Volume 24, No 2 | International Journal of Radiation Research, April 2026

Histomorphology of liver tissue and electrical impedance changes due to rats exposed to Ionizing radiation above a specific dose

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► ***Original article***

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Received: September 2025
Final revised: September 2025
Accepted: October 2025

Int. J. Radiat. Res., April 2026; 24(2): 521-526

DOI: 10.61186/ijrr.24.2.32

Keywords: *Electric impedance, radiation effects, liver, microscopy electron transmission.*

ABSTRACT

Background: This study investigated the relationship between histomorphological changes and electrical resistance in liver tissue following radiation exposure in a rat model. ***Materials and Methods:*** Five-week-old Sprague–Dawley rats were exposed to ionizing radiation at doses of 1, 5, 10, and 20 Gy. Impedance changes were measured using alternating current (AC) frequencies ranging from 1 kHz to 1 MHz. Gross physical signs were monitored four days post-irradiation. Transmission electron microscopy (TEM) was employed to assess histological alterations in liver tissue. ***Results:*** As the radiation dose increased, notable gross changes in general signs were observed. Impedance characteristics varied according to dose, reflecting underlying histological alterations. Ultrastructural examination revealed damage to intracellular organelles, likely resulting from disrupted mitotic activity in irradiated liver tissue. ***Conclusion:*** Radiation exposure induces dose-dependent histomorphological changes and corresponding alterations in electrical impedance in rat liver tissue. These findings provide insights into the relationship between tissue structure and bioelectrical properties under radiation stress.

INTRODUCTION

Living organisms, including rats, have individualized electrical properties owing to various differences, such as the arrangement of cells and shape of tissues. Biological changes in the tissues and changes in intrinsic electrical properties occur when ionizing radiation is applied to rat bodies [1-3]. In rats, organ cells that are highly radiosensitive, such as liver tissue, are more seriously affected by exposure to ionizing radiation than cells that are resistant to radiation. When rats are irradiated, initial damage is caused by the ionization of intracellular atoms and molecules. This interaction occurs in both living and dead cells; however, atoms and molecules ionized in living cells exhibit more chemical responses [4]. When rats are exposed to ionizing radiation, the incidence of vascular damage, hypoxia, and cell necrosis increases with radiation dose.

Liver tissue undergoes various histomorphological alterations when exposed to high doses of ionizing radiation. According to previous studies, exposure to doses above 2-8 Gy has been associated with ballooning degeneration of hepatocytes, nuclear chromatin condensation (pyknosis), diffuse necrosis, and vascular dilation and fibrosis [5]. In particular, mitochondrial damage and endoplasmic reticulum swelling within hepatocytes disrupt intracellular energy metabolism, leading to functional impairment. The biological effects of radiation are mediated through multiple pathways, including apoptosis, increased oxidative stress, and the release of pro-inflammatory cytokines, all of which are clearly reflected in histopathological findings.

When examining how ionizing radiation interacts with living organisms, it is essential to account for multiple parameters, including impedance. The overall electrical resistance of biological tissue arises from extracellular and intracellular resistances together with the capacitance of the cell membrane. Irradiation-induced injury alters these bioelectrical

[DOI: 10.61186/ijrr.24.2.32]

properties. More specifically, exposure to ionizing radiation within living systems provokes both physical and chemical damage, which subsequently triggers diverse intracellular biochemical events. These include DNA injury and repair processes, cell death, arrest of the cell division cycle, modifications in signaling pathways, and responses related to oxidative stress [4].

Ionizing radiation is an oxidative accelerator that produces free radicals, thereby increasing oxidative stress in tissues. Free radicals are short-lived but biologically important. Physical and chemical changes in the body occur within microseconds of exposure, and *in-vivo* impedance varies as the physical properties of tissues are influenced by both the radiation dose and the specific organ type [6].

Few studies have investigated the relationship between electrical signals and biological alterations induced by whole-body exposure to ionizing radiation is of particular importance. Most studies have focused on local irradiation at doses of 15 Gy or more in specific organs [7]. Research on whole-body irradiation in rats has advanced only modestly, with impedance investigations being especially limited. Because systems of living organisms are extremely complex, studying the correlation between electrical signals and histological changes induced by radiation exposure remains challenging. It is difficult to capture all the damage to various organ types. In other words, the effects of radiation on living organisms differ depending on the organism's type, tissue, or cell, even if the same radiation is applied under the same conditions.

