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Abstract

The aim of our study was to examine the effects of hypofunction of the pituitary gland previously irradiated with a total dose of x-rays of 27.92 Gy, applied in eight sessions in the period from 8 to 63 days of age on growth of rat’s craniofacial and stomatognathic system. In order to differentiate the effects of the irradiated pituitary from the direct effects of X rays on the head the experiment was set as a double study. One group had the pituitary gland protected with a lead plate set beneath the projection of the pituitary, while the second group was irradiated with the same dose, but without the protection.

Growth measurements of numerous parameters of the craniofacial system in rats with entire head locally irradiated showed a statistically significant delay compared both to not irradiated control group and to the group with protected pituitary gland. It can be concluded that the damage on the stomatognathic system is greater due to pituitary hypofunction compared to direct exposure to x-rays. The growth of the craniofacial system in rats in the sagittal direction is most sensitive to radiation and growth hormone deficiency. Pituitary hypofunction and irradiation showed a stronger effect on lower jaw growth rate impairment compared to the upper jaw.

Nowadays, irradiation is a standard procedure in medicine and the dangers related to it are ever growing. This brings up a need for further studies in order to examine the mechanisms of action, as well as the means of protection.

Apstrakt

Cilj našeg istraživanja je da ispitamo efekte hipofunkcije hipofize ozračene ukupnom dozom X zraka od 27,92 Gy aplicirane u 8 seansi u periodu od 8–og do 63–eg dana starosti, na rast kraniofacijalnog i stomatognatog sistema pacova. U nameri da u merenju brojnih parametara pomenutih sistema razdvojimo efekte ozračene hipofize od direktnih efekata X zraka koje smo primenili na glavu, postavljen je eksperiment sa ozračivanjem glave dvema grupama životinja istim dozama X zraka, ali je jednoj zaštićena hipofiza olovnim pločicom postavljenom u polje ispod koga leži hipofiza.

Zaostajanje u rastu stomatognatog sistema u pacova kojima je lokalno ozračena cela glava u odnosu na kontrolne životinje predstavlja zbirni efekat delovanja hipofunkcije hipofize i direktnog delovanja X zračenja, dok zaostajanje u rastu stomatognatog sistema u
pacova sa ozračenom glavom i zaštićenom hipofizom od zračenja predstavlja isključivo efekat X zraka. Iz ovoga se može izvući opšti zaključak da su oštećenja stomatognatog sistema veća kod hipofunkcije hipofize nego kod direktnog izlaganja X zračenju. Rast kraniofacijalnog sistema u sagitalnom pravcu je najosetljiviji na zračenje i nedostatak hormona rasta. Veći zaostatak u rastu su hipofunkcija hipofize i zračenje izazvali na donjoj nego na gornjoj vilici.

Obzirom na sve veću primenu jonizujućeg zračenja u medicini, opasnosti od dejstva ovog zračenja su u stalnom porastu. Ovo iziskuje stalna ispitivanja sa ciljem da se utvrde mehanizmi dejstva, kao i mogućnosti eventualne zaštite.

Introduction

A reaction of an organism to radiation depends on the level of irradiation and the sensitivity of the affected tissue cells. The biological effects on the cells and tissues are proportional to the absorbed radiation energy. A marked damage, with the same dose, is evident in tissues made up of cells with a high replication index, or occurs at an undifferentiated embryonic level. This is one of the milestones of radiobiology, set up by Tribondeau and Reaminer in 1905.

One of the most marked negative effects of irradiation of the neck and head region is pituitary hypofunction. There is a number of opposed opinions regarding the sensitivity of the pituitary gland to radiation. In some studies, it was considered that the pituitary gland of adults is resistant to radiation and that high doses are needed in order to induce hormonal changes. Nowadays it is thought that the hypothalamic pituitary unit is a particularly radiosensitive region, but due to a high hormonal reserve, the negative effects become evident after a longer latent period. It is established that the growth hormone secreting acidophilic pituicytes are sensitive at a single dose greater than 30 Gy, and this is often associated with impact on growth, sexual function and physical and psychological health. Consequently, hypopituitarism develops after radiotherapy for sellar neoplasms, brain tumors and head and neck tumors.

