Amelioration Effect of *Emblica officinalis* Extract on Ovary in Endosulfan Induced Swiss Albino Mice

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**Authors’ contributions**

*This work was carried out in collaboration between both authors. Authors PS and AK jointly designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors PS and AK managed the analyses of the study. Author AK managed the literature searches. Both authors read and approved the final manuscript.*

**Article Information**

DOI: 10.9734/BJPR/2016/17669

**Editors:**

(1) Othman Ghribi, Department of Pharmacology, Physiology & Therapeutics, University of North Dakota, USA.
(2) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
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(4) Anonymous, University of Tunis El Manar, Tunisia.

Complete Peer review History: [http://sciencedomain.org/review-history/13934](http://sciencedomain.org/review-history/13934)

Received 23rd March 2015
Accepted 17th February 2016
Published 30th March 2016

**Original Research Article**

**ABSTRACT**

**Background:** In the present time use of pesticides has become very common by the farmers for the better yield of crops. Endosulfan, a chlorinated cyclodiene insecticide, is widely and very liberally used in agricultural sector. But it has created deleterious effects on human health.

**Objective:** In the present study amelioration effect of *Emblilca officinalis* (*E. officinalis*) extract on ovary in Endosulfan induced Swiss albino mice was studied.

**Materials and Methods:** Three groups were formed, each with six mice. The control group of mice received distilled water as drinking water. The second group was exposed to Endosulfan @ 3 mg/kg body weight daily for 6 weeks by Gavage method. The third group was exposed to Endosulfan @ 3 mg/kg body weight daily for 6 weeks followed by 6 weeks exposed to *E. officinalis* (100 mg/kg body weight) extract. Animals were sacrificed after the schedule treatment.

**Results and Discussion:** Light microscopic observations showed various histopathological deformities in sections of treated ovary and improper development of follicle such as detached ova...
and degeneration of follicular cells due to Endosulfan exposure were also found. The present studies also indicate that alterations were found to be significantly ameliorated in *E. officinalis* extract treated group. **Conclusion:** It may be concluded from this study that *E. officinalis* have a potent capacity to control Endosulfan induced toxicity.

**Keywords:** Emblica officinalis; ovary; Endosulfan; mice.

### 1. INTRODUCTION

Pesticide poisoning is an important health issue. Their extensive use and wasteful application increases the risk of cancer, infertility, hormonal disorder [1,2,3,4] neurological disorders, congenital birth defects, chromosomal abnormalities, mental retardation, impaired learning and memory loss [5]. In present times use of pesticides has become very common by the farmers for the better yield of crops to overcome the increase pressure on agricultural land due to growing population. Endosulfan (polycyclic organochlorine) is one of the most toxic pesticides on the market today which is used for many pests like aphids, thrips, beetles, white flies, peach twig foliar feeding larvae, termites, tsetse fly, borers, mites, bugs leaf-hoppers etc [6,7]. Hence, it is effective against a wide range of pests of cereals, coffee, cotton, fruits, oilseeds, potato, tea and vegetables. So, it is the world’s largest consumable pesticide. Pesticide safety is classified by the World Health Organization (WHO) according to the results of LD$_{50}$ tests (which document the amount of a chemical required to kill 50% of a population). Under this system, Endosulfan is currently classified as Class II – moderately hazardous to human health. The US Environmental Protection Agency (USEPA) classifies it category Ib (highly hazardous). Endosulfan is still widely used and pollutes environment [8]. After the Stockholm Convention, Endosulfan is banned in more than 75 countries. India completely banned the Endosulfan in May 2013 and agreed to phase out use of Endosulfan by 2017.

