Fucus vesiculosus Ameliorates Histological and Biochemical Changes in Thyroid Gland and Ovary of Irradiated Rats

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ABSTRACT

The present study was designed to determine the possible protective effects of Fucus vesiculosus, a brownish-green seaweed, against gamma-radiation-induced oxidative damage in thyroid gland and ovary tissues of female albino rats. Fucus vesiculosus (100 mg/kg body weight/day) was given to rats, via gavages for 30 consecutive days prior exposure to irradiation (4 Gy) and the last dose of Fucus vesiculosus was administered 60 minutes before irradiation. Thyroid gland and ovary were taken for histological study and blood samples for biochemical analysis on the 7th and 15th day post irradiation. In the irradiated group, the histological observations of thyroid gland sections showed distortion of the thyroid follicles together with apparent swelling of the follicular cells, vacuolated cytoplasm, and ill-defined cell boundaries of the follicular epithelium. Observations of ovary sections showed dissolution of the majority of primary follicles, immature follicles and increased fibroblasts and inflammatory cells surrounded the dissolved follicles. Biochemical analysis in the blood showed a significant decrease in serum triiodothyronine (T3), thyroxin (T4) follicular stimulating hormone (FSH) and luteinizing hormone (LH). Also, a significant decrease was observed in serum superoxide dismutase (SOD), and catalase (CAT) activities. Moreover, blood glutathione (GSH) content was decreased. Treatment with Fucus vesiculosus (100 mg/kg) was found to offer significant protection against gamma radiation-induced toxicity in the tissues, which was evident by improved status of most investigated parameters. These results suggest that Fucus vesiculosus could increase the antioxidant defense systems and may protect from adverse effects of whole body gamma radiation.

Key words: Fucus vesiculosus, gamma irradiation, thyroid gland, ovary, blood, hormones, antioxidants.

INTRODUCTION

There is a large body of evidence indicating that we are constantly exposed to ionizing radiation, which is both natural and artificial. Thus radiation-related disorders are one of the challenging current health problems. Radiation exposure produce highly reactive and dangerous molecular species called free radicals in cells and tissues, which have high energies and can break chemical bonds. Free radicals may be formed within cells as well as in the extra cellular medium and can interact with membrane lipids, nucleic acids, carbohydrates and proteins. These reactions disturb membrane structure and transport processes which leads to histological and biochemical disorders and ultimately resulted in acute and chronic disease (1, 2).

Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Superoxide dismutase (SOD) activity catalyzes the reduction of superoxide anion to hydrogen peroxide (H₂O₂), the majority of which is broken down to oxygen and water by catalase (CAT)
activity. In addition to CAT activity, glutathione peroxidase in presence of adequate amount of reduced glutathione (GSH) can also break down H₂O₂ (3). However, under abnormal conditions such as exposure to ionizing radiation the antioxidant defense system is not full operative. Therefore, the development of radioprotective agents is important for protecting patients from the side-effects of radiotherapy, as well as occupational workers in nuclear and radiation plants. Natural compounds have been evaluated as radioprotector and it seemed that they exert effects through antioxidants content and immunostimulant activities.

Therefore, seeking for radioprotectors derived, from traditional foods and medicinal plant sources, is worthy to receive great attention, it is estimated that about 80% of the world population relies on potential preparations as medicine to meet their health needs (4). Brown algae have attracted an emerging interest for their high content of the phenolic compounds phlorotannins (5). Phlorotannins have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (6). Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins which only have three to four rings (7). Interest in Fucus vesiculosus; a brownish-green seaweed or algae is growing rapidly after publications implicating it in the treatment of hypothyroidism and goitre (8),(9) as well as to help women with abnormal menstrual cycling patterns and menstrual-related disease histories (10).

Fucus vesiculosus, also known by the common name bladder wrack, found on the coasts of the North Sea, the western Baltic Sea, and the Atlantic and Pacific Oceans is rich in polysaccharides and polyphenolic antioxidants (phlorotannins) (11). Moreover, significant biological constituents of Fucus vesiculosus include mucilage, algin, mannitol, zeaxanthin, iodine, bromine, potassium, volatile oils and beta-carotene as well as many other minerals. Fucoidan, a complex sulfated polysaccharide, derived from Fucus vesiculosus, has been shown to possess a variety of significant biological effects (12) including antioxidant activity (13),(14).

