



Di-(2-ethylhexyl) phthalate (DEHP) and ovarian physiology: A brief review of therapeutic potential of *Asparagus racemosus* on DEHP- induced toxicity

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ABSTRACT

Exposures to environmental contaminants have been shown to disrupt endocrine function by inhibiting or stimulating the hormone production and thus affecting the functions that these hormones control. Di-(2-ethylhexyl) phthalate (DEHP) is an endocrine disrupting chemical (EDC) and a potent environmental as well as reproductive toxicant that poses a great impact on human health severally affecting fertility in both male and female. This review discusses the adverse effect of selected EDCs that is DEHP on the ovarian physiology. Furthermore, this review also highlights one of the herbal drugs, that is, *Asparagus racemosus* (Shatavari) that can be utilized to minimize the DEHP induced toxicity. This approach appears to hold great promise for treating many reproductive abnormalities as common people cannot afford the cost of assisted reproduction techniques (ARTs) such as Di-(2-ethylhexyl) phthalate (IVF), intercytoplasmic sperm injection (ICSI) procedures. In addition, it will delineate a roadmap for using herbal based therapies to cure various incurable reproductive diseases.

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INTRODUCTION

In-utero exposure of environmental contaminants is the important risk factors for many preterm diseases including epigenetic changes in developing fetus. It is well known that most of environmental contaminants are able to cross the placenta and can interact with genetic and epigenetic mechanisms to alter the course of normal development. These contaminants are also able to disrupt endocrine function by inhibiting or stimulating the hormone production that ultimately leads to incidence of endocrine-related disorders in

humans, including pregnancy complications, genital malformations and cancer. Basically, environmental contaminants are endocrine disrupting chemicals (EDCs) that have recently received considerable attention due to their ubiquitous presence in our surrounding environment. EDCs could be natural or synthetic chemical compounds which are widely used as plasticizers to soften and increase the flexibility of plastics and cosmetics products. Mammals are exposed to EDCs mainly due to their use in food storage cans, bottles, wrapping materials and in food processing (Warmuth et al., 2006). According to a report, in India, 13% of the ever-married women who were aged 15-49 years were childless in 1981 (rural 13.4% and urban 11.3%), which had increased to 16% in 2001 (rural

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15.6% and urban 16.1%). Further, over half of the married women who were aged 15-19 years were childless in 1981, which had increased to 70% in 2001 (Dey, 2010). It has been demonstrated that long term exposure of EDCs mainly phthalates are linked with decreased embryo survival and risk of clinical pregnancy loss (Schmidt et al., 2012; Mu et al., 2015a, 2015b). Recently, in a study it is reported that exposure of benzyl butyl phthalate (BBP), a wide spread EDC, increased adipogenesis and metabolic dysregulation via impairing vital epigenetic regulators (Zhang et al., 2017). These reports are suggesting that exposure to EDCs could be the main culprit for reproductive related problems. Therefore, it seems impossible to avoid daily exposure to EDCs for many mammalian species including human. Despite this, very little is known about precise mechanisms, timing and toxicity of EDCs which are responsible for several aberrations in metabolism and reproductive functions in several mammalian species.

METHODOLOGY

Informations about EDCs mainly DEHP were collected from different scientific search engines viz. Google Scholar, Pubmed, Science Direct and Scopus. All the published informations about the impact of DEHP on ovarian physiology in indexed journals was included and cited in the text. Literatures available in the Ayurveda books, Indian Ayurvedic system were systematically examined to retrieve the beneficial effect of the medicinal plant *A. racemosus* (Shatavari). Further, authors have also searched the keywords like DEHP, ovary, infertility, herbal drugs and Shatavari using scientific internet browsers to gather more information's about the topic. All the available information's about the DEHP and *A. racemosus* were cited throughout the text. Pharmacological data of *A. racemosus* was collected from different *in vivo* and *in vitro* studies using the key words *A. racemosus*, reproduction, infertility, ovary and women reproductive health. Pharmacokinetics and clinical data of the *A. racemosus* was limited and data published in last one decade were considered for this review.

Di-(2-ethylhexyl) phthalate (DEHP)

DEHP is one of the most common EDCs, and is widely used to make vinyl plastics softer and more flexible. DEHP is one of the highest volume chemicals produced with an estimated 5% annual growth due to increasing manufacturing demand worldwide. More than 10 million tons of DEHP, an estrogen-like unbound chemical, are used worldwide every year to increase