By contrast, changes in the intrinsic electrical properties of rats exposed to radiation provide valuable information about the destruction of radiation-sensitive liver tissue. Owing to histological changes in rats exposed to radiation above the critical dose, changes in electrical properties, such as impedance, can be clearly identified. We aimed to examine membrane destruction in liver tissue by comparing various conditions before and after radiation.

Previous our studies [8, 9] investigated the critical radiation threshold in porcine tenderloin tissue and rat smooth muscle. We quantitatively analyzed the correlation between histological damage in their tissue and electrical impedance. This study presents a novel investigation linking histomorphological alterations in liver tissue with sharp changes in electrical impedance following exposure to ionizing radiation above a critical threshold dose, offering a unique quantitative approach to assess early tissue damage that has not been systematically explored in prior radiation biology research. This phenomenon is attributed to a combination of physical factors, including increased ionic mobility due to cell membrane disruption and altered water distribution within hepatocytes. Notably, the abrupt change in impedance values beyond a critical radiation threshold suggests its potential utility in quantitative modeling for predicting radiation-induced tissue damage. Additionally, the extent of liver destruction could be inferred from the morphological appearance of rats exposed to radiation above a critical dose.

MATERIALS AND METHODS

Animal groups and extraction of rat liver tissue

All animal procedures were reviewed and authorized by the Institutional Animal Care and Use Committee of Pusan National University (PNU-2017-1470) and were carried out in accordance with the university's guidelines for scientific research. Male Sprague-Dawley rats (n = 15; 5 weeks old; Crj:CD, Orient Bio Inc., Korea) weighing between 144 and 157 g were used for the study. This outbred strain is widely utilized in biomedical research. The animals were randomly assigned to five groups, with three rats housed together per cage. Before the start of the experiment, they were allowed to acclimate for 7 days in a controlled environment (23 ± 2 °C, 55% relative humidity, 12 h light/12 h dark cycle). Standard chow and water were provided ad libitum. For both irradiation and impedance measurements, anesthesia was achieved with isoflurane delivered via inhalation (5% for induction, 2% for maintenance) in oxygen. The rats were placed in a closed space, subjected to carbon dioxide suffocation, with their abdomens facing up to fix their arms and legs with pins. The leather on the lower abdomen was held with tweezers and stomach was cut so that the intestines were not injured. Liver tissue was extracted after cutting the diaphragm and ribs.

Irradiation and electrical impedance measurement

The rats were irradiated with radiation doses of 1, 5, 10, and 20 Gy using a linear accelerator (Versa HD, Elekta AB, Stockholm, Sweden) with 6 MV X-ray. The entire body of each rat was irradiated using a collimation field of 100 × 100 cm^2 and an SAD of 100 cm. The impedance of each group was measured using an impedance/gain-phase analyzer (4194A, Agilent, USA) before and after irradiation [8]. An AC frequency ranging from 1 kHz to 1 MHz was applied using an impedance-measuring instrument. The measurement system and data processing were automatically controlled by monitoring changes in absolute impedance values in conjunction with a real-time interface developed in LabVIEW (National Instruments, Austin, TX, USA).

Sample of TEM

The rat liver tissue (1 × 1 × 1 cm^3) was collected and prefixed with 2.5% glutaraldehyde (4°C, phosphate buffer, pH 7.2) and 1% osmium tetroxide (4°C, phosphate buffer, pH 7.2). After the fixed tissues were washed with the same buffer, they were

[DOI: 10.61186/ijrr.24.2.32]

dehydrated stepwise with alcohol at low-to-high concentrations and embedded in an Epon 812 mixture. After embedding, the tissues were sectioned to a 1-μm thickness, stained with 1% toluidine blue; a specific area was determined, and the rest of the tissue was trimmed; lead citrate was applied; and observations were made using a TEM (JEM 1200EX-II, JEOL).

RESULTS

Gross observation of general sign 4 days after irradiation

Irradiation of the whole body damages the hematopoietic organs, gastrointestinal tract, and vascular system, which are highly sensitive to radiation [10]. Previous work has shown that the $LD_{50/30}$ for whole-body irradiation in Albino Wistar rats is approximately 6.6 Gy when treated with a linear accelerator, as reported by Challapalli *et al.* [11]. In contrast, another study found a slightly higher $LD_{50/30}$ value of 7.5 Gy in rats [12].