Moreover, in an elegant study, on the antioxidant activity of Fucus vesiculosus in rats Zaragoza et al. (15) demonstrated that an extract containing 28.8% polyphenols lacks any relevant toxic effects and exhibited antioxidant activity in plasma and erythrocytes, after a week treatment. In view of these considerations the objective of this work is to elucidate the role of Fucus vesiculosus on radiation-induced structural changes in the thyroid gland and ovaries. In parallel the level of T3 and T4, markers of thyroid functions, and FSH and LH, markers of ovaries function were determined in the serum of irradiated female rats. The SOD and CAT activities and GSH content were estimated also to investigate the antioxidant status.

MATERIAL AND METHODS

Experimental animals:

All animal procedures were carried out in accordance with the Ethics Committee of the National Center conformed to the “Guide for the care and use of Laboratory Animals” published by the US National Institute of Health ( NIH publication No. 85-23, 1996). Female albino rats (120 -150g) obtained from the Egyptian Holding Company for Biological Products and Vaccines were used as experimental animals. The animals were housed in cages and maintained under standard conditions of ventilations, temperature and humidity. Food and water were available ad libitum.
Irradiation processing:

Whole body gamma-irradiation of rats was performed using a Canadian Gamma Cell-40(Cs^{137}) located at the National Center for Radiation and Technology, (Cs^{137}) located at the National Center for Radiation Research and Technology, Cairo, Egypt. Rats were whole body γ-irradiated with 4 Gy at a dose rate 1.5 Gy/min.

Treatment:

*Fucus vesiculosus* purchased from the International Company for Science and Medical Import was administered to female rats at a dose of 100 mg/kg body weight/day (Lee et al.) (16) by gavages for consecutive 30 days before irradiation. The last dose was given 60 minutes before irradiation.

Animals were categorized into 4 groups (n=12). Control group: rats not subjected to any treatment. *Fucus vesiculosus* group: rats received *Fucus vesiculosus* 100 mg/kg body weight/day for 30 consecutive days. Irradiation group: rat whole body gamma irradiated with 4Gy a single dose. *Fucus vesiculosus + Irradiation* group: rats received *Fucus vesiculosus* for 30 consecutive days before irradiation and the last dose given 60 minutes before irradiation.

The animals were sacrificed 7 & 15 days post-irradiation. Blood samples were collected and serum obtained by centrifugation at 3500 rpm for 15 minutes.

Histological studies:

The thyroid and ovaries were immediately excised for histological investigations. The tissues were fixed in buffered formol, processed routinely for paraffin embedding and sectioned at 6 μm. All sections were simultaneously stained with haematoxyline and eosin (H&E) and mounted with Canada balsam. Sections were examined by Zeiss light microscope (James)(17).

Biochemical analysis:

Reduced glutathione (GSH) content was determined spectrophotometrically according to the method of Beutler et al.(18). Superoxide dismutase (SOD) and catalase (CAT) activities were determined following the method of Minami and Yoshikawa (19) and Aebi (20), respectively. The levels of total T₃, T₄ were determined using Enzyme Immunoassay Kit produced by Diagnostic system Laboratory, Inc.USA (Ellis and Ekin) (21). The levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined using radiimmunoassay(RIA) methods and followed the procedure instruction of the corresponding Kits.

Statistical analysis:

Data were analyzed using one- way analysis of variance ANOVA. The results obtained were expressed as mean ± standard error of the mean. Differences were considered significant at t P<0.05.
RESULTS

Histological Observations:

Histological and histopathological observations of thyroid gland sections of control and Fucus vesiculosus treated rats showed that the gland is enveloped by a definite capsule of loose connective tissue underlined by an inner layer of fibro elastic tissue (Fig. 1a&b). From this layer, connective tissue septa extend into the gland dividing it into a large number of lobular structures. Each lobule consists of a number of follicles disposed in groups separated by partitions of connective tissue, containing capillary network. These follicles; the functional units of thyroid gland, exhibit marked variations in both shape and size; some of them have rounded shapes while others were either oval or elongated in shape. Each thyroid follicle is formed of an outer cuboidal epithelial layer (parafollicular cells) enclosing an internal lumen. The parafollicular cells are responsible for synthesis and secretion of the iodine-containing hormones (T₃ and T₄).

The internal of these follicles is occupied by a homogenous colloidal material, namely thyroglobulin, which exhibited a pink stain ability with haematoxylin and eosin (H&E), reflecting the presence of thyroid hormones when stored prior to secretion. In addition to the principle follicular cells or c-cells being present on the outer aspects of the wall of the follicles, parafollicular cells are detected either singly or in clumps. These cells are larger in size and usually have paler cytoplasm than follicular cells (Fig.1a&b). Whole body □-irradiation of rats with 4Gy induces obvious pathological changes. On the 7th day, distortion of the thyroid follicles together with apparent swelling of the follicular cells are observed, the majority of which contained vacuolated faintly stained cytoplasm, thus exhibiting prominent features of vacuolar degeneration.

