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Original article

PROTECTION AGAINST RADIATION-INDUCED TESTICULAR INJURY IN RABBITS BY *HABERLEA RHODOPENSIS* (A BALKAN RESSURECTION PLANT) EXTRACT

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Summary

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The aim of this study was to determine the deleterious effects of nonlethal gamma radiation on testes and their possible inhibition by *Haberlea rhodopensis* extract (HRE). For this goal, 20 male New Zealand rabbits were divided into 4 groups: group I – untreated, group II – treated with HRE extract, group III – exposed to 2 Gy gamma radiation, group IV – treated with HRE (0.24 g/kg b.w.) two hours before irradiation with 2 Gy. Exposure of animals to 2.0 Gy gamma radiation resulted into significant decrease in tubular diameter and the area of the seminiferous tubules on the 15th day after irradiation. HRE pretreatment resulted in significant increase in tubular diameter and the area of the seminiferous tubules as compared to the irradiated group. Radiation-induced histological lesions in testicular architecture were more severe in irradiated only than in HRE-treated irradiated rabbits. HRE administration before irradiation significantly attenuated radiation-induced histological damages in testes. These observations indicate the radioprotective potential of *Haberlea Rhodopensis* leaves extract against the effects of whole body gamma irradiation on rabbit testicular histostructure.

Key words: gamma radiation, *Haberlea rhodopensis,* rabbit, radioprotection, testicular histostructure

INTRODUCTION

Ionising irradiation has been extensively studied, and is well known to affect testicular function, histostructure and spermatogenesis (Sapp *et al.*, 1990; 1992). Depending on the administered dose, radiation of the testes can produce reversible or permanent sterility in males (Cordelli *et al.*, 2003).

Oxidative stress is an important factor in the etiology of male infertility. Spermatogenic cells are particularly susceptible to radiation-induced reactive oxygen species (ROS) since they are permanently under mitosis or meiosis. This makes testes a highly radiosensitive organ with a wide range of radiosensitive germ cells (Agarwal *et al.*, 2006).

There is an increasing interest among scientists to testing a variety of chemical and biological compounds in both *in vitro* and in *vivo* models as radioprotectors effective to preserve the testes from the noxious effect of ROS offense (Landauer *et al.*, 1997; Weiss, 1997; Links & Lewis, 1999; Kumar *et al.*, 2002). The synthetic radioprotectors such as the aminothiols are characterised by high protection factor, but they are usually more toxic (Rades *et al.*, 2004) than naturally occurring protectors (Weiss & Landauer, 2003). As a result, different plant extracts have been tested against radiation-induced effects and showed radioprotective activities due to their ability of scavenging the free radicals and adapting the antioxidant defense system by regulation of the antioxidant gene expression (Jagetia, 2007).

Haberlea rhodopensis Friv. (*Gesneriaceae* family) is a Balkan endemite belonging to the group of extremely desiccation-tolerant (ressurection) plants, which are capable of withstanding long periods of almost full desiccation and to recover quickly on water availability (Markovska *et al.*, 1994; Georgieva *et al,* 2008). Ethnobotanical data show that *Haberlea* leaves are used for the treatment of wounds and diseases of livestock in the Rhodope region of Bulgaria. One of the local plant names in the Rhodopes mountains is "shap" (foot and mouth disease) which is considered as confirmation that the local people have used the plant against animal diseases. Alcoholic extracts of *H. rhodopensis* (HRE) possess strong antioxidant and antibacterial activities (Ivanov *et al.*, 2009; Radev *et al.*, 2009). A variety of bioactive constituents, with antioxidant and free radical scavenging properties, have been isolated from *H. rhodopensis* (Markovska *et al.*, 1992; Berkov *et al.*, 2011; Ebrahimi *et al.*, 2011).

Our previous results, obtained after treatment of rabbits with different doses of HRE and gamma radiation *in vivo* and *in vitro*, showed antioxidant, antimutagenic and immunomodulatory properties of the total extract (Popov *et al.*, 2011; 2013; Georgieva *et al.*, 2013a,b; Dobreva *et al.*, 2015).

The present study was undertaken to evaluate the possible radioprotective potential of *H. rhodopensis* extract against radiation-induced testicular injury in New Zealand rabbits.

