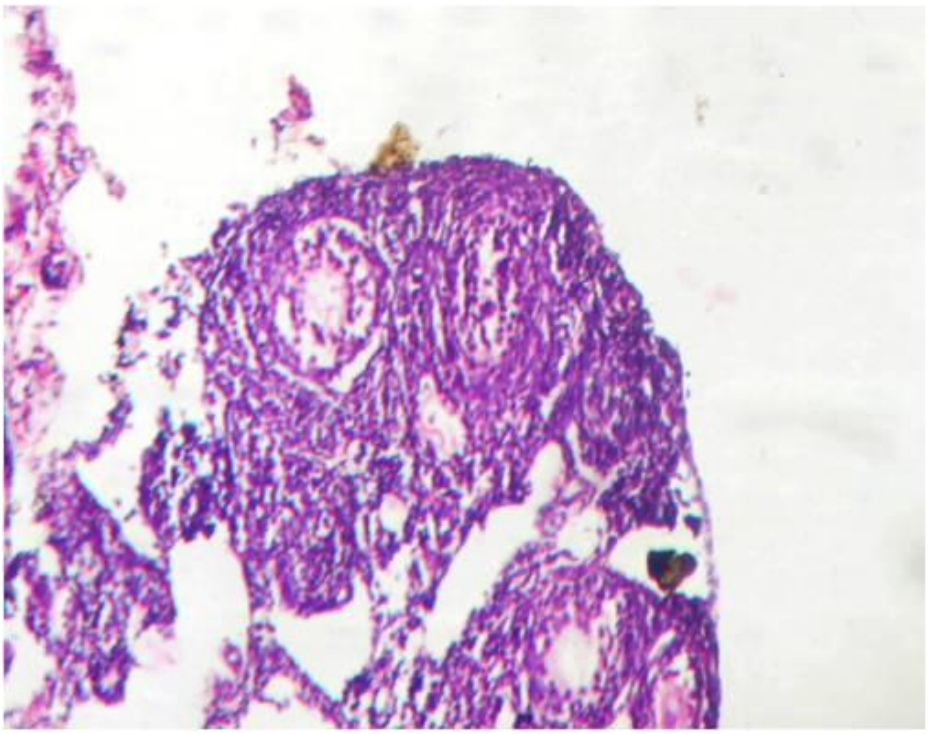

Figure .2: Ovary of chlorpyrifos four weeks administered mice shows degenerated mature Graffian follicle. Ova were not distinct. Many vacuolated spaces were observed in medulla of ovary. Degenerated germinal epithelium was observed with degenerated cytoplasm. X100

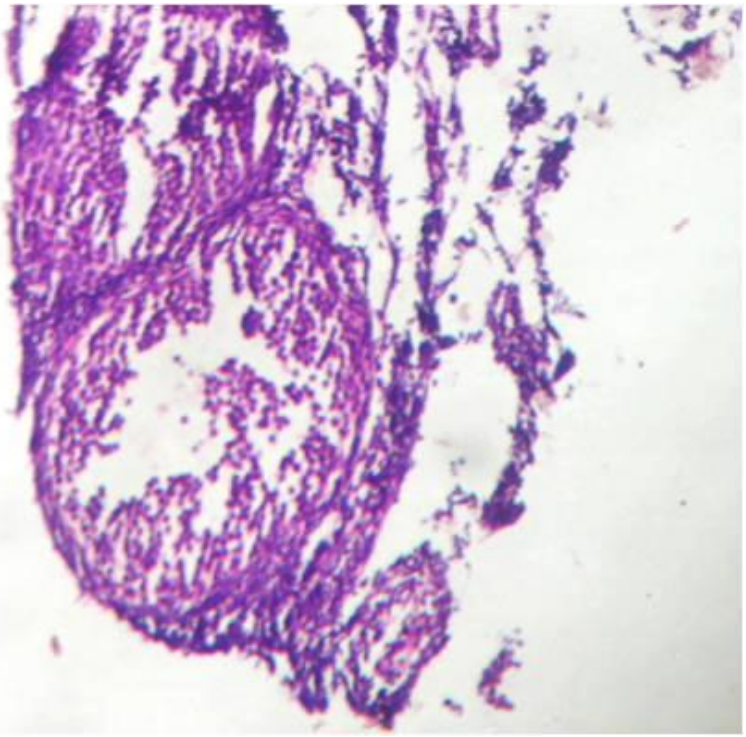

Figure.3: Ovary of chlorpyrifos four weeks administered mice shows degenerated ova in mature Graffian follicle. Degenerated germinal epithelium was observed. X200