the softness of plastics or liquefy materials (Zhang et al., 2013a, 2013b, 2013c). The ubiquitous presence of DEHP is invited to expose human as well as animal through their food materials (Figure 1). Widespread and continuous daily exposure to DEHP is believed to arise in general population primarily through the diet, as well as from drinking water, use of plastic medical devices for feeding, medicating and assisting the breathing of newborn infants (Li et al., 2015). Due to the toxicological nature of DEHP exposure, assimilation and disposition of DEHP have been analytically investigated in different species. One of the preliminary study showed that ¹⁴C-labeled DEHP disappeared rapidly from the blood and approximately 57% of the total dose was recovered in the feces and 42% in the urine after intravenous administration to the rats (Daniel et al., 1974). It is estimated that daily exposure of DEHP individually ranges from 0.21 to 2.1 mg/day for the general population (Tickner et al., 2001; Huber et al., 1996). Further, urinary metabolite concentrations of DEHP (3-30 mg/kg/day) (Koch et al., 2006) suggest that even little exposure of DEHP in daily life from any sources may reflect a potential risk factor. Since major route of exposure to DEHP is food and water intake in general population, the exposure of DEHP is a serious public health concern however, data concerning about the amount of its absorption as well as major pathway of action is not well understood.

Although, mechanism of action of DEHP is not well defined but it is predicted that toxicity of DEHP is mediated by its bioactive metabolite mono(2-ethylhexyl) phthalate (MEHP) which is derived from the hydrolysis of DEHP monomers that lead to its release into food and liquid that gets integrated as a part of food chain of human (Lovekamp-Swan et al., 2003; Heudorf et al., 2007; Helal et al., 2014; Zhang et al., 2013a, 2013b, 2015). Once ingested, MEHP is easily absorbed by intestinal cells and start to interfere with the biosynthesis, storage, release, transport, and/or receptor binding of endogenous hormones, ultimately interfering with the proper functions of these hormones. This statement is supported by the presence of MEHP in human tissues such as blood (Hogberg et al., 2008; Hannon et al., 2015), urine (Marsee et al., 2006; Heudorf et al., 2007), amniotic fluid (Huang et al., 2009), breast milk (Hogberg et al., 2008) and ovarian follicular fluid (Krotz et al., 2012). Furthermore, it is estimated that 3-30 µg of DEHP is exposed to human via food and water on daily basis (Kavlock et al., 2002; Hannon et al., 2015; Hannon et al., 2014; Li et al., 2015), that shows its ubiquitous presence and thus it seems very difficult to escape ourselves from DEHP toxicity.

Recently, our preliminary findings also indicated that exposure of DEHP impaired the folliculogenesis in rat ovary (Tripathi et al., 2018, unpublished observation)

