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ANTI- TOXICITY EFFECT OF *SARGASSUM WIGHTII* EXTRACTS AGAINST CADMIUM IN THE OVARY OF FEMALE ALBINO RATS

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Abstract:

Heavy metals are increasing ecological and global public health concern associated with environmental contamination by several industrial, agricultural, domestic and technological applications. Its toxicity related to factors like the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of individuals. Cadmium has detrimental effects on the reproductive system of human. It is an endocrine disruptor which interferes with hormonal action. Seaweed research has been carried by many research workers more than seven decades. Seaweeds are used in nutritional, pharmacological, clinical, biochemical, industrial uses and its application to human welfare. The objective of the present work was to evaluate the anti-toxicity response of seaweed *Sargassum wightii* against cadmium in the ovary of rat. An extract derived from *Sargassum wightii* was administered orally every day at a dose level of 200mg/kg of body weight to the rats exposed to 50ppm cadmium for 30 days. An administration of S*argassum wightii* extract prevented the histopathological inflictions and enhancing endocrine functioning.

Key Words: Heavy metal; Cadmium; Sea weed; Endocrine function

Introduction:

Heavy metals related with other poisonous chemicals, from natural or industrial sources, cause serious threats to human life (Rafati Rahimzadeh et al., 2017). Cadmium (Cd) is a highly toxic heavy metal and has spread generally in the world in recent years. Cadmium is discrete into the environment through various anthropogenic processes, such as mining, smelting, nickel and cadmium batteries, metal plating, pigments, plastic stabilizers, sewage sludge disposal, and the use of phosphate fertilizers (Nordberg et al., 2018). Long-term contacts to cadmium through air, water, soil, and food directs to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous, and respiratory systems (Ramberg and Nelson 2010). Cadmium exposed through the ingestion of food, drinking water or contaminated soil and dust and inhalation of tobacco smoke (ATSDR 2012). Cadmium has significant role in endocrine disruption capacity and deregulating all pituitary hormones (Jimenez-Ortega et al., 2012). Cd exposure is damage to the blood-testis barrier, decreasing germ cell adhesion leading to germ cell loss, reduced sperm count and sub-fertility or infertility in male rats (Gunnarsson et al., 2004).

Seaweed is widely used functional foods and medicinal herbs in Asian countries. The medicinal use of seaweeds goes back at least 5,000 years to ancient China (Liu et al., 2012). Seaweeds are used in the treatment of cancer, many kinds of crude extracts from various brown and red algae were tested for their property and showed antitumor activity against experimental tumour (Rodriguez et al., 2008). The protective effects of dietary algae against skin, intestinal, and mammary cancer supported by many epidemiological data in animal model studies (Namvar et al., 2012). An algal antioxidant-mediated mechanism (Tierney et al., 2010) improves the host's defense system by increasing natural killer cell activity (Myers et al., 2011) triggering of nonspecific immune system (Mohamed et al., 2012) inhibiting the cell growth in the G1 phase, inducing terminal differentiation inhibiting the complex process of angiogenesis (De Sousa et al., 2007) regulating the endogenous oestrogen biosynthesis and initiating apoptosis (Namvar et al., 2012). To the best of our facts, this is the first report on the toxic effects of Cd in the reproductive system and the detoxifying and curing property of Seaweed extract.

Materials and Methods:

Extract Preparation:

Sargassum wightii were collected from Rameshwaram Algal research center .The plant materials were allowed to sun shade dry and powdered. . A 200 g of Algal powder was soaked in 1L of distilled water for 48h at room temperature. The mixture was filtered by Whatman filter paper into a 500ml conical flask. Afterwards the extract was centrifuged at 4250 rpm for 5 minutes and filtered with Whatman number1 filter paper. The supernatant was collected as a 100% algal liquid extract. The concentration of the extract was made by dilution with distilled water into 2.25mg/kg body and administered to the animals.