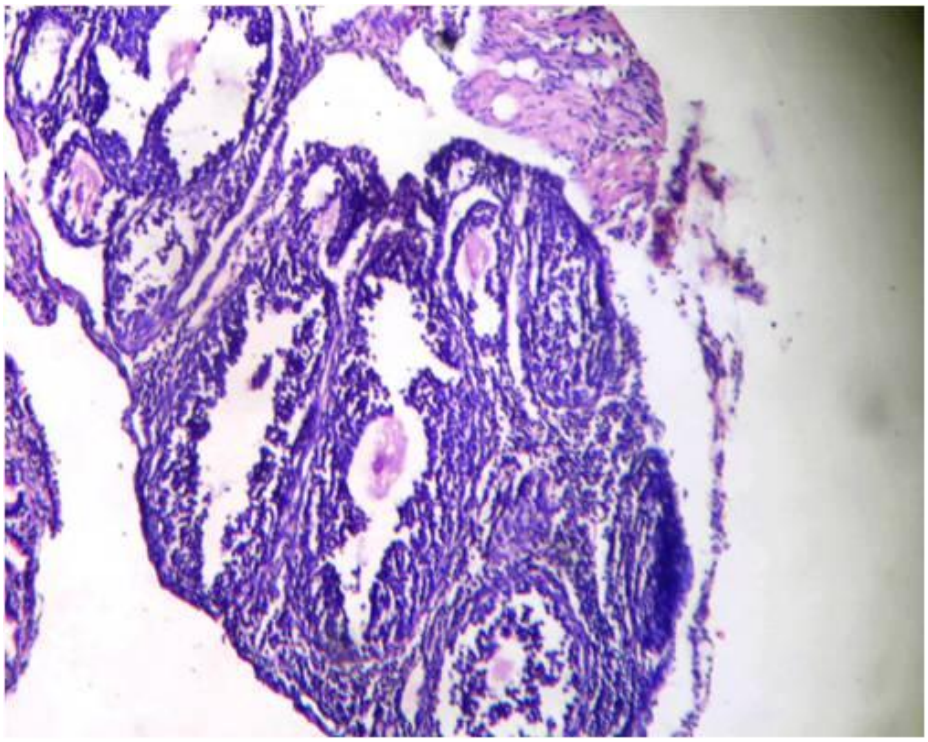

Figure.4: Ovary of chlorpyrifos eight weeks administered mice shows many vacuolated spaces with degenerated cytoplasm of ovarian cortex as well as medulla. Degenerated ova were also observed. Corpus luteum was also degenerated. X100