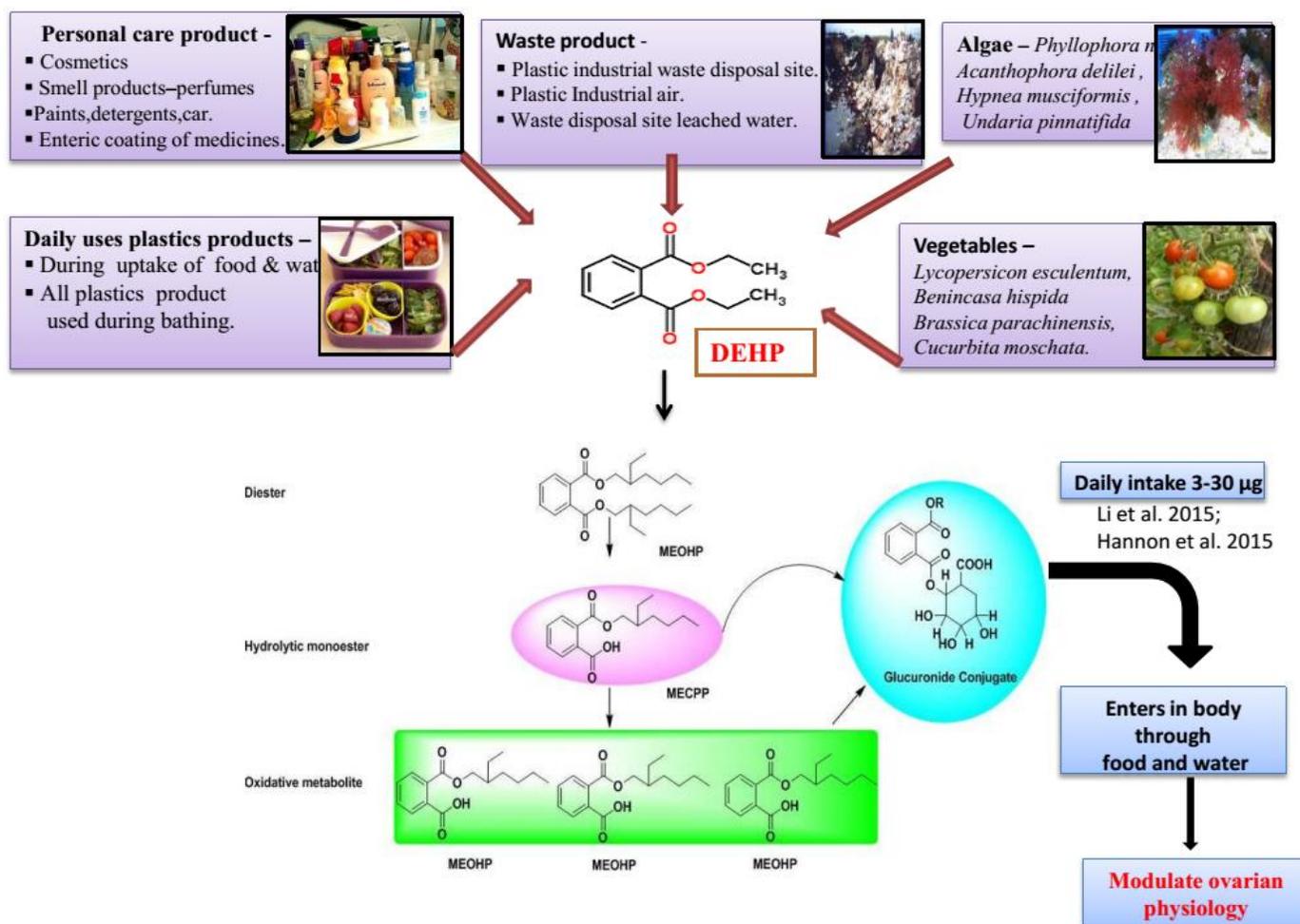


Figure 1. Generation of DEHP from different sources and mechanism through which it affect mammalian reproductive physiology.

suggesting that DEHP is having ability to disturb the reproductive physiology. Although, exact mechanism by which DEHP disrupt ovarian physiology remains unknown therefore, it is necessary to understand the effects of EDCs mainly DEHP on ovarian physiology.

DEHP and ovarian physiology

In mammals, ovary is metabolically active organ and the site of egg production via folliculogenesis. Folliculogenesis is a very complex process in which approximately 99% of follicles undergo follicular atresia and only few follicles reach to the ovulatory stage. During this process, any functional disruption may lead to reproductive problems which ultimately affect the reproductive potential of an individual. Interestingly, it is reported that DEHP has the potential to target the ovary at all stages of development. It may affect the female

reproductive development including early puberty, causes delay in menstrual cycle, and poses infertility even at low levels of exposure which could extend to future generations. Studies in animals suggest that DEHP exposure impairs the mouse primordial follicle assembly (Zhang et al., 2013a, 2013b, 2013c; 2014), DNA methylation in mouse oocytes and heritable modifications in imprinted genes (Li et al., 2014). Recently, a study has shown that DEHP exposure to newborn mice leads to the inhibition of oocytes, nest breakdown and primordial follicle assembly, and these kind of impairments were mediated by modulation of estrogen hormone level and their receptors (ERs) (Mu et al., 2015; Li et al., 2016). Further, Hannon and his colleagues found that *in vitro* exposure of MEHP increased the transition of primordial follicles to primary follicles via over-activating the PI3K signalling pathway (Li et al., 2016; Huang et al., 2009). Furthermore, a study reported that *in vivo* exposure of DEHP for 10