Endosulfan is acutely toxic and it is readily absorbed by the stomach, lungs and through the skin also. It acts via GABA receptor system and Ca$^{2+}$, Mg$^{2+}$ ATPase inhibitor; hence it has neurotoxic effect to both insects and mammals. It acts as an endocrine disruptor interfering with function of estrogen, testosterone and other steroid hormones. Its exposure through any route can be hazardous. Symptoms of acute endosulfan exposure include dizziness, vomiting, diarrhea, breathing difficulties, convulsions, and loss of consciousness etc. In extreme cases, death can result. Indeed, the chemical has been linked to dozens of accidental deaths in the USA, Colombia, Benin, India, Malaysia, Sudan, and the Philippines. In New South Wales, Endosulfan has been found a contaminant in rivers near the cotton – growing areas in late 1997 [9]. Abirami et al. [10] also reported in 2012 that the pesticide especially the organochlorine compounds have adverse impact on fish communities since these compounds exert the toxic effect even at lower concentrations. Endosulfan also caused deleterious effect at cellular and subcellular levels on animal model [11,12]. It may also cause Infertility [13].

One another important thing is ‘cure of the toxicity’. Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. The fruits of the *Emblica officinalis* are used in Ayurveda as a rasayana [14]. *E. officinalis* has an important position in Ayurveda due to strong antioxidant and prevents innumerable health disorders as it contains essential nutrients and highest amount of vitamin C [15]. *E. officinalis* fruit is used in traditional medicines for the treatment of diarrhoea, jaundice and inflammations [16]. Earlier studies have demonstrated potent anti-microbial [17], antioxidant [18,19], adaptogenic [20], hepatoprotective [21], anti-tumor [22], anti-ulcer activities [23] and cytoprotective [19] of the fruits of *E. officinalis*. The aim of the present study is exploring the deleterious effect of Endosulfan in the ovary of female Swiss albino mice and the curative effect of fruits of *E. officinalis* extract as amelioration. So, the author studied the ovarian histology through light microscopy and expected to help in enriching the database of toxic effect of pesticide on ovary and reduce the toxicity by naturally occurring herbal plant fruits.

### 2. MATERIALS AND METHODS

#### 2.1 Pesticide

Endosulfan (EC-35%) was obtained from local market (manufactured by Excel Industries LTD,
Mumbai, India). It was dissolved in distilled water to prepare sub-lethal concentration 3mg/kg body weight and administered to mice orally according to their body weight.

2.2 Identification and Collection of Plant Material

The fruits of *Emblica officinalis* were collected from local market and identified by botanist, Department of Botany, A.N. College, Patna.

2.3 Preparation of Extracts

The fruits were shaded dried with occasional shifting and powdered with mechanical grinder passing through sieve and stored in air-tight container. The crude extract was made up by powder. During experiment the crude extract was diluted with distilled water just before administration to animals. It was dissolved in distilled water. The dose was made to 100 mg/kg body weight for oral administration.

2.4 Animal Model and Grouping

Female Swiss albino mice (8-10 weeks old) were selected for this study from an inbreed colony. The average body weights of mice were 30 g. The animals were kept under standard laboratory conditions, maintained on 12 h light/dark cycle at 22±2°C and had free access to water & food. Animals were acclimatized to laboratory conditions before experiment. The animals were maintained in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India for laboratory animal facility.

Three groups of mice, six mice in each group received the following treatment schedule.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control (only vehicle distilled water; orally)</td>
</tr>
<tr>
<td>II</td>
<td>Endosulfan (3 mg/kg body weight/day for 6 weeks; orally)</td>
</tr>
<tr>
<td>III</td>
<td>Endosulfan (3 mg/kg body weight/day for 6 weeks; orally) followed by <em>E. officinalis</em> (fruit extract; 100 mg/kg body weight/day for 6 weeks; orally)</td>
</tr>
</tbody>
</table>

2.5 Weights and Histological Studies

Animals were individually weighed before the experiment and after the completion of the experiment in order to detect the changes in their body weights. The mice were sacrificed on the day next to the last day of treatment. Ovaries were collected from each experimental group. Ovary was washed in isotonic saline (0.85 w/v %) and weighed after cleaned the fat tissue and wiping them dry with blotting paper. Ovaries were fixed in the 10% formalin and tissue processing was done as per routine method and cut in section of 5 micron and stained with Haematoxylin & Eosin for Light Microscopy.

