Curative Potentials of Garlic (*Allium sativum*) Extract against Di-(2-Ethylhexyl) Phthalate Induced Reproductive Toxicity in Female Mice

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Abstract: The present study was intended to find out the curative potentials of Garlic (*Allium sativum*) against di-(2-Ethylhexyl) phthalate (DEHP) induced toxicity in the reproductive system of female mice. Forty female mice were divided into four groups (n=10) as the (a) control group was given normal feed and drinking water, (b) aqueous garlic extract treated group (500 mg/kg), (c) DEHP group received 500 mg/kg in corn oil, and (d) DEHP + aqueous garlic extract each at a dosage of 500 mg/kg body weight. All treatments were given daily through oral gavage for 28 days. After completion of the experiment, all the animals were dissected through cervical dislocation to get reproductive organs. Collected organs were weighed and processed through the conventional histology technique of staining with eosin and hematoxylin. This study indicated that DEHP exposure caused a significant decrease (P<0.001) in body weight and weight of the complete female reproductive tract as compared to the control group, while the garlic co-administered group showed prominent improvement in body and organ weight when compared to only DEHP treatment. The adverse effect of DEHP on the histology of the ovary such as a decreased mean number of developing follicles, thin and irregular corona radiata, disruption of cumulus oophorus, and reduction in the size of the antral cavity of the tertiary follicle was observed. However, a significant recovery in the development of follicles was seen in DEHP plus garlic-treated group. DEHP plus garlic extract treatment showed protective effects on the uterus, such as a significant increase in the diameter of the uterus (P<0.001), muscularis, mean number of endometrial glands (P<0.001), and endometrial epithelial heights as compared to only DEHP exposed group. Hence, garlic extract showed significant ameliorative potential against DEHP-induced reproductive anomalies in female mice.

Keywords: DEHP; reproduction; toxicity; garlic extract

1. INTRODUCTION

Di-(2-Ethylhexyl) phthalate (DEHP) is extensively utilized in the plastic industry for enhancing the durability, transparency, and flexibility of polyvinyl chloride (PVC) based polymers [1, 2]. DEHP is widely used in non-polymeric substances such as solvents in cosmetics, perfumes, insect repellents, inks, paints, adhesives, additives in hair sprays, building materials, and lubricating oil in the industry [3]. As DEHP is weakly associated with plastics and consequently easily releases and pollutes the environment by leaching, abrasion, and evaporation from the products [4]. The mode of introduction of phthalates in people can be oral, dermal or inhalation [1]. The presence of DEHP metabolites in several human urine samples has raised concerns about its harmful effects on human populations [5-7]. As previous studies have reported DEHP metabolites in reproductive fluids like amniotic and follicular [8-10] fluids, suggesting their ability to assimilate from circulating plasma.

Meanwhile, limited shreds of evidence suggest that exposure to DEHP may have adverse female reproductive outcomes including, decrease ovulation [11], reduced size of follicular granulosa cells [12], inhibition of synthesis of estradiol [13],...
accelerated apoptosis [14, 15], delayed follicular development [16], extended estrous cyclicity [17], reduced folliculogenesis [12], endometriosis [18], and delayed puberty onset [19]. Moreover, DEHP had also been associated with failure of implantation and fetal loss [10].

Several studies have shown that mono (2-Ethylhexyl) phthalate (MEHP), a major metabolite of DEHP, reduces the meiotic ability of oocytes and affects the blastocyst phase. In vitro and in vivo studies suggest that DEHP or MEHP can induce metaphase II spindle anomalies, negatively modulate the transition of prophase I to metaphase II, and thereby affect meiotic development [14, 15, 20]. Furthermore, these metabolites can also cause prolonged cumulus cell-oocyte aggregates development to the blastula stage [21, 22]. Previous studies suggested that DEHP levels detected in ‘everyday’ environments (200 μgKg\(^{-1}\)day\(^{-1}\)) and work-related environments (2000 μgK\(^{-1}\)day\(^{-1}\)) cause zygote division and arrest zygote growth from the second-cell stage [23].