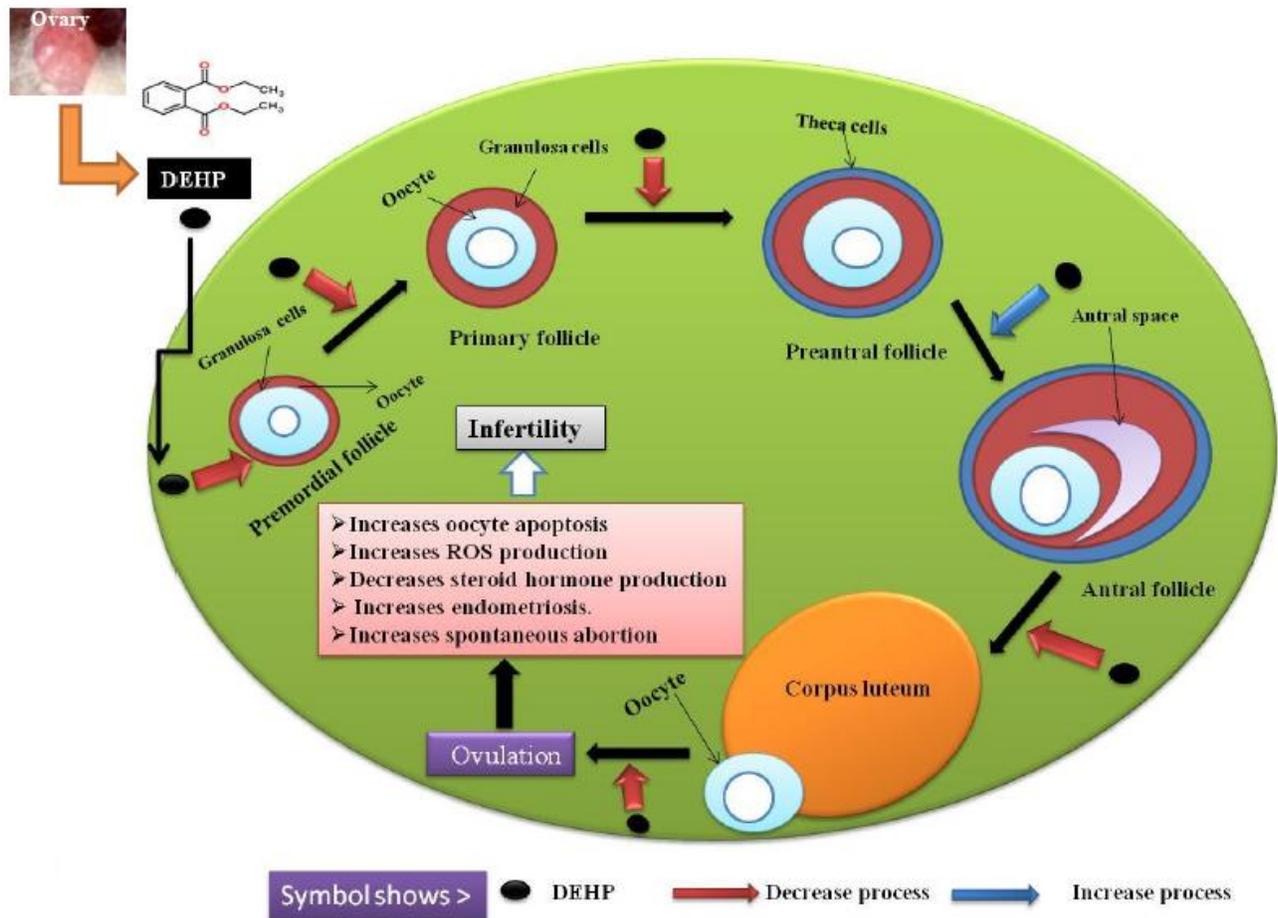


Figure 2. Mechanism of action of DEHP on the process of ovarian folliculogenesis.

days enhanced the recruitment of primordial follicles into the growing pool in adult ovaries (Li et al., 2016; Zhang et al., 2014). In addition, it is demonstrated that MEHP, but not DEHP inhibits antral follicle growth, induces atresia, and inhibits steroidogenesis in mice *in vitro* (Huang et al., 2009; Li et al., 2016). Moreover, Xu et al. (2010) showed that *in vivo* treatment of DEHP at 300 and 600 mg/kg significantly decreased levels of E₂, aromatase mRNA and protein in rat and the result is further supported by Craig et al. (2014).

Entertainingly, it has also been seen that when the follicles are co-cultured with MEHP and estradiol, it partly rescued the toxic effects of MEHP (Li et al., 2016; Craig et al., 2014). These studies together suggested that DEHP directly acts on ovarian granulosa cells and thereby inhibit estradiol production by blocking estradiol receptor in mammals that leads to increase oxidative stress and ovarian somatic cell apoptosis. If so, it is a serious concern for public health because DEHP adversely affect the two essential ovarian processes like folliculogenesis and steroidogenesis which are

essential for maintenance of appropriately timed reproductive cycle, senescence and fertility (Figure 2). However, we still have limited information about the impact of DEHP and their mechanism by which it exerts ovarian toxicity in women and in animal models. Thus, future studies are needed to understand the mechanism of action of DEHP in mammalian system.

It is believed that DEHP may induce its toxicity in two ways; either by disturbing the levels of some hormones like estrogen or progesterone and thus reduces fertility probably by inhibiting follicular development or by inducing oxidative stress via reactive oxygen species (ROS) generation and hence decreases the fertility. ROS are known to play important roles in many physiological processes (Ames, 1999; Erkekoglu et al., 2010). It has been reported that oxidative stress may be an important mechanism underlying the toxic effects of DEHP (Santhosh et al., 1998; Manojkumar et al., 1998). It is reported that DEHP induces ROS generation and alters oocyte maturation suggesting the role of DEHP-mediated ROS involvement in the ovarian physiology

(Suna et al., 2007). It has been shown that the oral administration of DEHP in rats increased the generation of ROS such as the superoxide radical and H_2O_2 (Suna et al., 2007). Moreover, MEHP, the active metabolite of DEHP is also reported to selectively induce oxidative stress and is responsible for the release of cytochrome c from mitochondria, thereby inducing apoptosis in germ cells (Suna et al., 2007; Kasahara et al., 2002). These results suggest that DEHP-mediated ROS generation might be one of the mechanisms underlying the inhibition of the process of ovarian folliculogenesis and steroidogenesis, and in turn, the reproductive toxicity of DEHP.