2.6 Statistical Analysis

For three - groups comparison, data were analyzed by one-way analysis of variance (ANOVA) using the software “Graph Pad Prism 5.0”. The p-value was considered significant when p≤0.05 at the significant level of 95%.

3. RESULTS

3.1 Body and Ovary Weights

The final average body and ovary weight, both control and experimental groups were given in Table 1. Group of mice that received Endosulfan for 6 weeks showed significantly decreased in body weight as well as reduction in the weight of ovary when it was compared to the control group. It was found that there was no significant change in the body weight whereas progressive decrease in ovary weight after administration of aqueous extract of *E. officinalis* when compared to the Endosulfan treated mice.

3.2 Histological Studies

Light Microscopic observations showed normal folliculogenesis in control group of mice. Their

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Body Weight (g)</td>
<td>30±1.095</td>
<td>27.34±0.816</td>
<td>27.17±1.169</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>2</td>
<td>Ovary weight (mg)</td>
<td>16±0.867</td>
<td>11.11±0.960</td>
<td>9.37±0.972</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Data represented as Mean ± SD; N=6
Oocytes were characterized by thick layers of Follicular cells. Follicular antrum, Cumulus oophorus Membrana granulosa and Theca interna are distinctive (Plate 1, Fig. 1). Ovaries of 6 weeks Endosulfan administered mice showed medullary region with frequent vacuolization. Degenerated germinal epithelium was observed with clustered nuclei (Plate 2, Fig. 1). Degenerated Graafian follicle was observed with vacuolated spaces in granulosa cells. Degenerated cytoplasm was also observed with clustered nuclei in Corpus luteum. Frequent vacuolization's were observed in Corpus luteum (Plate 2, Figs. 2, 3 and 4).

Plate 1. Microphotographs section of Ovary of Control mice stained with H & E

**Fig. 1.** Mature Graafian Follicle of ovary of control mice (x 400) - Oocytes (O) is surrounded by thick layers of Follicular cells (FC); well defined follicular antrum (FA) and well developed membrana granulosa (MG) clearly visible; stromal cells form a distinctive layers theca interna (TI).

Ovaries of 6 weeks Endosulfan administered mice after receiving 6 weeks of treatment with *E. officinalis* showed restoration in germinal epithelium to some extent. However degenerated follicular structure with frequent vacuolated spaces in Graafian follicle was observed. Degenerated corpus luteum with less vacuolization was observed. A vacuolated space in stroma was also observed (Plate 3).

4. DISCUSSION

Endosulfan is an organochlorine insecticide used on a variety of field. As in the cases of most other pesticides, Endosulfan can cause acute toxicity in animals and human beings due to overexposure [24]. Several studies have been reported that Endosulfan has adverse effects on health [1,11,25,26]. In a previous study, the adverse effect of Endosulfan was also reported on follicular development of BALB/c mice [27]. Hiremath and Kaliwal [28] observed that Endosulfan treatment caused a significant decrease in compensatory ovarian hypertrophy, an increase in the number of atretic follicles and disruption of the estrous cycle. The results of the present study suggested that Endosulfan cause ovarian damage and it indicates toxicity reached at cellular level, which affects the follicular functions and ultimately affect the reproductive function and fertility. The relationship between female fertility and ovarian follicle development is well recognized [29]. Pathak et al. [30] suggested that higher levels of some of the organochlorine pesticide like Endosulfan may be associated with preterm delivery and increased oxidative stress.

In the present experiment, Endosulfan treated mice showed reduction in the body weight during all days of observation, as compared to the control group. Gradual declination in the body weight of mice may be attributed to different side effects of inability to use carbohydrates including lipolysis, glycogenolysis and acidosis. It also may be attributed to disturbances in one or many metabolic pathways and due to deficiency of protein or disturbances in different enzymatic activities. Similar observations were reported by earlier workers [31,32]. According to Abirami et al. [10] the protein, carbohydrate and lipid content of fish tissue decreases at different concentration with increased concentration of Endosulfan.