Modern scientific studies highlighted the wide usage of plants as a source of herbal medicine. Conventionally Garlic (Allium sativum) is utilized against protozoans, toxins, viral, fungal, and bacterial agents [24].

As garlic has different composites, due to multivitamins, but sulfur component mainly represents the biological and medicinal properties of this plant [25]. It has several therapeutic effects on different vital organs and tissues [26-35]. Garlic oil thickens the zygotic wall, which demonstrates that garlic oil has a positive effect on ovarian function and increases the secretion of estrogen [36]. Garlic extract triggers the emission of gonadotropic hormones and ovarian hormones via stimulation of the pituitary gland, increasing the Golgi complex with drawl rate, cell cycle, and accelerating binding to estrogen receptors [37]. Many plastic industry female workers across the world are vulnerable to heavy amounts of phthalates in daily life. The use of utensils, food wrappers and packing made of phthalates made the general population prone to exposure to phthalates. Microwave application is responsible for the leeching and mixing of phthalates in food. Female reproductive disorders are increasing day by day, so this research was premeditated to investigate the adverse effects of DEHP exposure on the female reproductive system and to check the ameliorative effects of commonly used cheapest spice i.e garlic extract against DEHP-induced reproductive deformities in adult female albino mice as an experimental model.

2. MATERIALS AND METHODS

2.1 Animals

Laboratory-raised Mus musculus (female albino mice) were utilized as experimental animals in the current research. Animal House of the Department of Zoology, University of Sargodha, Sargodha was used to take care of the animals under standard environmental conditions such as 24-hour day and night cycles, temperature 25±2 °C, and 45 % humidity. Steel cages bedded with wood shavings were used to keep animals. Feed and water were provided ad-libitum.

2.2 Treatment

Forty female albino mice aged 10-11 weeks and weight 28-30 gm were used in this research and divided into the following four groups=10).

1. Control Group received only standard feed and water.
2. Garlic Group received 500 mg/kg aqueous garlic extract in 0.2 mL distilled water.
3. DEHP Group was given 500 mg/kg body weight DEHP dissolved in 0.2 mL corn oil. 4-DEHP+Garlic Group was administered with 500 mg/kg DEHP in corn oil (0.2 mL) followed by (gap of 2 hours) 500 mg/kg body weight garlic extract. Doses were given by oral gavage once daily for 4 weeks [1, 40,41].

2.3 Organ’s Recovery

After the completion of the experimental time, all the animals were dissected after a cervical dislocation [38] to obtain the ovary, oviduct (fallopian tube), and uterus. Organs were placed in saline solution and the fats that cover the reproductive organs were removed. Fat-free organs were weighed on a digital weight balance (HI-500) and fixed in Conroy’s fixative for further processing [1].
2.4 Histological Preparation

After gradual alcoholic dehydration (50 %, 70 %, 90 %, and 100 %), and clearance in xylene, the organs were embedded in paraffin wax to obtain serial sections of 5µ thickness through rotary microtome (ERMA TOKYO 42). Sections on frosted slides were deparaffinized in xylene, hydrated in descending grades of alcohol, stained by dipping in hematoxylin, washed by tap water, counter stained with 1 % Eosin, dehydrated by ascending grades of alcohol, cleared in xylene and mounted with Canada balsam. Microphotographs of Hematoxylin and Eosin-stained sections were taken by using a digital camera of Samsung company (Model no. SM-J260F/DS-8.00 megapixel) attached to the trinocular microscope (Labomid CXR2) at 100× and 400× magnification [39]. The photomicrographs of the concerned histological portion were processed in Coral DRAW 7 for sharpness, shading, and editing.