The cells bounders of the follicular epithelium were markedly ill-defined. Abundant degenerated cell structures were observed in most of the inter-follicular areas. Moderate edema in some areas was also observed (Figure 1c). On the 15th day thyroid follicles exhibited various shapes and sizes and some of them were closely adherent together whereas others were rather separated from each other. The cytoplasmic portions of the follicular epithelial cells have almost vanished and their nuclei were noticeably pyknotic. The stroma lying in-between the majority of follicles showed abundant infiltrative lymphatic cells as well as pronounced edema (Fig.1d). Microscopic examination of the thyroid gland of rats treated with Fucus vesiculosus (100 mg/kg body weight/day) for 30 days before irradiation showed improvement of follicular cells and the follicles exhibit marked variation in both shape and size. The follicles are filled with colloid and regenerated parafollicular cells or c-cells in the outer wall of the follicles are detected (Fig.1e&f).

Ovary sections of control and Fucus vesiculosus treated rats showed normal developing ovarian follicles, primordial follicles with one layer of flattened follicular cells and normal corpus luteum with granulosa cells and theca cells (Fig.2a&b). The effect of irradiation on ovarian tissue 7 and 15 days post-irradiation appears in the form of dissolution and ill-defined shaped follicles, immature follicles and abundant infiltrative fibroblasts and inflammatory cells surrounded the dissolved follicles (Fig.2c&d). A significant protection was afforded when animals were supplied with Fucus vesiculosus before irradiation. The ovary sections 7 & 15 days post irradiation showed well defined germinal epithelium, normal tunica albuginea, regeneration of different stages of follicles formation such as primordial follicles, primary follicles and secondary follicles with granulosa cells and regeneration of theca cells (Fig.2e&f).
Fig 1: Photomicrographs of sections in the thyroid gland.

a) control, b) *Fucus vesiculosus*: showing normal thyroid tissue architecture with normal follicular epithelial cells (f), normal parafollicular cells (Pf) and normal colloid (c), c) Irradiated (7th day): showing ruptured and ill-defined follicular epithelial cells (f), disappearance of parafollicular cells (pf), ruptured colloid (c) and edema (o), d) Irradiated (15th day): showing disappearance of parafollicular cells (pf), necrosis of the majority of follicular cells (f), vacuolated colloid (c) and edema (o), e) and f) *Fucus vesiculosus* + irradiated (7th & 15th day): showing regenerated follicular epithelial cells (f), regenerated colloid (c) and regenerated parafollicular cells (pf) (H & E) (x 400).
a) control & b) *Fucus vesiculosus* showing ovarian follicles (p), with normal stratified follicular epithelial (granulosa) (g) and mature corpus luteum (m), zona pellucida (z) and developing theca (th), c) & d) Irradiated (7th day and 15th day): showing dissolution of ovarian follicles (1), increased fibroblasts (2), and inflammatory cells around dissolved follicles, e) & f) *Fucus vesiculosus* + Irradiated (7th & 15th day): showing well defined germinal epithelium (1), normal tunica albuginea (2), normal primordial follicles (3), normal primary follicles (4) well-defined corpus luteum with normal granulosa cells and theca cells (5). (H & E) (x 400).

Fig 2: Photomicrographs of sections in the ovary.
**Biochemical Results:**

Data in table (1) demonstrate that irradiation induced a significant decrease (p<0.05) in serum T3 and T4 compared to the control level after 7th and 15th day post – irradiation. These levels were considerably ameliorated when irradiation was combined with *Fucus vesiculosus* showing significant difference compared to the corresponding irradiated groups.