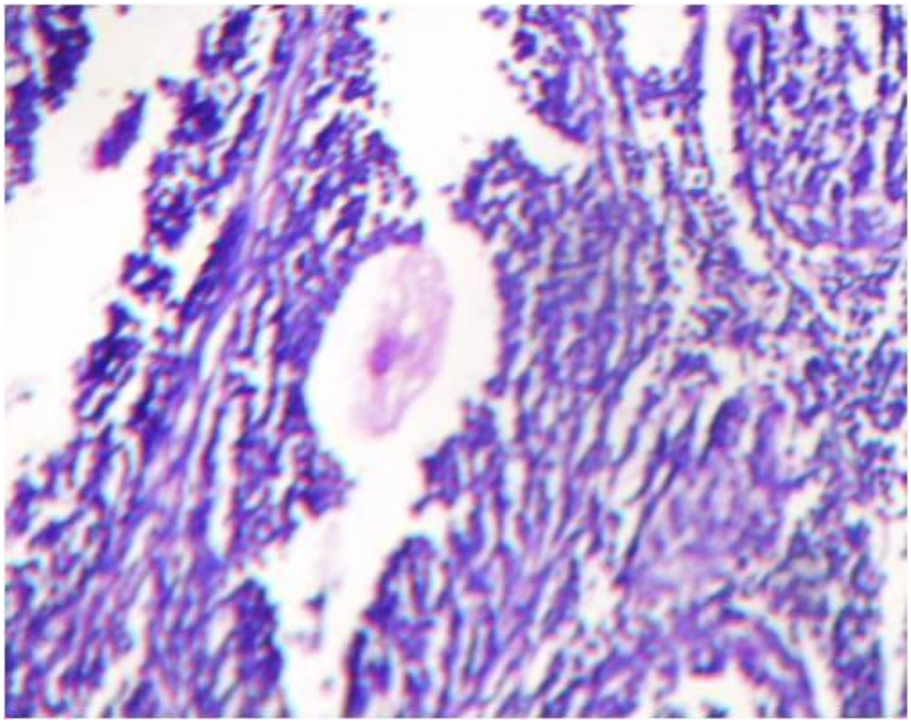

Figure.5: Ovary of chlorpyrifos eight weeks administered mice shows degenerated ova with degeneration of granulosa cells of mature Graffian follicle. Many vacuolated spaces were also observed.X300

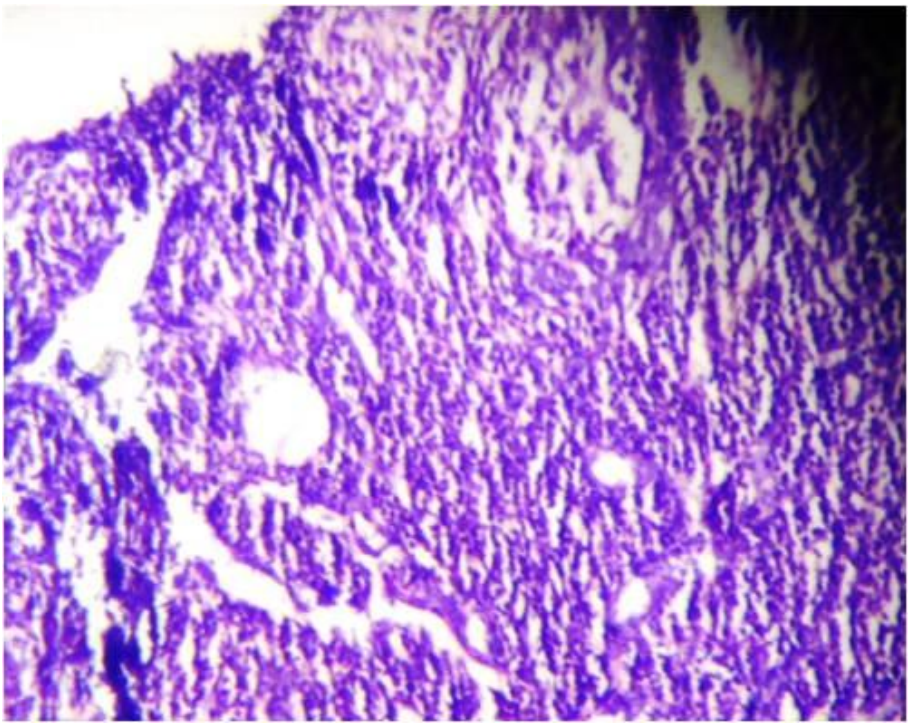

Figure.6: Ovary of chlorpyrifos eight weeks administered mice followed by two weeks administration of *Withania somnifera* shows restoration in cytoplasm of ovarian medulla with few vacuolated spaces.X200