2.5 Micrometry

The readings were taken from five arbitrarily chosen areas on the computer screen utilizing a pre-aligned advanced scale in Coral DRAW 7 from digital photographs. The calibrations were made from computerized photograph shots of the stage micrometer on a similar magnification. The mean number of mucosal folds of the ampulla, and the number of primary, secondary, and tertiary follicles of the ovary were taken at 100× objective of a microscope. Diameter of the primary and secondary follicles, the average cross-sectional area of tertiary follicles, the epithelial height of the mucosal fold, the muscular thickness of the ampulla, number of uterine glands, diameter of round uterine glands, the diameter of the uterus, endometrium and myometrium thickness, and epithelial height of uterus were measured at 400 × magnifications.

2.6 Statistical Analysis

Results were analyzed using One-Way Analysis of Variance (ANOVA) and post hoc Tukey’s tests in the IBM SPSS version 23.

2.7 Ethical Statement

This study was approved by the Biosafety and Ethical Review Committee of the University of Sargodha, 40100 Pakistan (Ref: SU/Acad/1167/2, Dated: November 06, 2019).

3. RESULTS

3.1 Body Weight

Statistically, no difference was observed in the mean initial body weight of animals belonging to different groups. While the mean final body weight of the DEHP-treated group was significantly (P<0.001) reduced as compared to the control, garlic extract, and DEHP+ Garlic (P<0.01) given group (Fig. 1).

3.2 Mean Weight of Female Reproductive Tract

Highly significant decrease was found by statistical analysis in mean weight of complete female reproductive tract of the DEHP treated group as compared to control (P<0.001), garlic (P<0.01) treated and DEHP+Garlic (P<0.01) administered group (Figure 2). Effect of garlic in attempt to reduce DEHP toxicity was obvious in co-administered group.

3.3 Histological Observations

Histological study of ovary of the control group showed developing primary, secondary, tertiary follicles and corpus luteum in the cortex of ovary, blood vessels, and normal stroma in the center (Figure 3A). Ovary of garlic extract-treated group (Figure 3B) indicated proper ovarian follicles with normal blood vessels and stromal cells resembling that of the control. Histological examination of ovaries of mice given DEHP (Figure 3C) showed a significantly reduced number of ovarian follicles and that of corpus luteum, blood vessels appear congested and dilated. In follicles, oocytes (O) became distorted and granulosa cells were irregular. Whereas DEHP+Garlic treated group (Figure 3D) indicated an improvement in the ovarian structure with decreased number of atretic follicles.

Histological analysis of primary follicles of the control group and garlic extract-treated group at higher magnification (Figure 4) showed normal structure having a central oocyte surrounded by granulosa cells encapsulated by theca cell. DEHP
Fig. 1. Effect of 28 days oral exposure of 500 mg/kg b.wt. garlic extract, DEHP and DEHP+garlic extract (500 mg/kg each) on mean body weight of female albino mice. Data is represented as Mean±SEM, **P<0.01, ***P<0.001

Fig. 2. Effect of oral intake (28 days) of garlic extract, DEHP and DEHP+garlic extract (500 mg/kg b. wt. each) on the mean weight of the complete reproductive system of female albino mice. Data is represented as Mean±SEM, a = control vs treated groups, b = Garlic extract group vs other treatments and c=DEHP treated group vs DEHP+garlic group. **P<0.01, ***P<0.001
Epithelial sloughed cells were present in lumen. In the DEHP+Garlic treated group, cuboidal shaped principal cells were present with hypertrophy in round basal cells. Detaching epithelial cells can be seen toward lumen (Figure 7L).

Fig. 3. Hematoxylin and Eosin-stained sections of adult female mice ovary exposed to garlic extract (500 mg/kg), DEHP (500 mg/kg), and DEHP+ garlic extract (500 mg/kg each) through gavage for 28 days (10X). Control (A) and garlic extract (B) groups showing normal ovary with well follicular development such as primary follicle (PF), secondary follicle (SF), Graafian follicle (GF) and corpus luteum (CL). DEHP group (C), ovary has reduced number of all stages of the follicle. While ovaries of animals treated with DEHP+garlic extract (D) showed improvement in the number of ovarian follicles.

exposed group represented primary follicles with a lesser number of granulosa cell and distorted theca cells. However, the DEHP + Garlic extract group indicated a normal-sized primary follicle with compact granulosa and theca covering.