The results of the pilot study confirmed that Sprague–Dawley rats died on the fifth day in the group irradiated with more than 10 Gy, whereas those in the group irradiated with 5 Gy died after 10 d. Therefore, the stable period for confirming the acute-phase effect and whole-body electrical resistance change simultaneously in the group below 5 Gy and group above 10 Gy was 4 d.

Table 1 shows the general signs observed on the fourth day after whole-body irradiation with ionizing radiation. Compared with the normal group, the 1 Gy group showed no external changes; however, in the 5, 10, and 20 Gy groups, hair loss, diarrhea, fatigue, reduced food intake, and marks on both forelimbs from electrode clip pressure were observed.

Table 1. Gross observation of general sign 4 days after irradiation.

	n	Control	1Gy	5Gy	10Gy	20Gy
Alopecia	3	-	-	+	++	++
Diarrhea	3	-	-	+	++	+
Fatigue	3	-	-	+	+	++
Food intake	3	-	-	+	++	++
Wound Healing Ability	3	-	-	+	++	++

General sign and behavior were assessed in a similar manner using the following classification. -, none; +, moderate; ++, severe. n = number of animals.

Daily gross examination of each group (table 1) revealed signs of acute radiation syndrome as the radiation dose increased when ionizing radiation was applied to the entire body. Compared with the normal group, there was no significant difference in appearance between the 1 Gy and 5 Gy groups, and no clear signs were found except for diarrhea until 4 d after irradiation. Hair loss was observed in the 10 and 20 Gy groups, accompanied by little activity. In addition, it was possible to confirm that food intake did not recover and diarrhea persisted. As the radiation dose increased, the damage became more severe, recovery became more difficult, and activity was reduced [13]. The low-dose (<5 Gy) and normal groups showed no visible signs of damaged wounds. However, in the high-dose group of 10 Gy or more, the forelimbs were damaged by contact with the electrode clip, and wounds around the mouth caused by mastication were observed. Wounds caused by these physical stimuli could not be identified in groups receiving 5 Gy or less. We confirmed that body weight on the fourth day after irradiation increased in the 1 Gy group. However, in the 5, 10, and 20 Gy groups, it was observed that as the dose increased, weight loss increased, and the body appeared smaller.

Table 2. Percentage differences in body weight (g) before and 4days after irradiation.

	n	Before irradiation [a)]	4days after irradiation [b)]	%Differences
Control	3	169.2	214.0	26.5
1Gy	3	174.1	202.0	16.0
5Gy	3	164.9	150.8	-8.6
10Gy	3	166.5	133.9	-19.6
20Gy	3	164.4	123.9	-24.6

Table 1 shows the percentage difference in body weight (g) before and 4 d after irradiation. After exposure to 5 Gy of radiation, a significant decrease in body weight was observed. These results suggest that the higher the dose, the more severe the gastrointestinal syndrome, indicating that normal food intake and digestive ability could not be achieved, resulting in decreased body weight and physique. Therefore, it can be considered that the decrease in body weight and physique of rats due to acute radiation syndrome causes structural changes in the body and acts as an important factor in the reduction of electrical impedance.

TEM

In this study, we observed the destruction of liver tissue, which is a vital organ, with increasing radiation exposure using TEM. Microstructural changes in the liver tissue were histologically observed using TEM. Electron microscopic examination of the liver tissues from rats exposed to 1 Gy of radiation did not reveal any histological changes.

Figure 1 (a–c) shows the TEM micrograph of the rat liver in the control group (1 Gy), and figure 1 (d-f) shows the TEM images of the rat liver at 5 Gy. After 24 h, we systematically observed the TEM changes in the liver. Compared with the control group (0 Gy), TEM performed after 1 Gy irradiation revealed no noticeable histological alterations, as shown in figure 1 (a–c). We observed similar aspects in the nuclear and cell membranes at 5 Gy, but the number of mitochondria seemed to be somewhat higher than in the control group (figure 1-a). The cytoplasm was similar to that of the control group, but the endoplasmic reticulum was divided and somewhat

[DOI: 10.61186/ijrr.24.2.32]

scattered (figure 1-e). The tight junctions maintained the junctional complex without any unusual changes compared with those in the control group (figure 1-f).