MATERIALS AND METHODS

Animals

The animal care and handling were performed according to the guidelines set by the World Health Organisation, Geneva, Switzerland and the Trakia University, Stara Zagora, Bulgaria. New Zealand rabbits, 5-months old, weighing 3.5–4.0 kg, were used in the present study. They were maintained under controlled conditions of temperature and light. The animals were provided with standard rabbit feed and water *ad libitum*. The Institutional Animal Ethical Committee approved the study.

Irradiation

A cobalt teletherapy unit (Rocus M, ^{60}Co) at the Inter-District Cancer Dispensary, Stara Zagora, Bulgaria, was used for irradiation. Each rabbit, in a wooden container, was exposed to 2.0 Gy gamma rays, at a dose rate of 89.18 cGy/min.

Preparation of the plant extract

Fresh leaves of *Haberlea rhodopensis* (Friv.) were collected from their natural habitat (the vicinity of Asenovgrad, Bulgaria) at the period of flowering in May-June. They were botanically identified in Department of Pharmacology and Pharmacognosy, Medical University, Sofia, Bulgaria by a botanist-phytotherapist.

Leaves were cut into small pieces and dried at room temperature for 1 month. After grinding the dry matter was macerated for 6 h in 70% ethyl alcohol and percolated for 48 h. The primary extract was concentrated by evaporation of ethanol in a vacuum environment to obtain a ratio of 5% ethanol and 95% water. The extract was filtered through filter paper to remove emulsified substances, chlorophyll and other particles. The extract was standardised in accordance with the method for determining the relative density (Bulgarian Pharmacopoeia Roll 2, p.19). The average amount of extracted substances was 0.120 g/cm^3.

Experimental design

To evaluate the adverse effects of gamma rays and the possible radioprotective effectiveness of HRE extract, male rabbits were randomly divided into following groups. Group I – sham-irradiated rabbits (n=5); Group II (negative control; n=5) – treated with HRE; Group III (irradiated control; n=5) – exposed to 2.0 Gy gamma radiation; Group IV (experimental; n=5) – treated with HRE (0.24 g/kg body weight) two hours before irradiation with 2 Gy.

The animals from Groups I and III were injected (i.m.) with double distilled water (DDW) and after two hours, those from Group III were irradiated. The animals from Groups II and IV were injected (IM) with HRE and 2 h later, the rabbits from IV group only were exposed to 2.0 Gy gamma radiation.

Light microscopic observation

Testes from all rabbits were surgically removed (after anaesthesia with Thiopental, 10 mg/kg b.w., as 5% solution i.v.) 15 days post irradiation. Samples were fixed in Bouin's fluid for 24 h, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin and sectioned at 5 µm thickness. Sections were stained with haematoxylin and eosin for general histology (Kiernan, 2008). The tubular diameter of 25 seminiferous tubules in the testes of each group was measured with the help of an ocular micrometer. Area of the seminiferous tubules was calculated as per Avtandilov (1990):

$$S=0.25\pi\times d^2$$

Statistical analysis

The results obtained in the present study were expressed as the mean ± SD. Student's *t*-test was applied to find significant differences between the groups.

RESULTS

Tubular diameter and area of the seminiferous tubules

After exposure to 2.0 Gy gamma rays, the diameter of seminiferous tubules in testes exhibited a significant reduction as compared to animals treated with either DDW or HRE alone. A significantly higher tubular diameter was recorded in the HRE pretreated group. The results obtained for the area of the seminiferous tubules in all groups correlated to the data about tubular diameters (Table 1).

Table 1. Tubular diameter and area of the seminiferous tubules in New Zealand rabbits. Group I: sham-irradiated, Group II: negative control, treated with *Haberlea rhodopensis* extract, Group III: irradiated control, Group IV: experimental, treated with *Haberlea rhodopensis* extract before exposure to gamma radiation. Data are presented as mean ± SEM (n=5)

Parameter	Group I	Group II	Group III	Group IV
Tubular diameter (μm)	253.95± 11.56	249.44± 10.12	200.32± 8.89 ^{A}	229.32± 12.57 ^{B}
Area of seminiferous tubules (μm^2)	50505.26± 2503.9	48920.29± 3970.8	31561.42± 2819.6 ^{A}	40878.66± 4237.8 ^{B}

^{A} P<0.001 versus groups I, II and IV; ^{B} P<0.01 versus groups I, II and III.