In the control and garlic extract groups, secondary follicles presented normal structures having central oocyte surrounded by compact zona granulosa. Between the zona granulosa and oocyte, a thick glycoprotein coat of zona pellucida was present (Figure 4). Whereas in DEHP exposed group, the secondary follicles were filled with autophagic granulosa cells with a thin thecal layer. Toxic effect was evident in the combined treatment group as the secondary follicles were surrounded by vacuolated granulosa layers (Figure 4).

While the tertiary follicle of control group showed a normal mature oocyte surrounded by granulosa layers with normal corona radiata and cumulus oophorus. Animals treated with garlic extract represented the normal structure of the preovulatory follicle. The most evident histological changes in DEHP exposed group include vacuolation in granulosa cells of preovulatory follicles. Moreover, other changes indicated thin and irregular corona radiata, disruption of cumulus oophorus, and reduction in the size of the antral cavity of the Graafian follicle. Animals given DEHP+Garlic showed the ameliorative effect of garlic against toxicant on the mature follicle as shown in Figure 4.

Histological analysis of oviduct section of control group presented normal anatomical structure as branched and highly invaginated mucosa surrounding the oviduct lumen. A compact and organized epithelium of the oviduct was observed with normal-sized ciliated principal cells and richly populated basal cells (Figure 5A&B). Oviduct of garlic extract-treated group indicated normal organization of mucosal folds with deep grooves and prominent epithelium with fine ciliated principal cells and round basal cells near lamina propria (Figure 5C&D). Histological deformities were seen in mucosal folds and epithelial layer of the DEHP exposed group as compared to the control. Branching invaginations of mucosal folds were decreased and a wider lumen was prominent. DEHP also showed disorganized loosely packed epithelium where basal cells were more abundant as compared to columnar cells and exfoliation of principle cell was the prominent feature in epithelium (Figure 5E&F). The DEHP+Garlic treated group showed tall mucosal folds with a
Fig. 4. Photomicrographs of all stages of follicles, secondary oocyte and corpus luteum of control and treated groups at higher magnification. Effect of Phthalate is prominent on all follicles as hypertrophy in granulosa (Gc) and theca cells (Tc), congested antrum (A), reduced corona radiata (C), disrupted zona pellucida (Zp) and regressed oocyte as compared to control and other treatments. Garlic coadministration can be seen as a rescuing treatment against DEHP intoxication. H&E staining.
slight portion of lumen. Epithelium clearly showed rehabilitative potential of garlic as size and shape of principle and basal cells resemble to that of control but still, exfoliation can be seen (Figure 5G&H).

In control and garlic-treated groups, uterus had a very dense external muscular layer, the endometrium, and myometrium. Deep invaginations were observed within the endometrial stroma (Figure 6 A&B). Histomorphological alterations were observed in uteri of DEHP-given animals as compared to the garlic and control groups. The size of uterus was decreased having less invaginated epithelium, and endometrium was thicker with marked proliferation and hypertrophy in the stromal cell (Figure 6C). While the DEHP+Garlic extract treated group showed normal anatomical structure of uterine layers and lumen as garlic seems to attenuate the effect of toxin in this group (Figure 6D).

In control and Garlic treated sections of uterus, densely populated and well-organized (circular and elongated) endometrial glands were present in the uterine endometrium (Figure 7B&E). However, comparatively small-sized, undeveloped and congested endometrial glands were observed in the DEHP-treated group (Figure 7H). While small circular and glands were prominent in the DEHP+Garlic extract treated group where the effect of toxin was prominent (Figure 7K).