Experimental Design:

Thirty days old female albino rats (*Rattus norvegicus*) weighing 70 ± 10 g used for the present investigation. Rats were maintained in a separate animal house and were fed standard rat pellet diet and drinking water by libitum. The animals with were separated into the following groups,

Group I: Control

Group II: 50 ppm (Treated with Cd)

Group III: Treated with Sea weed extract

The minimum (50 ppm) doses of cadmium were selected (Daliah Roopha and Padmalatha 2013) and the female animals (Group I) were treated with cadmium in the form of cadmium chloride through drinking water for 30 days. After 30 days the Cd treatment was stopped and the animals were treated and *Sargassum wightii* extract through drinking water for 30days. Trunk blood was collected and sera estranged out and used for hormone assays and the ovary was dissected out and fixed in fixative for Histopathological studies.

Analytical Methods

Approximately 10 mL of blood was collected from each animal group and 3 to 5 mL of blood was shifted to serum separation tubes and permitted to stand for 30 minutes. Samples were centrifuged (2500 rpm for 30 minutes), serum poured into siliconized polystyrene vials, and stored at -70c before analysis.

Enzyme Immunoassay for the quantitative determination of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Estradiol (E2) and Progesterone, in Human Serum or plasma (Hall 1988) by CLIA kit.

Histology Study:

Immediately after dissection, the ovary was removed surgically and washed with ice cold physiological saline. Zenker‟s fixative was used for the fixation of tissues. The tissues were fixed in the fixative for 12 hours and cleaned in the running tap water overnight and dehydrated in the ascending grades of isopropyl alcohol. The tissues were put in 1% celloidin dissolved in methyl benzoate. The celloidin in filtered tissues was left in toluene till they became translucent. After moving toluene, the tissues were entrenched in wax. The sections were cut at a thickness of 6µ in a rotary microtome. The sections were permitted to expand by gently warming the slide on a hot plate maintained around 50°C. When the sections became flat and expanded, the water was exhausted. The slides were left overnight on the hot plate maintained at constant temperature of 45°C. The sections were stained by haemotoxylin to get cellular as well as sub-cellular structural details Baker (1955).

Statistical Analysis:

All the data are presented as means standard error of the mean (SEM). Statistical significance was calculated using student's t' test to test the significance of individual variations. Where n_1 and n_2 are the number of observations in the two classes being compared (Ostle 1966). The value of probability was obtained from the degree of freedom by using standard table value, given by Fisher and Yates (1948).The level of significance was assessed at $P< 0.05$.

Results:

Body Weight and Ovary Weight:

Cd exposure significantly decreased the body weight and ovary weight in 30 days Cd-treated rats. However the body weight and ovary weight were significantly ($p <0.05$) increased by *Sargassum wightii* extract (Table 1).

Table 1: Effect of S*argassum wightii* extract on Body and Ovary weight of Cd treated Rats.

	Control	Cd (50ppm)	S*argassum wightii* extract
Body weight	87.5000±3.23	75.0000±2.04	95.0000±2.04
Ovary weight	0.5300±.01472	0.4700±.01732	0.5100±01080

Each value represents the mean and SEM of 15 female rats. Cd: Cadmium chloride

Changes in Hormone Level:

Pituitary hormones LH and FSH were decreased 50 ppm Cd treated rats. Steroids hormone Estradiol and Progesterone also decreased in Cd treated when compared to control. The present study was carried out to estimate the anti-toxicity effect of a seaweed extract. The seaweed extract significantly increased the LH, FSH, Estradiol and Progesterone hormone levels (Figure 1, 2 3, 4).