On the other hand, administration of *E. officinalis* fruit extracts did not show much ameliorating impact on the body and ovary weight of Endosulfan treated mice. This finding is supported by Guruprasad et al. [33] who reported absence of significant body weight changes in Brahma rasayana treated male Swiss albino mice. In Brahma rasayana extracts of *E. officinalis* are one of the main ingredients. Administration of extract to 6 weeks showed slight declination in ovary weight. Also,
Anilakumar et al. [34] reported that treatment with *E. officinalis*, either at 5 or 10% did not result in any change in the food intake pattern and organ weights (kidney and liver) of rats.

**Plate 2. Microphotographs section of ovary of Endosulfan treated mice stained with H & E**

**Fig. 1.** Showing degeneration in follicular structure with vacuolated (V) stroma. Clustered nuclei (Cn) are observed in germinal epithelium (GEp). (200x)

**Fig. 2.** Showing vacuolization in corpus luteum (CL) with clustered nuclei. Degenerated Graafian follicle (GF) are observed with large vacuolated spaces in stroma. (200x)

**Fig. 3.** Showing enlarged view of corpus luteum with frequent vacuolization. Degenerated cytoplasm is observed with clustered nuclei. (600x)

**Fig. 4.** Showing enlarged view of mature Graafian follicle with vacuolated spaces in granulosa cells. Clustered nuclei are also observed in germinal epithelium. (800x)
Plate 3. Photomicrograph of section of ovary of 6 weeks Endosulfan followed by 6 weeks *E. officinalis* administered mice with H&E staining

**Fig 1.** Showing degenerated mature Graafian follicle (GF). Corpus luteum (CL) is also degenerated. Germinal epithelium (GEp) is restored to some extent. (200x)

**Fig. 2.** Showing degenerated corpus luteum with less vacuolization. Degenerated follicular cells are also observed. More vacuolated space (V) in Stroma. (200x)

**Fig 3.** Showing enlarged view of Fig 1 with degenerated corpus luteum. Vacuolated spaces are observed in corpus luteum. (600x)

**Fig. 4.** Showing degenerated follicular structure with frequent vacuolated spaces in Graafian follicle. Vacuolated spaces in stroma are also observed. Granulosa cells (GC) is restored to some extent. (600x)

In a recent study, it was found that the iron content in *E. officinalis* has sperm enhancing properties. *E. officinalis* has also been proved to increase the motility of sperm and increase sperm production in men suffering from Oligospermia (a condition where a man has a low sperm count). Al-Rehaily et al. [35] reported that oral administration of *E. officinalis* ethanolic extract at dose levels of 250 mg/kg and 500 mg/kg significantly possessed cytoprotective
properties in rats. Dutta and Sahu [36] reported that *E. officinalis* play a core role to reduce testicular toxicity induced by chlorpyrifos in male rats. In the present study, *E. officinalis* fruit extracts at 100 mg/kg body weight was administered to Endosulfan pretreated female mice. The findings showed restoration in germinal epithelium to some extent. However, degenerated follicular structure and corpus luteum with less vacuolization were observed after 6 weeks of herb administration. May be possible restoration will be more after longer duration of herb administration. De et al. [37] reported that *E. officinalis* extract inhibits growth of ovarian cancer cells *in vitro* and *in vivo*, perhaps through the activation of autophagy and inhibition of angiogenesis. In another study, efficacy of herbal product of *E. officinalis* (fruit) has been evaluated against Carbon tetrachloride (CCl₄) and thio-acetamide (TAA) induced changes in rat liver [38].

5. CONCLUSION

Thus, the significance of these findings indicates changes in ovarian follicles caused by Endosulfan can be ameliorated by the aqueous fruit extract of *E. officinalis*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been examined and approved by the appropriate ethics committee, Mahavir Cancer Sansthan & Research Centre, Patna, Bihar, India.

COMPETING INTERESTS

Authors have declared that no competing interests.

REFERENCES


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Peer-review history:
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