In the control and garlic extract-treated uterine sections, the epithelium was well-defined, and thick having compact tall columnar ciliated cells with a prominent basement membrane on which basal nuclei were lying (Figure 7C and F). However, in the DEHP group, the organization and shape of epithelium, and basement membrane were so distorted that stromal tissues and endometrium epithelium were lacking a clear demarcation (Figure 7I) Epithelial sloughed cells were present in lumen. In the in DEHP+Garlic treated group, cuboidal shaped principal cells were present with hypertrophy in round basal cells. Detaching epithelial cells can be seen toward lumen (Figure 7L).

### 3.3 Micrometric Analysis of Ovary

#### 3.3.1 Mean Number Ovarian Follicles and Corpus Luteum

Statistically, no significant difference was noted in mean number of primary follicles in ovaries of treated females as compared to control group. Whereas, mean number of secondary and tertiary follicles was reduced (P<0.05) by DEHP treatment as compared to control and garlic extract given group. DEHP treatment also caused a reduction in mean number of secondary follicles when compared to the co-exposed (P<0.01) group as given in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
<th>Corpus luteum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.33±0.33</td>
<td>3.50±0.23</td>
<td>1.00±0.00</td>
<td>3.50±0.28</td>
</tr>
<tr>
<td>Garlic</td>
<td>5.00±0.44</td>
<td>3.50±0.23</td>
<td>1.00±0.00</td>
<td>3.83±0.40</td>
</tr>
<tr>
<td>DEHP</td>
<td>3.83±0.47</td>
<td>2.83±0.16 (a^b)</td>
<td>0.66±0.14 (a^b)</td>
<td>2.00±0.24 (a^b)***</td>
</tr>
<tr>
<td>DEHP+Garlic</td>
<td>4.83±0.30</td>
<td>3.55±0.12 (c^)**</td>
<td>0.83±0.11</td>
<td>3.66±0.22 (c^)**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM and comparison is made by Tukey’s test. a = Control vs treated groups, b = DEHP group vs Garlic and DEHP+Garlic group and c = Garlic vs DEHP+Garlic group. \(^*P<0.05, \quad ^{**}P<0.001, \quad ^{***}P<0.001\)

Table 1. Mean number of primary, secondary, tertiary follicles, and corpus luteum in control and treated groups after 28 days oral exposure to DEHP and garlic extract alone and in combination.
Fig. 5. Hematoxylin and Eosin-stained sections of Ampulla from the oviduct of control and treated mice at low and high magnification. Control group (A) shows branched, tall mucosal folds with deep grooves, muscle layer, and epithelium (B) containing ciliated columnar principal cell lying on round basal cells. Garlic extract-treated group (C) shows many mucosal folds, thick muscle layer, and epithelium (D) with an organized adluminal layer of ciliated principal cells and a dense layer of basal cells. While DEHP exposure (E) resulted in reduced muscle layer thickness, less branches and disorganized epithelium (F) with loose flaking principal cells and hypertrophy in basal cells. Combined group (G) showing increased mucosal folds, and epithelium (H) presented much improved signs as compared to only toxin-treated epithelium as regular-sized principal cells and basal cells was prominent.

Values are expressed as Mean ± SEM and data was compared by Tukey’s test. a = Control vs treated groups, b = DEHP treated vs Garlic and DEHP+Garlic treated groups and c = Garlic vs DEHP+Garlic group. ***P<0.001, **P<0.01, *P<0.05

Fig. 6. Hematoxylin and Eosin-stained uterine longitudinal sections of adult female control and treated mice. Control (A) and garlic extract group (B) sections exhibited prominent invagination and branching in endometrium (E), well-organized myometrium (M), and clear lumen (L). While the DEHP group (C) showed congestion in all layers of uterus with a significantly reduced lumen. DEHP+Garlic extract group (D) showed improvement signs in uterine tissue as compared to only DEHP given group.
Fig. 7. Photomicrographs taken from sections of endometrium and uterine epithelium (Ep) of adult female mice of control and treated groups at different magnifications. Control (A, B, C) and garlic extract (D, E, F) group show regular-sized uterine glands (Gl) embedded in rich stromal tissue, while in epithelium columnar cells with prominent basal nuclei on a clear basement membrane were obvious. While DEHP exposed group (G,H,I) present distorted glands in the disorganized stroma, epithelium with shorter principle cells and irregular-shaped cells sloughed in lumen. DEHP+Garlic treated group (J,K,L) showed improvement in columnar epithelium with normal basement membrane and seems to lessen the effect of DEHP but exfoliating cells are also prominent. A,D,G,I at 10X and all other at 100X.