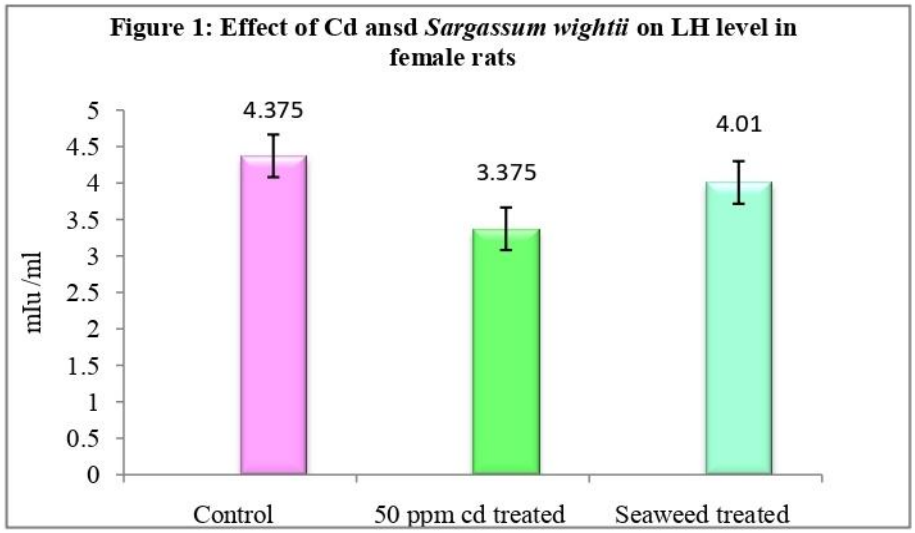

Figure 1: Effect of Cd and S*argassum wightii* extract on LH level in Female rats.

Each bar represents the mean, and the vertical line above denotes SEM (n=4) Statistical significance of difference among groups at p< 0.05; Control versus Cd Treated p < 0.05 (** significant); Cd Treated versus Seaweed Treated p < 0.05 (**significant); Control versus Seaweed Treated p < 0.05 (** significant).

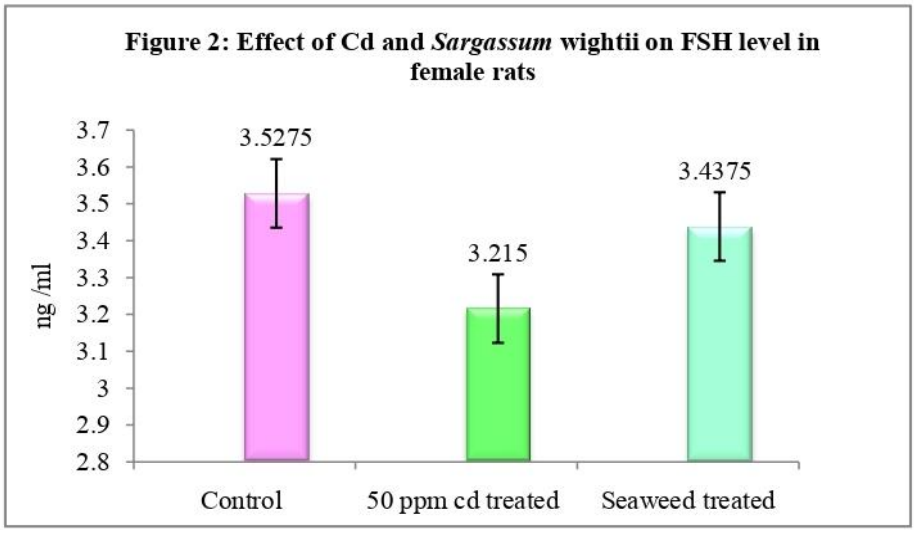

Figure 2: Effect of Cd and S*argassum wightii* extract on FSH level in Female rats.

Each bar represents the mean, and the vertical line above denotes SEM (n=4) Statistical significance of difference among groups at p< 0.05; Control versus Cd Treated p < 0.05 (** significant); Cd Treated versus Seaweed Treated p < 0.05 (**significant); Control versus Seaweed Treated p < 0.05 (** significant).