On the basis of research findings and available literature, it is logical to state that DEHP might be one of the factors that are responsible for early puberty, disturbance in menstrual cycle, amenorrhea, and recurrent abortion. In addition, the problems are also associated with the individual's life style, living and working environment as well as society that ultimately lead to the overall reproductive health of an individual. It can be argued that reproductive health is one of most prevalent health care problems in India especially in rural areas because of the lack of adequate primary healthcare facilities, unhygienic conditions and direct exposure to various kinds of endocrine disruptors. It has also been seen that in any adverse case on women's reproductive health, clinicians depending on the case, prescribe wide range of medicines like nonsteroidal anti-inflammatory drugs (NSAIDs), dopamine agonists, diuretic agents, and selective serotonin reuptake inhibitors (SSRIs) which ultimately leads to infertility (Kelderhouse and Taylor, 2013; Imai et al., 2015). In addition, use of hormones and other medicines based costlier therapies for augmenting reproductive performance are economically not affordable by common man. Hence, it seems necessary to look for some other safe, cheap and locally available alternative therapy and in this context herbal based drugs could be one of the better options to protect against DEHP-induced reproductive toxicity.

In India, Ayurveda is one of traditional medicinal system that uses herbs from Vedic period to till date for the treatment of various kinds of diseases. Traditional Ayurvedic medicines still play a vital role in rural areas because of their easy access and are economically cheap. According to a study conducted by Mugomeri et al. (2015) majority of pregnant women use herbs for no particular reason, other than because it is a tradition. Therefore, traditional herbal medicine can be considered to provide holistic treatment and could be one of the better alternative approaches to cure or at least minimize the toxicity of EDCs. In this paper, we have restricted the detailed discussion of Ayurvedic system, and only an inclusive overview of the medicinal herbs that can restore the reproductive health is provided in the

text. The purpose of this paper is to keep a view about medicinal herbs especially *A. racemosus* that can be useful to cure reproductive problems induced by DEHP.

DEHP and herbal drugs

There are many plants of medicinal value that may be used to minimize the toxicity of DEHP. It is reported that celery oil (*A. graveolens*) alleviated the testis damage induced by DEHP (Marsee et al., 2006; Piomboni et al., 2008; Shalaby et al., 2010; Wahba, 2011) by decreasing oxidative stress and enhancing the maturation of spermatozoa. It is suggested that protective effects of celery oil is due to its antioxidant properties and the androgenic activities of apigenin, limonene and phthalide glycosides-ingredients. It is also reported that plants like *Aloe vera*, *Foeniculum vulgare*, *Anethum graveolens*, *Portulaca oleracea*, *Phoenix dactylifera* and *Allium sativum* increase the estrogen level (Sarkar et al., 2006; Moshtagh et al., 2010; Poorfarid et al., 2013; Kooti et al., 2015; Heidarifar et al., 2015). In addition, *Aloe buettneri*, *A. berger* and *Justica insularis* have shown to have estrogenic and ovulatory effects (Telefoa et al., 2011). *Saraca asoca* (Ashoka) bark contains an oestrogenic compound and is reported to have stimulating effect on ovarian tissue that repair menstrual bleeding. In addition, *Viscum album* (Mistletoe) leaf extract increases ovarian hormones (LH and FSH) concentrations (Ofem et al., 2014). Furthermore, *A. graveolens* and *P. dactylifera* is reported to have effect on progesterone levels (Moshtagh et al., 2010; Heidarifar et al., 2015). *Ficus asperifolia* is also reported to have a pronounced effect against female infertility (Watchoa et al., 2009). Some of the other plants like, *A. graveolens* (Heidarifar et al., 2015), *F. vulgare* (Kooti et al., 2015) and *P. dactylifera* (Moshtagh et al., 2010) have more pronounced effects on reproductive female hormones via their antioxidant effects. *Lawsonia inermis* (Henna or Mehndi) has also been shown to have antioxidant and antifertility effects (Gagandeep et al., 2010).

It is surprising that the sales of herbal dietary medicine reached 6.4 billion in 2014, with women as the major users taking these herbals for a variety of ailments (Blumenthal et al., 2015) indicating the popularity of herbal medicines particularly for woman's health. These plants based scientific research suggest that medicinal herbs have the ability to increase estrogen or progesterone levels and might be useful to protect EDCs-induced reproductive toxicity. The mechanism behind minimizing the toxic effects of DEHP can be explained because herbal plants act as an antioxidant and thus may reduce ROS generation and thereby reduces oxidative stress. Although, the active components of the many herbal drugs are not known, and even today many drugs still need further exploration

for their active constituent characterization and elucidation of the exact mechanism of action.

DEHP and *Asparagus racemosus*

A. racemosus (Shatavari; family- Asparagaceae, Liliaceae) is a climbing plant which grows in low forest areas throughout India. The plant is popularly known as “shatavari” which means who possesses hundred husbands or acceptable to many (Shashi et al., 2013), implying its ability to increase fertility and vitality. In the Indian Ayurvedic system of herbal medicine, this amazing herb is mentioned as the “Queen of herbs”, because it promotes love and devotion.