Testicular histology

Light microscopic examination of sections of the testes from Group I (treated with DDW) displayed testicular tissue with densely packed seminiferous tubules and little interstitium, occupied by clusters of interstitial endocrine (Leydig) cells and blood vessels of different size. The seminiferous tubules were lined with stratified epithelium, composed of all generations of spermatogenic cells and tubular lumina containing spermatozoa (Fig. 1). In the seminiferous tubules of testes from the group treated with HRE the histological picture was almost the same (Fig. 2).

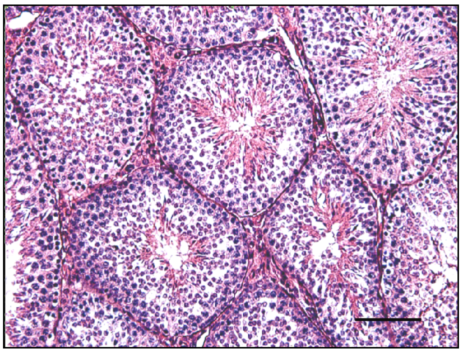

Fig. 1. Testis of rabbit treated with double-distilled water only. Normal histostructure. H/E; bar=100 μm.

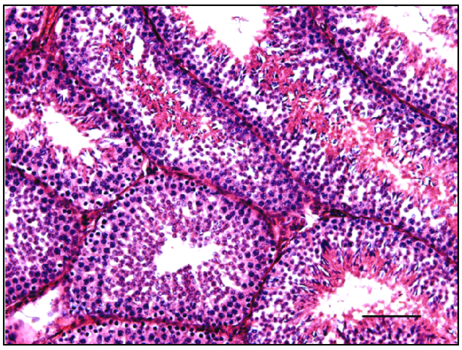

Fig. 2. Testis of a rabbit treated with HRE alone. Mild impairment of the histostructure. H/E; bar=100 μm.

Testicular sections of irradiated only rabbits showed pathological lesions in the tubular architecture when compared with rabbits treated with either DDW or HRE alone. Distorted histostructure of seminiferous tubules was represented by shrunken tubules, exfoliation and intertubular oedema (Fig. 3). Radiological symptoms in this group were demonstrated also with decreased populations of germ cells in some tubules (Fig. 4).

The HRE pretreatment was characterised by intact germinal epithelium, mild cytoplasmic vacuolisation with absence of exfoliation and intertubular oedema as well as increased germ cells number, and

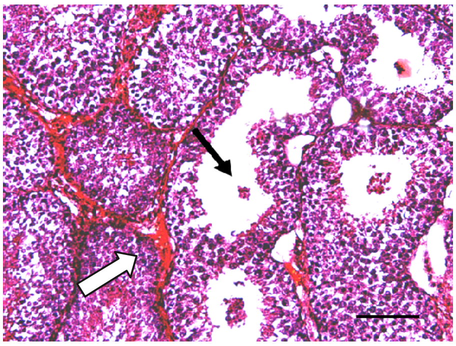

Fig. 3. Testis of an irradiated control rabbit with intertubular oedema (white arrow) and exfoliation of germ cells (black arrow). H/E; bar=100 μm.

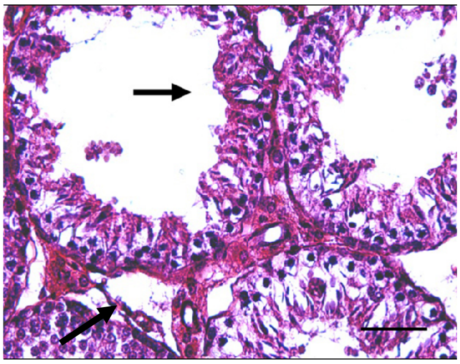

Fig. 4. Testis of an irradiated control rabbit showing reduction of seminiferous epithelium (arrows), H/E; bar=50 μm.