### 3.3.2 Mean Diameter of Ovarian Follicles

Statistical analysis indicated that combined treatment of DEHP+Garlic caused a significant (P<0.05) increase in mean diameter of primary follicles as compared to control, garlic and DEHP alone administered (P<0.01) females. Mean diameter of secondary follicles of mice given different treatments was not statistically different from that of control. Significant (P<0.05) decrease in the mean diameter of tertiary follicles was noticed in DEHP-exposed mice as compared to control and garlic-treated mice (Table 2).

### 3.4 Micrometric Analysis of Oviduct

Statistical analysis (ANOVA) showed that treatment of DEHP caused a highly significant (P<0.001) decrease in the mean number of mucosal folds as compared to the control and other treatment
groups. While mean number of mucosal folds was significantly (P<0.001) increased in DEHP+Garlic treated ampulla as compared to DEHP treated oviduct which showed the protective ability of garlic extract. Mean epithelial height was observed to be significantly (P<0.001) increased in garlic and DEHP+Garlic exposed group as compared to control. Whereas, the DEHP-given group displayed a considerable (P<0.001) reduction in mean thickness of ampullary epithelium as compared to control and other treatment groups. Toxic effect of Phthalate was prominent on epithelium of ampulla. Epithelial height was also reduced (P<0.05) in DEHP+Garlic treatment as compared to only garlic extract given group. Muscle layer thickness of the oviduct was not significantly affected by any of the treatments (Table 3) as compared to control.

3.5 Micrometric Analysis of Uterus

3.5.1 Uterine Diameter and Epithelial Height

According to one-way ANOVA, a highly significant increase was found in the mean diameter of uterus of female mice exposed to DEHP+Garlic when compared to the control while other treatments caused no noticeable difference when related to control. Among treated groups, significant (P<0.001) decrease was observed in the mean diameter of the uterus of DEHP exposed group as compared to the garlic and DEHP+Garlic administered group. Noteworthy (P<0.001) increase was seen in mean epithelial height of uterus in garlic extract-given group as compared to control and all other treatment groups. Whereas, DEHP and combined treatment caused a prominent (P<0.01) decrease in mean epithelial height as compared to control (Table 4).

3.5.2 Number of Uterine Glands

Data after statistical analysis revealed a significant (P<0.001) decrease in the mean number of uterine glands in Garlic and DEHP alone treatments as compared to the control group while co-administration of both increased (P<0.001) in mean number of glands in uterine stroma as compared to control. Among treated groups, the mean number of uterine glands was significantly (P<0.001) increased in DEHP+Garlic exposed group as compared to garlic and DEHP+Garlic extract given groups. However, combined exposure of DEHP+Garlic extract resulted in highest (P<0.001) number of uterine glands as compared to other treatments (Table 4).

3.5.3 Muscular Thickness: Endometrium and Myometrium

One-way ANOVA showed no significant effect on the mean thickness of endometrium by any treatment as compared to control. While a significant (P<0.05) reduction was observed in the mean thickness of

Table 2. Mean diameter of primary, secondary and tertiary follicles in control and treated groups after 28 days of oral exposure of DEHP and garlic extract alone and in combination.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diameter of Follicles (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>Control</td>
<td>1.34±0.11</td>
</tr>
<tr>
<td>Garlic</td>
<td>1.36±0.07</td>
</tr>
<tr>
<td>DEHP</td>
<td>1.20±0.05</td>
</tr>
<tr>
<td>DEHP+Garlic</td>
<td>1.74±0.14ab*c**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM and comparison is made by Tukey’s test. a = Control vs treated groups, b = DEHP group vs Garlic and DEHP+Garlic group and c = Garlic vs DEHP+Garlic group. *P<0.05, **P<0.01.
Our result clearly showed that the... features in ovarian tissue.