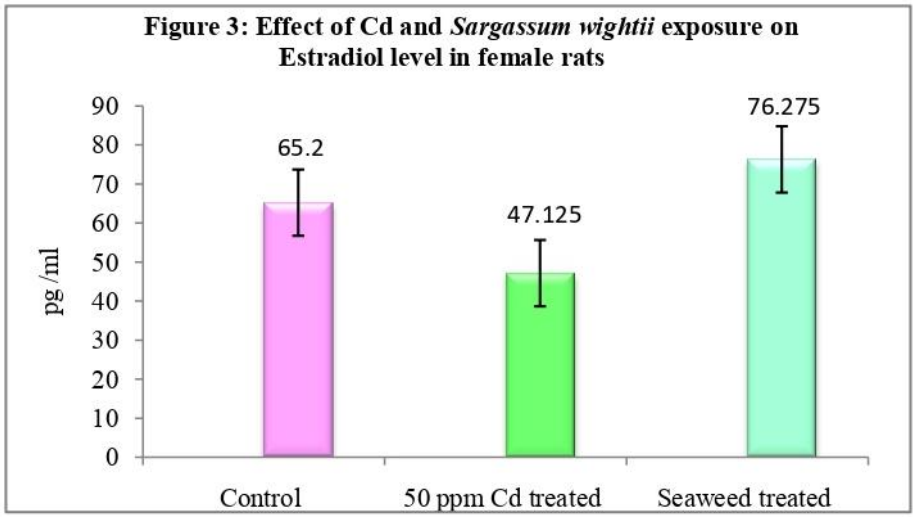

Figure 3: Effect of Cd and S*argassum wightii* extract on Estradiol level in Female rats

Each bar represents the mean, and the vertical line above denotes SEM (n=4) Statistical significance of difference among groups at p< 0.05; Control versus Cd Treated p < 0.05 (** significant); Cd Treated versus Seaweed Treated p < 0.05 (**significant); Control versus Seaweed Treated p < 0.05 (** significant).

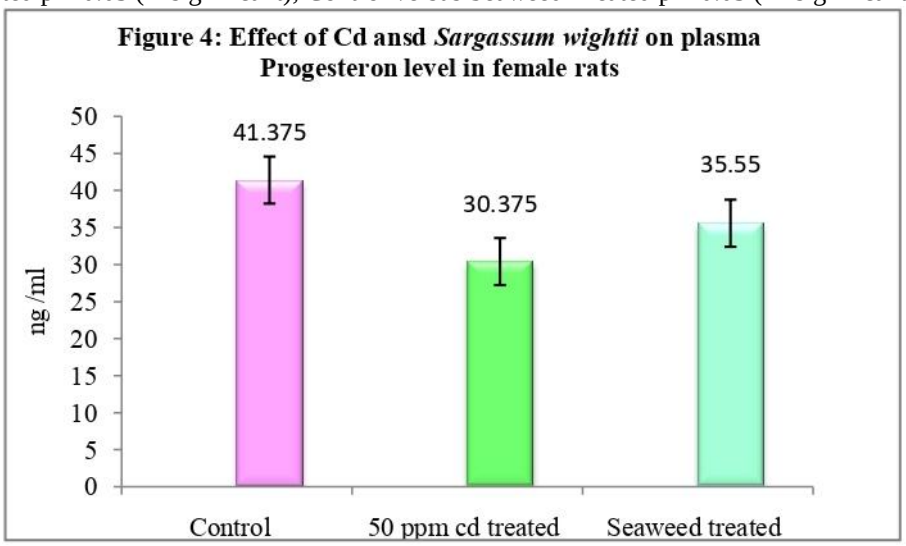

Figure 4: Effect of Cd and S*argassum wightii* extract on Progesterone level in Female rats

Each bar represents the mean, and the vertical line above denotes SEM (n=4) Statistical significance of difference among groups at p< 0.05; Control versus Cd Treated p < 0.05 (** significant); Cd Treated versus Seaweed Treated p < 0.05 (**significant); Control versus Seaweed Treated p < 0.05 (** significant).

Histopathological Study:

Figure 5 a, b showed an Ovary of Control Rats.
Figure 5 c, d showed an Ovary of Cd (50ppm) treated Rats.
Figure 5 d, e showed an Ovary of *Sargassum wightii* extract treated Rats.