Scientific classification:

Kingdom: Plantae
Class: Angiosperm
Order: Asparagales
Family: Asparagaceae
Subfamily: Asparagoideae
Genus: *Asparagus*
Species: *racemosus*

A. racemosus is well known for its beneficial effects specifically on the female reproductive system and it has been scientifically validated as reproductive system tonic, immunomodulator, antioxidant, and antistress (Kumar et al., 2008). It is used in all female related problems such as sexual debility (Frawley, 1989), ammenorrhoea, dysmenorrhoea, dysfunctional uterine bleeding (Swarup and Umadevi, 1988; Chopra and Simon, 2000) and gonorrhoea (Thomson, 2002). *A. racemosus* also works as a stimulant of endometrium and ovarian tissues, regulate menstruation and ovulation, balance hormonal levels (TSH, FSH, LH, estrogen) and improves the Conception rate (64% vs 28%) in women (Kumar and Singh, 2001). *A. racemosus* is well recognized for its phytoestrogenic properties and is used as a hormone modulator. Phytoestrogens are defined as a plant compound that is structurally and/or functionally similar to the ovarian and placental oestrogens and their active metabolites (Whitten and Patisaul, 2001). The phytoestrogenic activity in plants is due to the presence of steroidal saponins which exert hormone like actions in the body, and also due to the presence of isoflavones which have mild estrogenic activity that help to balance the estrogen levels. The root extracts of *A. racemosus* with estrogenic effects, is particularly related with estrogen receptor (ER) selectivity may offer safer alternatives for conventional hormone based therapy (Figure 3).

A. racemosus is further considered as a therapeutic agent because of the presence of glycosides,

diosgenins, cytoestrol and stigmaestend. The major active constituents of *A. racemosus* are steroidal saponins (Shatavarins I-IV) that are present in the roots. Shatavarin IV has been reported to display significant activity as an inhibitor of core Golgi enzymes transferase in cell free assays and to exhibit immunomodulation activity against specific T-dependent antigens in immuno compromised animals (Kamat et al., 2000). Shatavarin IV is a glycoside of sarsasapogenin having 2 molecules of *Asparagus* rhamnose and 1 molecule of glucose. The presence of sarsasapogenin and shatavarin I-IV in roots, leaves, and fruits of *Asparagus* spp. make this herb an amazing therapeutic agent and it could be useful against reproductive toxicity induced by EDCs (Table 1). However, there is a need to conduct clinical trial and proper validation to justify the therapeutic candidature of *A. racemosus* against reproductive toxicity induced by EDCs.

In Ayurveda, it is described that long term use of *A. racemosus* is absolutely safe, as well as even during pregnancy and lactation. Experimentally, it is proved that systemic administration of higher doses of *A. racemosus* extracts does not produce any abnormality in behavior of mice and rat (Jetmalani et al., 1967). Further, LD50 of the *A. racemosus* has not been assessed because it did not produce mortality even up to oral dosage of 64 g/kg (Narendranath et al., 1986). Furthermore, Prabha et al. (2004) assessed the safety level of *A. racemosus* by studying acute and chronic toxicity (1g/kg) on pre and post natal developments in rats. Both the studies indicated no changes in general behavior, gait, food and water intake on the body weight. Further no change in liver and renal function test parameters are also reported. Further, root extract of *A. racemosus* is prescribed in Ayurveda to increase milk secretion during lactation (Nadkarni, 1954). Lactogenic effects of *A. racemosus* has been investigated in different mammalian species like guinea pigs (Meites, 1962), humans (Sholapurkar, 1986), goats (Vihan and Panwar, 1988), buffaloes (Bhutada, 1999), and a significant increase in milk yield has been noticed with increased growth of the mammary glands, alveolar tissues and acini (Sabins et al., 1968). However, one of the studies did not observe any change in prolactin level in females after supplementation of *A. racemosus*, suggesting that it has no lactogenic effect (Pandey et al., 2001). Further, reversal effects of *A. racemosus* extract has been observed in case of cisplatin induced intestinal hyper motility (Regh et al., 1989). In recent past, it has been shown that liposomes made from the extract of *A. racemosus* possess huge anti-inflammatory activity and can be utilized for transdermal drug delivery (Plangsombat et al., 2016). On the basis of available literature it seems that *A. racemosus* possess great potential to enhance milk secretion or repair the alveolar tissue and could be useful in case of milk secretion