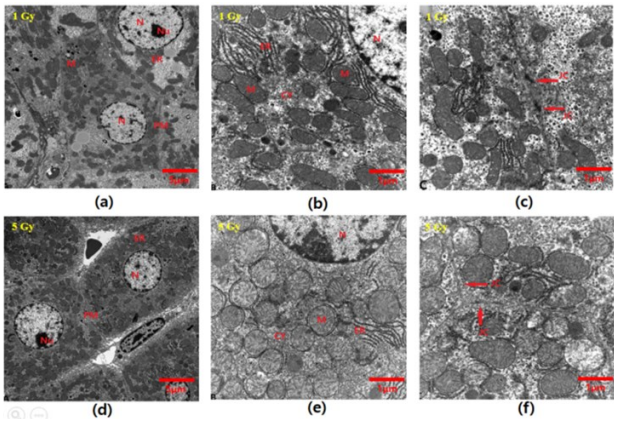

Figure 1. Electron micrograph of a control rat liver showing hepatocytes: **(a)** hepatocytes with smooth-contoured nuclei (N) with some clumped heterochromatin, one nucleolus (Nu), developed mitochondria (M), endoplasmic reticulum (ER), and plasma membranes (PM) (× 5000). **(b)** Normal structure illustrated in the homogeneous cytoplasm (CY) (× 20,000). **(c)** A coherent monolayer displaying an intact junctional complex containing tight junctions (arrows) **(d)** Hepatocytes with smooth contoured nuclei (N), nucleoli (Nu), mitochondria (M), endoplasmic reticulum (ER), and plasma membranes (PM) (5000 ×). **(e)** Normal structure illustrated in the homogeneous cytoplasm (CY) and fragmented endoplasmic reticulum (ER) with ribosome (× 20,000). **(f)** No significant alterations in the junction complex (arrows) (× 20,000).

Compared with the control group, TEM showed that the nucleus and cell membranes had similar patterns; however, the nuclear structure was not uniform, mitochondria were somewhat destroyed, and size was reduced at 10 Gy (figure 2-a). The density of the cytoplasm was generally uniform, but the vesicles were more scattered than those at 5 Gy (figure 1-e and f). At radiation doses between 5 and 10 Gy, hepatocyte replication and the number of mitochondria decreased, but similar fragmented structures increased. This plays a role in destroying tissue-level integrity in hepatocytes.

TEM can be used to observe the distortion of the circular quality of whole cells, where the nucleometric parameter is nuclear concentration. The mitochondria in these cells were observed to be smaller and denser. The overall density was observed at a dose of 20 Gy (figure 2-d). The density of the cytoplasm was reduced, and the mitochondria showed short and scattered shapes (figure 2-e). The tight junction was completely destroyed, and the junctional complex was not observed (figure 2-f).

A critical dose of 10 Gy or higher was estimated in rats using a single dose of radiation. After exposure to radiation at doses exceeding this threshold, there is a decrease in liver tissue replication and the number of mitochondria within several weeks. However, the mitotic index also increased. Using TEM, we observed alterations in the ultrastructure of the cells, which were believed to be due to altered mitotic activity and functions, leading to damage to the intracellular organelles of the liver tissues. Additionally, a decrease in the density of the cytoplasm was noted, and it appeared brighter, which was postulated to be the result of the destruction of cellular junctions in the cell membrane, leading to an influx of extracellular fluid into the cells.