**4. DISCUSSION**

DEHP, a potent toxicant, is widely used in various products and found everywhere in the environment. The initial studies suggest that the reproductive system is more vulnerable to phthalates as compared to other organs [42]. On the other hand, oxidative stress caused by environmental contaminants can be prohibited or reduced by dietary natural antioxidants through their capacity by scavenging these products [43]. This study was intended to investigate the lesser-known protective effects of aqueous extract against DEHP-induced reproductive toxicity in adult female mice.

Our study indicated that the number of developing primary, secondary, and tertiary follicles and corpus luteum were adversely affected by DEHP exposure in mice. DEHP treatment also caused histomorphological alteration such as degeneration of ovarian follicles, oocytes degradation, antral reduction and granulosa cells hypertrophy. It has been reported by Wang et al. (2012) [29] that antral follicle growth of mice was reduced by using all doses (0.1, 1, 10, 100 µg/mL) of MEHP and DEHP. Furthermore, DEHP can alter the antioxidant system causing the accumulation of superoxide (O$_2^-$) that in turn damage the antral follicle [44]. In the current study, DEHP-induced superoxide addition might be the cause of degenerative features in ovarian tissue.

myometrium in female mice treated with DEHP as compared to the control group (Table 4).

### Table 3. Mean number of mucosal folds, muscular thickness and epithelial height (µm) in ampulla region of oviduct in control and treated groups after 28 days of oral exposure to DEHP and/or garlic extract.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mucosal folds (µm)</th>
<th>Epithelial height (µm)</th>
<th>Muscularis (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.33±0.81</td>
<td>0.446±0.01</td>
<td>1.00±0.10</td>
</tr>
<tr>
<td>Garlic</td>
<td>28.16±0.70</td>
<td>0.457±0.01**</td>
<td>1.36±0.15</td>
</tr>
<tr>
<td>DEHP</td>
<td>17.33±0.71ab***</td>
<td>0.361±0.01ab***</td>
<td>1.06±0.11</td>
</tr>
<tr>
<td>DEHP+Garlic</td>
<td>26.75±0.70c***</td>
<td>0.455±0.02b***</td>
<td>1.16±0.13</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM and comparison is made by Tukey’s test. a = Control vs treated groups, b = DEHP group vs Garlic and DEHP+Garlic group and c = Garlic vs DEHP+Garlic group. *P<0.05, **P<0.01, ***P<0.001