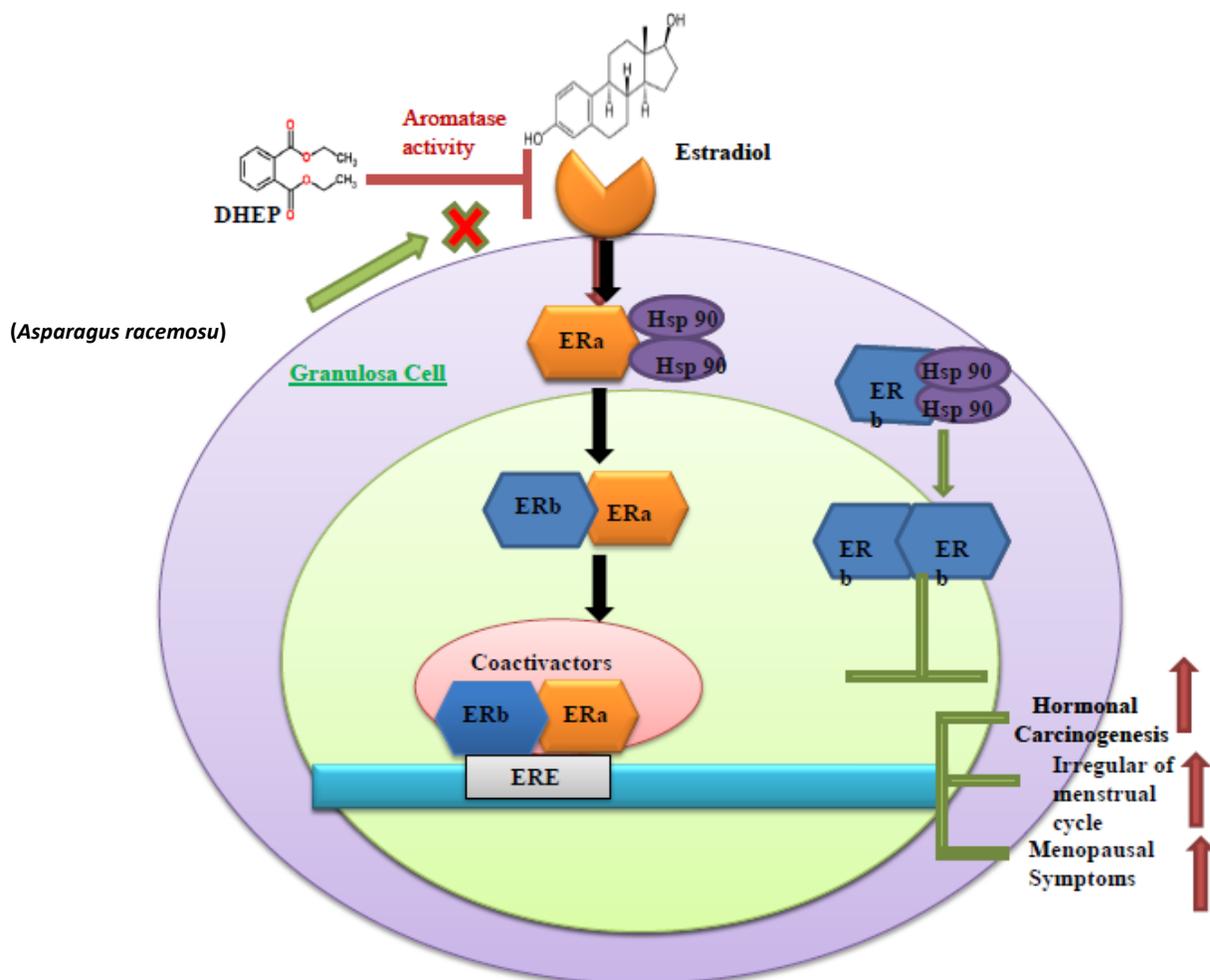


Figure 3. Effect of DEHP on synthesis of estrogen hormone (by interfering with estradiol receptor).

Table 1. Phytochemical constituents of Shatavari (*A. racemosus*) and their uses in different reproductive disorders.

Phytochemicals	Bioactive ingredients	Uses	References
Steroidal saponins	Shatvarin I–VI	Ammenorrhea, Dysmenorrhea, Hormonal imbalance	Swarup and Umadevi (1988), Chopra & Simon (2000) and Patricia et al. (2006)
Oligospirostanoside	Immunoside	Immunostimulant	Dahanukar and Thatt (1997) and Handa et al. 2003
Polycyclic alkaloid	Aspargamine A		Kukasawa et al. (1994)
Isoflavanoids	Isoflavones-8-methoxy-5, 6, 4-trihydroxy isoflavone-7-O-beta-D-glucopyranoside	Hormonal imbalance, Menstrual irregularities	Saxena and Chourasia (2001)
Flavanoids	Glycosides of quercitin, rutin and hyperoside	Ammenorrhea, Gonorrhoea	Sharma (1981) and Thomson (2002)
Cyclic hydrocarbon	Racemosol, dihydrophenantherene	Anobolic	Sekine and Fukasawa (1997)
Furan compound	Racemofuran	Ammenorrhea, dysmenorrhea,	Wiboonpun et al. (2004)
Sterols	Sitosterol, 4, 6-dihydroxy-2-O (-2-hydroxy isobutyl) benzaldehyde and undecanyl cetanoate	Thirst, Sunstroke	Singh and Tiwari (1991)