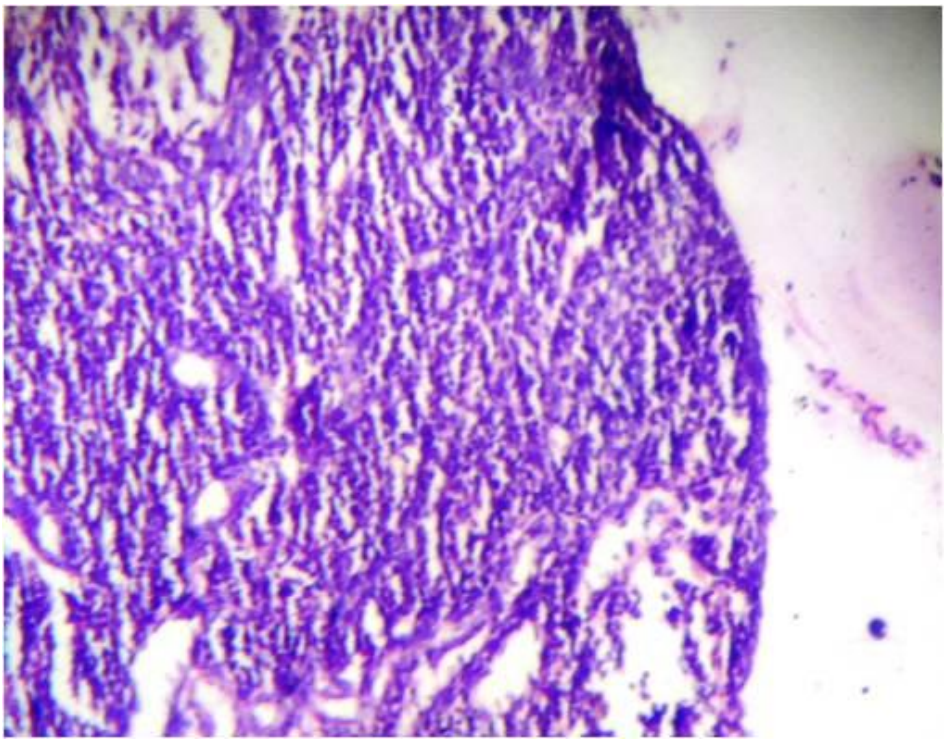

Figure.7: Ovary of chlorpyrifos eight weeks administered mice followed by two weeks administration of *Withania somnifera* shows restoration in germinal epithelium of ovary. Ovarian cortex was also restored to some extent. X200

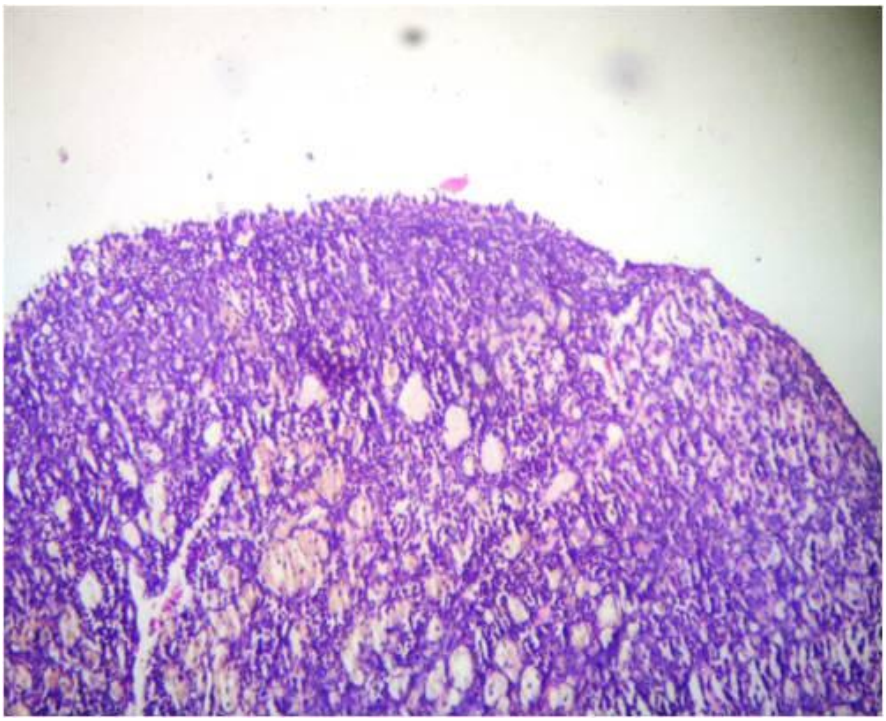

Figure.8: Ovary of chlorpyrifos eight weeks administered mice followed by four weeks administration of *Withania somnifera* shows restoration in germinal epithelium. Different stages of follicles were restored. Both ovarian cortex and medulla was restored in structure. Cytoplasmic material was well distributed in ovarian cortex. X100