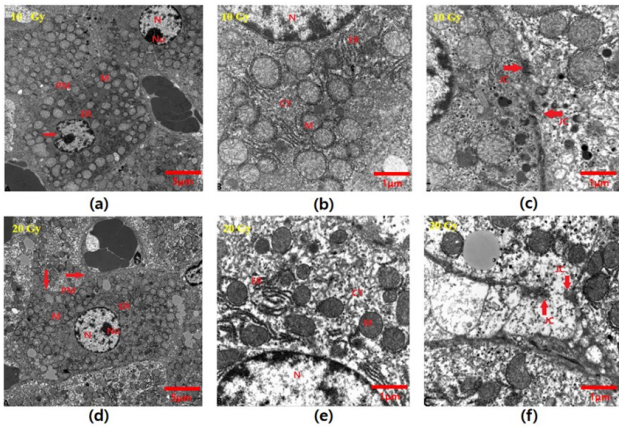

Figure 2. Electron micrograph of rat liver at 10 Gy: **(a)** Hepatocyte with indented (arrows) nucleus (N), nucleolus (Nu), few scattered mitochondria (M), endoplasmic reticulum (ER), and plasma membranes (PM) (× 5000) **(b)** Normal structure illustrated in homogeneous cytoplasm (CY), fragmented endoplasmic reticulum (ER) with ribosome (× 20,000) **(c)** Significant alterations in the junctional complex (arrows). Electron micrograph of rat liver at 20 Gy: The hepatocyte outlines were lost (arrows) **(d)** Hepatocyte with pyknotic nuclei (N), nucleolus (Nu), Mitochondria were darker (M), endoplasmic reticulum (ER), and plasma membranes (PM) (× 5000) **(e)** Areas of low cytoplasmic density in the hepatocytes (CY), fragmented endoplasmic reticulum (ER) with ribosome (× 20,000) **(f)** Monolayer were markedly dilated in the junctional complex(arrows) (× 20,000).

DISCUSSION

In-vivo electrical signal analysis can be performed easily and noninvasively at a low cost; therefore, it is widely used in various fields, such as in-body examination, electrocardiography, and electromyography [6, 14]. However, no investigations have yet addressed how alterations in electrical signals correspond to the biological effects induced by whole-body exposure to ionizing radiation. The effect of ionizing radiation on the living body can change the biological impedance by altering the physical characteristics according to the dose and type of organ [6]. In other words, it is difficult to reflect all the damage to various living tissues, and the organs of the whole body have different radiation sensitivities and extents of damage. Therefore, it was not possible to compare the impedance and histomorphological analyses of each organ separately. According to most

[DOI: 10.61186/ijrr.24.2.32]

previous studies, there are *in-vivo* electrical impedance measurements during and after electroporation of rat liver [15], electrical properties of extracted rat liver tissue [16], and electrical impedance spectroscopy study of biological tissues [17]. This study analyzed the impedance changes according to radiation dose following whole-body irradiation in live rats, and examined the irradiated liver tissue using TEM.

In our previous research, impedance changes were measured *in-vivo* across a frequency range of 1 kHz to 200 Hz in rats exposed to varying doses of whole-body radiation [8]. In this study, we analyzed the changes in the impedance values in response to frequency changes in the range of 1 kHz to 1 MHz in the beta range. This range reflects the tissue structure, as estimated by the cell-level relaxation phenomenon, which is related to the RC frequency. In the beta range, particularly in the early stages (1 kHz ~ 30 kHz), the frequency range does not penetrate the biological membrane. Instead, it reflects their surface effects, resulting in a sharp decrease in impedance values rather than a proportional relationship with the frequency [8, 9]. So, this region is not free of polarization and Warburg impedance of the electrode surface [18]. Particularly, at frequencies below the RC frequency, the characteristics of capacitance dominate.

In this study, we set the dose range from 1 Gy to 20 Gy (1Gy, 5Gy, 10Gy, 20Gy) to find an obvious difference in the electrical resistance and tissue changes induced by radiation in living Sprague–Dawley rats. Our data were analyzed by fitting them to an equivalent circuit model consisting of resistances and capacitances in series and parallel combinations [18, 19]. Other electrical models for biological cells and tissues have also been developed. As biological cells are extremely complex, several combinations of series and parallel resistors and capacitances are required. No unique equivalent circuit can describe any cell completely. The pattern of the impedance was shown to be similar to the results of our research, which investigated radiation on the whole body of rats [8].

We observed a rapid impedance change at 10 Gy, which was related to the gradual rupture of the liver tissue membrane during radiation exposure This phenomenon appears to be significant, similar to our previous experimental results on pork tenderloin tissue, where the spacing between sarcomeres and myofibrils widened at 10 Gy [9]. In this experiment, we observed that the density of the cytoplasm was reduced above 10Gy, caused by cell membrane destruction, allowing the cytoplasm to flow into the extracellular fluid. This indicates that the increase in electrolytes (ions) in the extracellular fluid after radiation exposure can be indirectly confirmed by TEM.