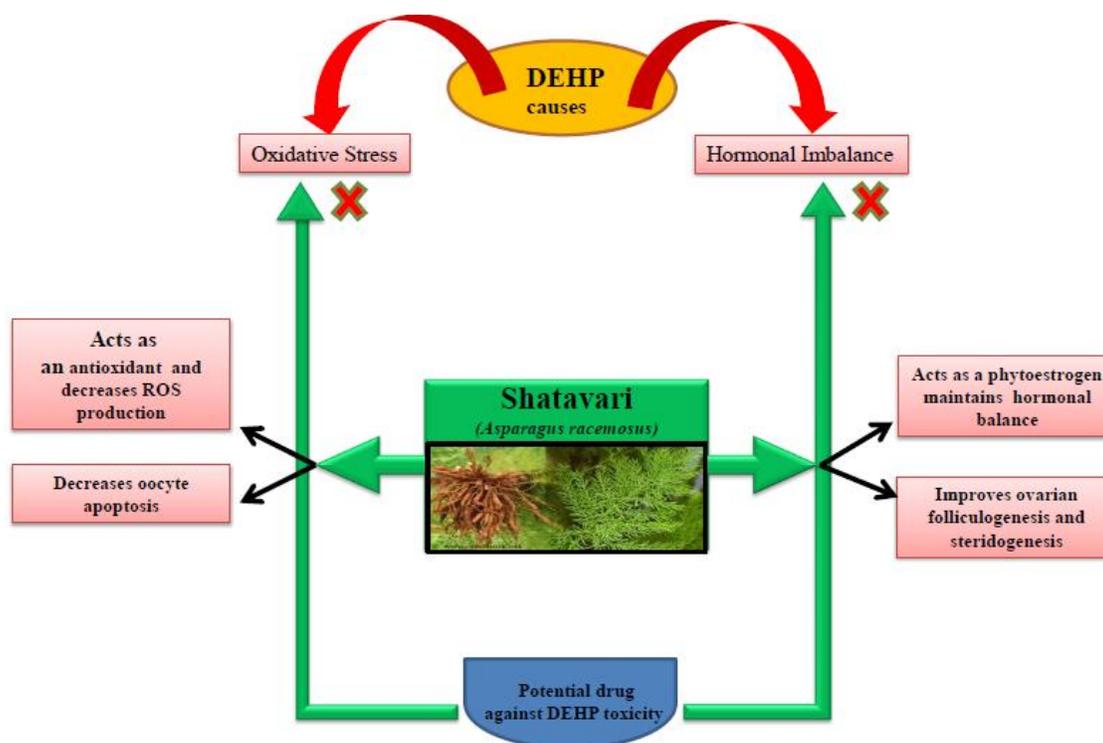


Figure 4. Possible approach to minimize DEHP induced reproductive toxicity using *Asparagus racemosus*.

during lactation period and as anti-inflammatory agent. *A. racemosus* has much pronounced beneficial effect on female reproductive system. However, the effect of *A. racemosus* on DEHP-induced toxicity has not been investigated so far. Indeed, *A. racemosus* (Shatavari) may be a potential herbal drug to treat against DEHP-induced reproductive toxicity.

CONCLUSION AND FUTURE PERSPECTIVES

DEHP is frequently used for packing food items and is a major constituent of the polythene. Higher level of DEHP in the circulation may cause several health related problems severely affecting the ovarian physiology causing a number of problems including premature ovarian failure, anovulation, infertility, and decreased steroidogenesis. Figure 4 summarizes the beneficial effects of *A. racemosus* on women's health. With the rising popularity of herbal medicine that is originated from natural sources, people believe that extract of *A. racemosus* can be useful for the treatment of UT infections, nausea associated with pregnancy, and lactation issues. However, the exact mechanism of action must be determined to avoid any potential toxic side effects or loss of activity of this herbal medicine during the treatment course. Furthermore, women must be

promoted to discuss about the use of herbal based medicine in their society, health care providers and with family members for managing all aspects of their health throughout their lives. This review may spread awareness about the harmful effects of plastic products and the use of cost-effective and cheap herbal drugs to overcome the DEHP-induced reproductive problems in comparison with the costly ART procedures which are unaffordable to the common population. Further, the use of *A. racemosus* will delineate a roadmap for using herbal based therapies to cure various incurable reproductive diseases.

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Abbreviations

ART, Assisted reproduction techniques; **EDCs**, endocrine

disruptors; **DEHP**, Di-(2-ethylhexyl) phthalate; **IVF**, *in vitro* fertilization; **ICSI**, intercytoplasmic sperm injection; **MEHP**, mono (2-ethylhexyl) phthalate; **ROS**, reactive oxygen species.

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