A section through the ovary of the control rat showed different phases of oogenesis. The cortex of the ovary is filled with group of follicles in various stages of development. Proliferation of follicle cells around enlarging Oocyte is prominent. Corpus albicans (old scar of early corpus luteum) is seen (CA) (Figure 5a). Another view of the ovary of control rat showed normal oogenesis. The nucleus looks granular (N) and dark nucleolus (NL) is prominent (Figure 5b). A section through the ovary treated with 50ppm of Cd with a degeneration of the follicles. The degenerated follicles looked irregular with a dense mass of tissue (MT). The ovarian follicles fail to ovulate and became atretic because of the loss of hormonal activity and the development of apoptosis (Figure 5 c). Oocyte is irregular and hypertrophied with an abnormal nucleus and nucleolus (AB). It also showed necrosis (N) (Figure 5d). The section through the ovary of rat supplemented with seaweed showing regeneration in damaged ovarian architecture. Section through the ovary showing corpus luteum with the ingression of granulosa cells. Corpus albicans (CA) is also seen (Figure 5e). Granulosa cells (G) got proliferated and enlarged to become granulosa luteum cells and filled the follicular cavity. This indicates the recovery of the pituitary LH functions (Figure 5f).

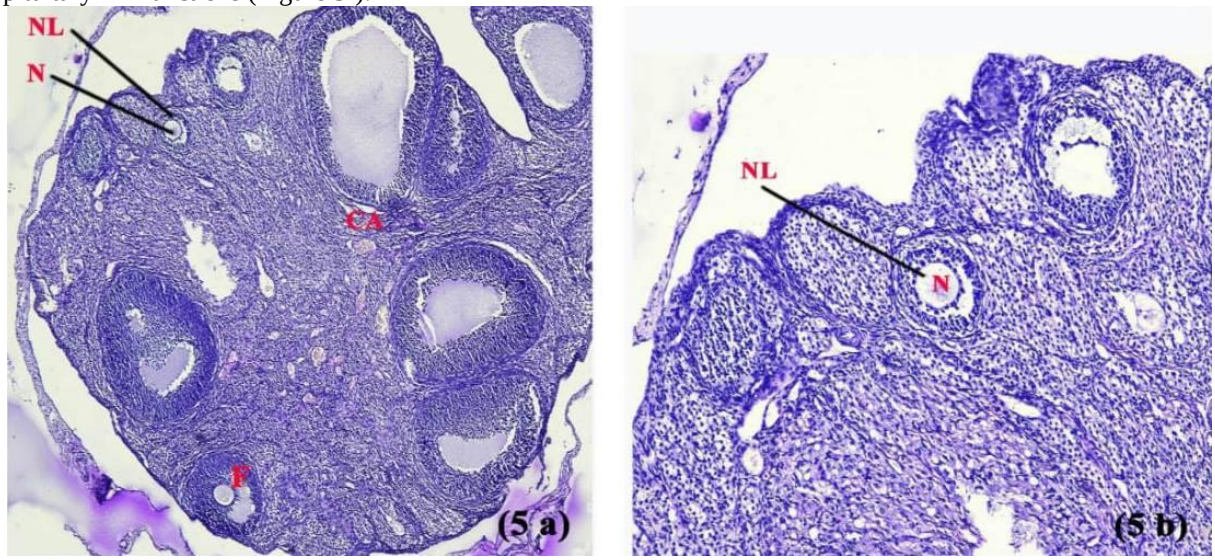

Figure 5 (a, b): Showed an Ovary of Control Rats.