**Table 1 : Effect of *Fucus vesiculosus* on serum tri-iodothyronine (T3) and thyroxine (T4) concentration in irradiated rats (4Gy), 7 and 15 days post irradiation.**

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>T3 (ng/dl)</th>
<th>T4 (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.860±0.140</td>
<td>10.20±1.100</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> (7th day)</td>
<td>0.856±0.150</td>
<td>10.02±1.100</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> (15th day)</td>
<td>0.845±0.140</td>
<td>10.21±1.152</td>
</tr>
<tr>
<td>Irradiated (7th day)</td>
<td>0.490±0.092</td>
<td>4.30±0.092</td>
</tr>
<tr>
<td>Irradiated (15 days)</td>
<td>0.438±0.089</td>
<td>4.51±0.071</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> + Irradiated (7th day)</td>
<td>0.726±0.130</td>
<td>8.61±0.320</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> + irradiated (15th day)</td>
<td>0.710±0.120</td>
<td>8.10±0.310</td>
</tr>
</tbody>
</table>

*Data are presented as Mean±SE (n=12). Means with different superscripts are significantly different at P<0.05*

Results in table (2) showed that FSH and LH levels were within normal range in *Fucus vesiculosus* group and data showed a significant decrease (P< 0.05) after exposure of rats to γ-radiation at the two time intervals. This decrease was improved in treated irradiated rats with *Fucus vesiculosus*.

**Table 2: Effect of *Fucus vesiculosus* on serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in irradiated rats (4Gy), 7 and 15 days post irradiation.**

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>LH (IU/L)</th>
<th>FSH (IU /L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.94±0.13</td>
<td>0.84±0.07</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> (7th day)</td>
<td>0.91±0.017</td>
<td>0.87±0.06</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> (15th day)</td>
<td>0.97±0.065</td>
<td>0.84±0.05</td>
</tr>
<tr>
<td>irradiated (7th day)</td>
<td>0.57 ± 0.078</td>
<td>0.66±0.05</td>
</tr>
<tr>
<td>Irradiated (15th day)</td>
<td>0.53 ± 0.029</td>
<td>0.64 ± 0.7</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> + irradiated (7th day)</td>
<td>0.69 ± 0.04</td>
<td>0.74±0.06</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em> + irradiated (15th day)</td>
<td>0.71±0.032</td>
<td>0.75±0.07</td>
</tr>
</tbody>
</table>
Legends as table 1

Data in table (3) showed that blood antioxidants which include serum CAT and SOD activities and blood GSH content decreased significantly 7th and 15th days post irradiation, compared to the control values. These decreases in blood antioxidants level were improved when Fucus vesiculosus was administered to rats before irradiation.

Table 3: Effect of Fucus vesiculosus on serum superoxide dismutase (SOD) and catalase (CAT) activities and blood glutathione (GSH) content in irradiated rats (4Gy), 7 and 15 days post irradiation

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>SOD (µg/ml packed RBCs)</th>
<th>CAT (µmole/ml)</th>
<th>GSH (mg/ml packed RBCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>228.32± 6.16a</td>
<td>29.74± 2.71a</td>
<td>16.56± 1.19a</td>
</tr>
<tr>
<td><strong>Fucus vesiculosus (7th day)</strong></td>
<td>225.33± 3.13a</td>
<td>28.31± 1.08a</td>
<td>15.59± 1.82a</td>
</tr>
<tr>
<td><strong>Fucus vesiculosus (15th day)</strong></td>
<td>227.54±4.11a</td>
<td>29.77±2.19a</td>
<td>15.48±1.51a</td>
</tr>
<tr>
<td>Irradiated (7th day)</td>
<td>117.82±2.23b</td>
<td>36.17±1.58b</td>
<td>10.61±1.80b</td>
</tr>
<tr>
<td>irradiated (15th day)</td>
<td>119.13±3.13b</td>
<td>39.53±1.72b</td>
<td>10.15±1.36b</td>
</tr>
<tr>
<td><strong>Fucus vesiculosus + irradiated (7th day)</strong></td>
<td>192.21±6.06ab</td>
<td>30.22±2.46ab</td>
<td>13.63±1.55ab</td>
</tr>
<tr>
<td><strong>Fucus vesiculosus + Irradiated (15th day)</strong></td>
<td>185.16±7.07ab</td>
<td>32.21±2.30ab</td>
<td>13.17±1.25ab</td>
</tr>
</tbody>
</table>

Legends as table 1

DISCUSSION

Ionizing radiation is an important environmental risk factor known to produce various types of reactive oxygen species in biological systems provoking oxidative damage, organ dysfunction and metabolic disturbances (22). The choice of the thyroid gland has been based on the fact that secretory disturbance of one ductless gland often has reflex effect on the structure and function of other ductless gland (23). The thyroid gland has been considered radiosensitive (24). Ionizing radiation is a well known mutagen and a risk factor for thyroid cancer because acute exposure of thyroid cells to gamma-radiation results in several specific patterns of miRNA response which may affect other cell functions such as DNA repair (25).