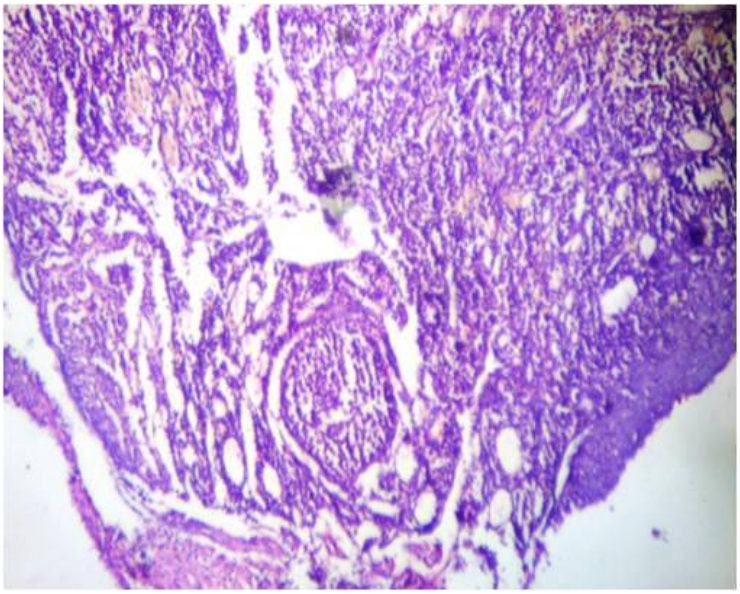

Figure .9: Ovary of chlorpyrifos eight weeks administered mice followed by four weeks administration of *Withania somnifera* shows restoration in ovarian follicle with restored germinal epithelium. Mature Graffian follicles were also observed. Least vacuolization were observed. X150

Chlorpyrifos causes degenerative effect on glomerulus, bowmen's capsule and ductal system of kidney of mice that lead to an increase in biochemical parameters of kidney also [12]. El-Deeb [13] reported that in chlorpyrifos administrated rats body weight, kidney weight and testis weight significantly decreased with decrease in the concentration of sperm cells, percentage of live sperms; but on the other hand, increase in the percentage of abnormal sperms. Joshi [14] reported that chlorpyrifos administrated male Wistar rats showed decreased testes weight with marked reduction in epididymal and testicular sperm counts. Kumar [15] reported that chlorpyrifos causes significant increase in serum LPO levels and decrease in FSH levels in mice. In the present study degenerative changes were observed in chlorpyrifos administered group of mice. With increased duration of chlorpyrifos administration, degenerative changes were increased.

Abdel Magied[16]reported that lyophilized aqueous extract of *Withania somnifera* had profound effect on testicular development including increase in testicular weight and on serum levels of testosterone, ICSH and FSH in immature male Wistar rats. Patil[17] reported that ethanolic extract of Withania somnifera causes restoration of degenerated testis and epididymis of D-galactose stressed mice with increase in sperm count and decrease in LPO levels. Sadia 18 demonstrated that root extract of Withania somnifera have nephroprotective effect against gentamicin induced nephrotoxicity in Wistar albino rats. Present study showed that W. somnifera restored ovary of mice effectively. Restoration increased with increased duration of Withania administration.

CONCLUSIONS

Thus it is evident from entire study that chloropyrifos causes degenerative changes in ovary, While *Withania somnifera* causes effective restoration in germinal epithelium, mature graffian follicles and ova. It is concluded that *Withania somnifera* restore normal fertility in female mice.

ACKNOWLEDGEMENT

The authors are thankful to Mahavir Cancer Institute and Research Centre, Patna for providing infrastructural facility and also to all research laboratory staff and animal house staff for their proper support during this study.

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