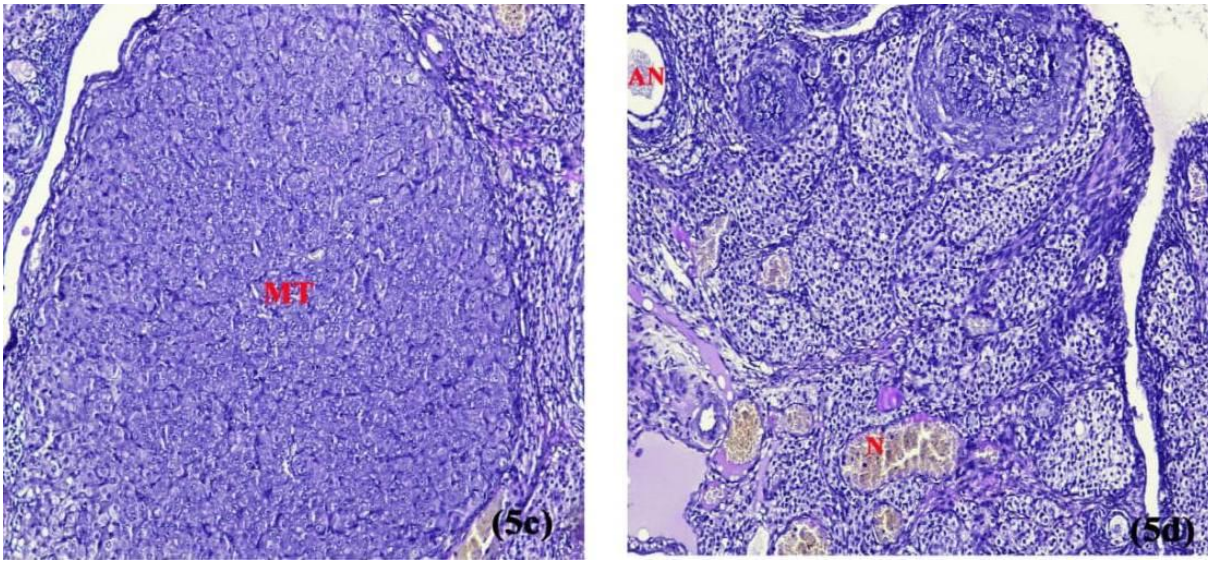

Figure 5 (c, d): Showed an Ovary of Cd (50ppm) treated Rats

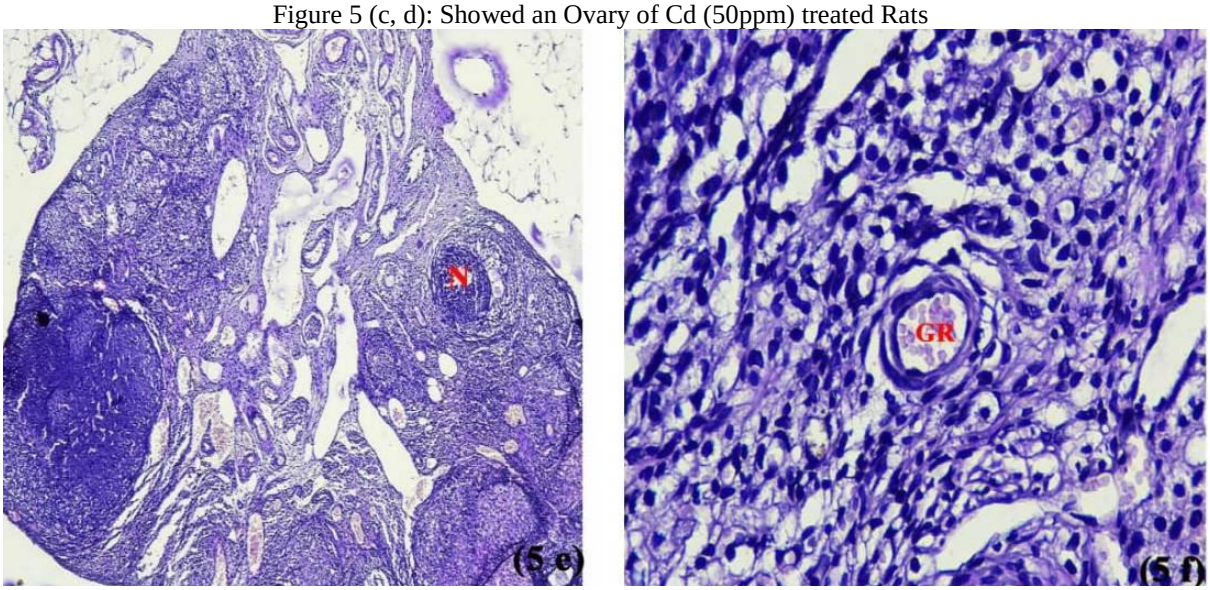

Figure 5 (e, f): Showed an Ovary of *Sargassum wightii* extract treated Rats

Discussion:

Cadmium, is one of the large amount of environmental and occupational metallic toxicants, has been proved to probably threaten human health (Zhou et al., 2016). The reproductive toxicity affected by heavy metal contamination has been a matter of increasing distress. Sorell and Graziano (1990) found that the reductions in both fetal and maternal weight in maternal consumption of drinking water containing Cd at 50 or 100 ppm. Lactational Cd exposure drastically reduced the body weight and ovary weight in PND 45 and PND 65 of Cd-treated rats (Daliah Roopha and Padmalatha 2013). In the rats treated with 50ppm cadmium the mean body weight and ovary weight was significantly decreased when compared with the control. The result indicates that the percentage decrease in body weight after 30 days was less. It may be due to some sort of metabolic alteration and physiological tolerance during chronic treatment with cadmium. The finding of present investigation was in accordance with previous workers.

The actual mechanisms of Cd effects on the female steroid production have still remained unelucidated. The intermission in the steroidogenic pathway by Cd toxic accomplishment explained in a few different ways. The E2 level decline and the Progesterone level increase may result from the destruction of steroidogenic enzymatic activities by Cd (Pillai et al., 2012). Cd affected progesterone production in JC-410 porcine granulose cells (Tierney et al., 2010). Banu et al., (2008) reported that the accumulation of chromium (Cr) in serum and ovary of rats exposed to hexavalent chromium originate the decline in the ovarian follicle number. The CrVI exposure-induced impairment of steroidogenic mechanisms have leaded to decrease the synthesis of steroid hormones. The effects of LH and FSH are mediate by their particular receptors LHR and FSHR. Earlier reports stated that the CrVI reduced LHR and FSHR levels both in vivo (Smida et al., 2008) and in vitro (Banu et al., 2008; Dailiah Roopha et al., 2012). The hormones concerned in reproductive system in the ovary, including LH, FSH, Estradiol, Testosterone and Progesterone which were connected with delayed puberty, Estrous cyclicity

were calculated in the control, cadmium-exposed rats without treatment and in the cadmium-exposed rats (Samuel et al., 2011b). In the present study confirmed Cd alters LH, FSH, Estradiol and Progesterone levels and make deleterious effects on female reproductive system

Several studies have pointed to the therapeutic properties of the plants which are used to traditional and recent medicine (Asadi-Samani et al., 2016). *Sargassum* has analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial properties as well as anti-tumor, fibrinolytic, immune-modulatory, anti-coagulant, hepatoprotective and has anti-viral activity (Chan et al., 2011). *Sargassum muticum*, might be an plentiful source of potential corresponding and substitute functional food for the prevention and treatment of cancer, and it is the most appropriate tool for further research to deal with selective antitumor active substances to human cancer especially breast cancer (Farideh Namvar et al., 2013). Based on the previous investigation, this is the first study clearly shows that, the extract of *Sargassum* is a restorative toxic functioning on the reproductive physiology of Wistar rats. The extract preparation rejuvenated the damaged reproductive functions and elevated the secretion of the reproductive hormones viz., LH, FSH, Estradiol and Progesterone.

The histological changes in ovarian follicles after administration of cadmium may also cause hormonal changes. It is well established that cadmium affects the plasma level of pituitary hormones (Maliheazaman Monsefi 2013). In previous study Massanyi et al., 2007 reported that Cd can cause vacuolation, congestion and necrosis in the ovary and uterus. Dailiah Roopha et al., (2008) stated that the Cd treatment had provoked apoptosis. In the rat enhanced with PR (Prasava Rasayanam), Cd could not impair the functioning of reproductive system and associated hormonal activities. An administration of PR along with Cd prohibited the histopathological inflictions in the ovary by rejuvenating the cellular function, withdrawing oxidative stress and enhancing endocrine function.

An administration of *Sargassum* prevented the histopathological alterations in the ovary by renewing the cellular function, diminishing oxidative stress and enhancing endocrine functioning. Beyond doubt the present study proved that *Sargassum wightii* plays a defensive role in preventing histological damages in the ovarian follicles and oviduct. The present study confirms the superiority of Seaweeds for gynecological problems in human beings.

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