In the present study, histological investigations in the thyroid gland of female rats 7 days post irradiation with 4Gy showed ruptured follicular epithelial cells, ruptured colloid, disappearance of parafollicular cells, and edema. Also, on the 15th day, most follicular cells were disappeared. The results corroborate the findings of El-Masry (26) who pointed out some distorted follicles with abnormal shapes and arrangement following 4Gy gamma radiation. This was accompanied by a noticeable increase in the interfollicular cells. Moreover, a decrease in the size of most thyroid follicles was observed and follicular lumen appeared empty of colloid. Pyknosis and karyolysis of some cell nuclei were also observed. (Moawad) (27) showed that rats under the influence of 2 Gy gamma-irradiation stress revealed distortion of the thyroid gland architecture, retraction of the colloidal material and appearance of vacuolar degeneration in some follicles, which were invaded by inflammatory cells. The results are also consistent with the findings of Ermakova (28) who observed that irradiation in low doses caused morphological disorders in the thyroid gland characterized by a
The thyroid gland, in response to stimulation by thyroid stimulating hormone (TSH) produces thyroxin (T4) and triiodothyronine (T3). Although T3 is secreted by thyroid gland, the majority is synthesized by the metabolism of T4 in peripheral tissues (28).

In the current study, the histopathological changes detected in the thyroid gland of whole body gamma irradiated female rats with 4 Gy were accompanied by a significant decrease in the level of serum T4 and T3 which corroborate that exposure to ionizing radiation induced hypothyroidism (29) (30). The results are consistent also with the findings of El-Masry (26) and Ibrahim & Kenaway (31) who reported a significant decrease in T3 level 7 days post irradiation of rats with 4 Gy and recorded a significant decrease in serum T3 and T4, 2 weeks post exposure of rats to 3 Gy gamma-irradiation. The decrease of T4 and T3 might be attributed to an altered rate of metabolism (32). In addition, the direct effect of radiation on thyroid cells decreases their ability to synthesize the hormone (33). The synthesis of thyroid hormones is greatly affected by the damage to the follicular DNA and the system through which hormones enter the blood (34).

It is well documented that the ovaries are radiosensitive organs (35), (36). In the present work histological investigations in the ovarian tissues of female rats 7 and 15 days post irradiation with 4Gy showed abnormal and dissolution of the majority of the follicles and absence of mature follicles. Furthermore, the theca interna and granulosa cells of the ovarian follicles and the corpus luteum had ruptured and ill-defined shape.

In the current study, the histopathological changes detected in the ovarian tissues of whole body gamma irradiated female rats with 4 Gy were accompanied by a significant decrease in the level of serum follicle stimulating hormone (FSH) and luteimizing hormone (LH). The results are consistent also with the findings of Cos et al. (37) who observed that exposure of rats to gamma radiation at 7 Gy (single dose) significantly diminished serum FSH and LH levels. They reported that radiation has particulary severe adverse effect on gonads in animals.

The present results indicated that whole body gamma irradiated female rats with 4Gy revealed alterations in the antioxidant status obvious by the significant decrease of SOD and CAT activities and blood GSH content. The depletion in GSH may be due to its reaction with free radicals resulting in the formation of thyl radicals that associate to produce oxidized glutathione (38). The decrease of SOD and CAT activities and GSH content might result from their increased utilization to neutralize the excess of free radicals generated in the body after exposure to ionizing radiations. One must consider, also, that the decrease of SOD and CAT activities is probably the consequence of proteins oxidation that contribute to the partial inactivation of enzymes (39).

According to the results obtained in the current study, the administration of Fucus vesiculosus to rats before irradiation provide substantial protection against the harmful effects of radiation on the thyroid gland and ovaries and biochemical parameters that might be attributed to its stimulatory effect on the antioxidant system. On the other hand, Fucus vesiculosus is rich in fucoidan, a polysaccharide reported to be a valuable radioprotector (40). Fucoidan has been shown to possess superoxide, and hydroxyl radicals scavenging activity, and to inhibit lipid peroxidation (41), (14), (42). In addition to fucoidan Fucus vesiculosus is rich in polyphenols (phlorotannins) both of them reported to possess powerful antioxidant and anti-inflammatory activities (Lee et al., (16); Diaz-Rubio et al. (13), Gupt and Abu-Ghamnam, (43). Moreover, Fucus vesiculosus is rich in iodine which might help in the metabolism of thyroid hormone (Bradley) (8).
CONCLUSION

It could be concluded that *Fucus vesiculosus* might protect female rats from radiation-induced damage in the thyroid gland, ovaries and in blood antioxidants and hormones.

REFERENCES