### Table 4. Mean diameter of uterus, epithelial height, mean number of uterine glands and thickness of endometrium and myometrium in control and treated groups after 28 days of oral exposure to DEHP and/or garlic extract.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diameter (µm)</th>
<th>Epithelial height (µm)</th>
<th>Uterine glands</th>
<th>Endometrium</th>
<th>Myometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.82±0.28</td>
<td>0.47±0.02</td>
<td>171.83±5.67</td>
<td>1.24±0.11</td>
<td>0.70±0.07</td>
</tr>
<tr>
<td>Garlic</td>
<td>5.65±0.29</td>
<td>0.58±0.02**</td>
<td>143.00±3.91***</td>
<td>1.20±0.08</td>
<td>0.64±0.06</td>
</tr>
<tr>
<td>DEHP</td>
<td>3.98±0.23b***</td>
<td>0.38±0.01a<strong>h</strong>*</td>
<td>106.66±10.47ab***</td>
<td>0.98±0.11</td>
<td>0.50±0.03a</td>
</tr>
<tr>
<td>DEHP+Garlic</td>
<td>6.01±0.24abc***</td>
<td>0.38±0.01a<strong>h</strong>*</td>
<td>192.83±12.93bc***</td>
<td>1.20±0.09</td>
<td>0.65±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM and data was compared by Tukey’s test. a = Control vs treated groups, b = DEHP group vs Garlic and DEHP+Garlic group and c = Garlic vs DEHP+Garlic group. ***P<0.001, **P<0.01, *P<0.05
histomorphology of the ovary was improved by garlic extract supplementation. Diameter and number of developing primary, secondary, antral follicles and corpus luteum were ameliorated upon garlic extract supplementation in DEHP-intoxicated mice. A previous study reported that garlic oil supplementation played a direct and indirect role in enhancing the activity of ovaries by stimulating the release of sex hormones together with estrogen [36]. Another research found that garlic extract induces secretion of ovarian hormones and gonadotropins through the stimulation of the pituitary gland, cell cycle, and accelerates estrogen receptors binding [37]. These reported effects of garlic on female reproduction are also established in this study in an improved form of ovarian histological architecture in garlic-exposed groups that might be due to enhanced gonadotropins input and boosted hormone receptor binding. All these reports point toward the importance of garlic intake as an important nutraceutical to nullify effect of environmental toxicants.

In the mammalian oviduct, the ampulla is a major anatomical region in which fertilization takes place and it is the main site of early embryo development [45, 46]. Results of our research indicated that DEHP exposure adversely affected the oviduct of mice by a reduction in the number of mucosal folds along with histological alteration in the ampullary region in comparison to the control and garlic extract-treated group. According to another research oviduct with a reduced number of ciliated cells [47] and low circulating estrogen [48] level would lead to ectopic pregnancies in comparison to normal intrauterine gestation. The structural changes in the ampulla of oviduct in this study might be attributed to DEHP interference in hypothalamic gonadal axis.

Our results demonstrated that histomorphology of oviduct was improved upon garlic extract supplementation in the co-administered group. It has been reported in previous studies that garlic has antioxidant properties and often prevents cell damage, aging and cancer [36]. These properties of garlic seem to be playing role in attenuating the toxic effects of DEHP in co-exposed ampullary tissue in this study.

In the uterus, a significant decrease overall diameter of uterus, muscularis, mean number of uterine glands, and epithelium heights were observed in DEHP exposed group as compared to control in the current study. Another similar research also emphasized on the toxic effects of DEHP on uterus which disturbed the morphology and physiology of the uterus by causing a change in stromal cell patterns comparable to changes observed in females on progestin contraceptives [49]. Altered patterns of cell proliferation were observed in the DEHP-exposed uterus leading to de-regulation of the normal oestradiol-progesterone responses in the uterus which in turn causing to an anti-estrogenic effect [50]. DEHP-imposed anti-estrogenic effects might be the reason behind histopathological alterations in uterine tissue in this study as uterus ia an estrogen-dependent organ. While our result dictated the ameliorative abilities of aqueous garlic extract on uterine histology and morphometry due to its antioxidant and gonadal axis stimulatory abilities.

5. CONCLUSION

Various histo-protective effects in reproductive organs were noticed in the combined group (DEHP+Garlic) as compared to only DEHP exposed group that reflected the antioxidant abilities of garlic extract. These findings indicated that garlic is a common, cheapest, and most effective remedy against reproductive toxicity induced by environmental pollutants. Many female workers of plastic industries and other industries where phthalates are used in abundance are exposed to heavy amounts of a mixture of phthalates and are at much higher risk of developing reproductive abnormalities. However, simple natural products such as garlic can be useful to reduce the damage caused by these environmental pollutants. As we know that exposure to toxicants generates reproductive abnormalities so daily intake of diet-based cheap and easily available remedies like garlic are suggested by this study.

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7. CONFLICT OF INTEREST

All authors unanimously declare no competing conflict of interest in any regard for this research work.

8. REFERENCES