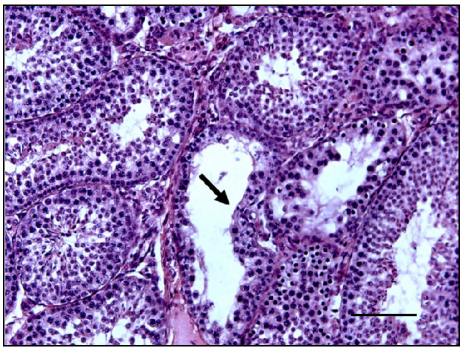

Fig. 5. Testis of a HRE pretreated and irradiated rabbit. Almost normal testicular architecture with lower number of mature spermatozoa in the lumen of some tubules (arrow), H/E; bar=100 μm.

an almost normal testicular architecture (Fig. 5).

DISCUSSION

The major purpose of the present investigation was to assess the possible radioprotective ability of *H. rhodopensis* extract against radiation-induced damages on the male reproductive system.

Radiation is a potent toxicant and whole-body exposure can change the general physiology of animal which might have an effect on the normal histology of testes. Radiation can also damage steroidogenic and spermatogenic activity through the generation of oxidative stress, suppressed antioxidant mechanisms and altered normal testicular architecture (Peltola *et al.*, 1992). In the present experiment, histological examination of seminiferous tubules of irradiated rabbits showed marked pathological alterations: shrinkage of tubules, distorted cellular arrangement, exfoliation and intertubular oedema. These results are in agreement with the report of Pareek *et al.* (2005), which documented the degenerative effects of gamma rays on spermatogenesis in lethally irradiated mice. Similar types of testicular injuries have also been reported by Eissa & Moustafa (2007), Koruji *et al.* (2008), Grewenig *et al.* (2015) in irradiated mice and by Akdere *et al.* (2015) in rats.

Microscopic analysis of the testes in both irradiated control and HRE-treated animals revealed that tubular diameter and area of seminiferous tubules decreased which might be caused by the spermatogenic cell loss and tubular disorganisation. In HRE-treated irradiated animals, pathological lesions showed a similar model of alteration as in the irradiated control group, but they were less prominent. HRE pretreatment resulted in an increased number of spermatogenic cells, and almost a normal testicular architecture, but some pathological lesions like mild cytoplasmic vacuolation and lower number of mature spermatozoa still persisted in the lumen of some tubules.

A similar observation was also reported while using *Panax ginseng* (Pande *et al.*, 1998), *Podophyllum hexandrum* (Samanta *et al.*, 2004), *Mentha piperita* (Samarth & Samarth, 2009), L-carnitine (Ramadan *et al.* 2013), *Echinacea purpurea* (Rezk *et al.* 2013) and *Prunus domestica* (Sharma *et al.* 2015) as radioprotectors against testicular injuries after irradiation.

The exact mechanism of the protective action of HRE is unknown. However, scavenging of free radicals and increased concentration of endogenous antioxidants may be important elements of protection provided by HRE against radiation-induced damage of the testicular tissue. Earlier, Berkov *et al.* (2011) reported the free-radical scavenging ability of methanolic extract of *H. rhodopensis*. The protection of HRE against radiation-induced reproductive disorders may be due to the presence of several bioactive components like phenolics acids, flavonoid aglycones and glycosides etc. (Ebrahimi *et al.*, 2011; Mihaylova *et al.*, 2011). According to Weiss & Landauer (2003) and Hou *et al.* (2004) polyphenols act as radical scavengers, which is due to their antioxidant properties. Radioprotective effectiveness of plant extracts may be due to the synergistic action of many constituents rather than to a single factor (Piya *et al.* 2011).

Correspondingly, a number of plant extracts have also been reported to react with free radicals and modulate free radical-mediated reactions mainly through their polyphenolic and flavonoid composition (Prabhakar *et al.* 2007; Li *et al.* 2008). Other studies are necessary to explain the mechanism of such plants against oxidative stress damage to make their effectiveness better.

In conclusion, based on the reported favourable results, it can be suggested that the extract of *H. rhodopensis* has the potential to diminish the testicular injuries induced by non-lethal doses of gamma radiation.

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