When radiation is absorbed by living rats, the initial damage arises from the ionization of intracellular atoms and molecules and subsequent events. Although such interactions can occur in both living and deceased tissues, the ionized atoms and molecules in living cells tend to be more chemically reactive. Ionizing irradiation acts as an oxidative promoter, generating ROS, which in turn elevate oxidative stress in the liver tissue. This results in the immediate generation of highly reactive free radicals, leading to rapid protein modification and damage to DNA, RNA, and cell membranes [16]. In this study, we hypothesized that exposure to ionizing radiation leads to changes in cell size, spacing, composition, intracellular and extracellular matrices, and damage to cell membranes, resulting in an increase in hydrated substances and a decrease in tissue electrical resistance [8, 20]. With this change, if the hydrating substances increase and support structure between the cells loosens, the impedance decreases.

For whole-body radiation, we expected the electrical resistance to decrease owing to the change in the amount of water and decrease in physique above 10 Gy. This is particularly related to the characteristics and conditions of the liver tissue. In addition, the frequency-dependent relation between impedance (Z), conductivity (σ), and relative permittivity (ε_r) was as follows [7]: ($\mathbf{Z} = \frac{1}{\sigma + j\omega\omega_0\varepsilon_r}$). As shown in this equation, the impedance is affected not only by the frequency but also by the permittivity and conductivity. Both conductivity and relative permittivity vary widely among different biological tissues, and these parameters also vary with the alternating current frequency. Therefore, the destruction of cell membranes by radiation exposure appears to cause a significant difference in impedance values.

The mechanism linking changes in tissue and electrical impedance of the whole body caused by ionizing radiation is complex and difficult to fully understand. Body fat and changes in extracellular and intracellular fluids should be considered [6].

The destruction of cell structures, such as nuclei, cytoplasm, and mitochondria, is related to changes in impedance. The density of the cytoplasm decreased and appeared as bright areas, which were attributed to the destruction of cell junctions in the cell membrane, allowing extracellular fluid to flow into the intracellular space. Exposure to ionizing radiation provokes an acute inflammatory reaction in rat tissues, accompanied by alterations in the extracellular matrix toward a more hydrated and less compact structure, which promotes cellular infiltration and subsequent recovery.

By establishing a correlation between alterations in these cellular components and whole-body electrical resistance, and by systematically collecting the data, radiation dose could be directly estimated from resistance changes. Results indicated that higher radiation doses were associated with more

[DOI: 10.61186/ijrr.24.2.32]

pronounced alterations in the nucleus, cytoplasm, and cell membrane, reflecting structural damage at the tissue level. When the cell membrane was damaged, the intrinsic capacitance of the cell membrane decreased. In addition, a reduction in body weight, cell size, or depletion of parenchymal cells may lower the impedance by geometrically changing the living body. However, to accurately understand the relation between whole-body irradiation and changes in electrical resistance, it is necessary to measure and compare real-time irradiation and time-lapse data. In addition, to investigate the relation between changes in the electrical resistance of the whole body and tissue damage caused by ionizing radiation, additional analyses of other tissues are required.

CONCLUSION

This study demonstrated that histomorphological and electrical resistance changes occur in rat liver tissue after critical radiation doses, alongside gross biological responses. Future research will expand to highly radiation-sensitive organs and explore impedance changes, even at low doses, focusing on nuclear and mitochondrial DNA effects.

Acknowledgments: This work was supported by a Research Institute for Convergence of Biomedical Science and Technology (30-2024-014), Pusan National University Yang-san Hospital.

Funding: None.

Conflicts of interest: The authors report no conflicts of interest in this work.

Ethical compliance: All animal procedures were reviewed and authorized by the Institutional Animal Care and Use Committee of Pusan National University (PNU-2017-1470) and were carried out in accordance with the university's guidelines for scientific research and ethical standards as required for humans or animals.

Authors Contribution: S.K.S., conducted the experiment. G.R.K., and M.S.L., conducted the data analysis. G.D.K., C.H.L., and H.J.C., provided consultation on the experimental results. J.H.K., and S.H.K., reviewed and discussed the overall content of the manuscript.

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[DOI: 10.61186/ijrr.24.2.32]