Postradiation therapy hypopituitarism shows more damage in younger patients. Growth failure develops after radiotherapy tumor doses of 46 Gy. Growth hormone insufficiency of 50–100% is usually the only abnormality after irradiation of hypothalamic-
pituitary axis with doses <30 Gy\textsuperscript{10}. Children irradiated for head and neck tumors have significant alterations in some skeletal measurements, such as asymmetry, potential deformity\textsuperscript{11} and deviations in craniofacial structures\textsuperscript{12}. The mandible is more sensitive to radiation compared to the maxilla, and especially the condylar region as the center of growth. Doses of 30 to 40 Gy and above are particularly harmful regarding facial bone growth\textsuperscript{13,14}.

The majority of bones of rat’s neurocranium grow by apposition along the sutures, and except for the frontonasal bone, this ends by the age of 34 days. The scull must be observed as a whole, as each part affects the development of not only adjoining structures, but also distant structures. Differential growth along the sutures gives the final shape to the scull\textsuperscript{15}.

Not many authors have researched scull and jaw growth in rats\textsuperscript{16,17,18}, although in examining the adverse effects on rat’s head and jaw growth many authors also recorded values from control, untreated groups\textsuperscript{19,20,21}.

The pituitary gland in rat is located ventral to the diencephalon and caudal to the \textit{chiasma optici} in a shallow groove at the base of the sphenoid bone\textsuperscript{22}. The adenohypophysis is a heart-shaped disk joined to the neurohypophysis at the medial line. The pars intermedia separate these two parts. The horizontal cleft of the hypophyseal space is between the neurohypophysis and the pars intermedia and persists throughout the lifespan\textsuperscript{23}.

Since the 1980s, extensive studies have been carried out on the effects of irradiation on development and growth of rats. Some researchers irradiated whole animals, and some only certain parts of their bodies. Studies on the influence of limb irradiation on the growth of long bones showed a significant, but reversible delay in growth of femur or tibia as early as at doses of 5 to 7 Gy\textsuperscript{24,25}. At a dose of 17.5 Gy, a greater delay in tibial growth in width than in length was observed\textsuperscript{26}. Severe damage in femur osteogenesis was caused by a dose of 20 Gy, while three doses of 10 Gy each caused mild, but significant changes\textsuperscript{27}.

Different doses were also used to irradiate rat’s heads in examining the effect on skull and jaw growth. Neurological and cognitive impairment have also been reported\textsuperscript{28,29}, as well as a decline in immunity\textsuperscript{30}. 
Demajo (alone as well as with associates) have studied the effects of irradiation on the growth of the skull and teeth in rats. Their results indicate that doses above 4.8 Gy (maximal dose 9.6 Gy) result in measurable effects on decreasing of jaw parameters\textsuperscript{31,32}. A dose of 20 Gy caused a significant delay in the growth of the lower jaw\textsuperscript{33}.

Irradiation of the rat’s head with doses of 20, 22, and 24 Gy caused a significant decrease in the secretion of the pituitary hormone, the most sensitive being the growth hormone\textsuperscript{34}. A dose of 10 Gy of $\gamma$-radiation applied to the whole body, abdomen and head caused increased hypothalamic-pituitary axis activity and greater hormone secretion in order of protecting the body from radiation\textsuperscript{35}.

The aim of our study was to examine the effects of hypofunction of the pituitary gland previously irradiated with a total dose of x-rays of 27.92 Gy, applied in eight sessions in the period from the eighth to 63rd days of age on rat’s growth, as well as on growth of the craniofacial and stomatognathic system. In order to differentiate the effects of the irradiated pituitary from the direct effects of x-rays on the head, the experiment was set as a double study. One group of rats had pituitary gland protected with a lead plate set beneath the projection of the pituitary, the second group was irradiated with the same dose, but without the protection of a lead plate.

**Methods**

*Experimental animals*

In the experiment, Wistar rats (n=45) with a genetic uniformity of 98% were studied, thus ensuring a uniform response to irradiation. The rats were introduced in the trial at the age of eight days. Up to the age of 30 days, they were kept with their mothers in groups of 6–8 animals per cage. They were weaned at the age of 30 days and set in separate individual cages. All animals were kept under a uniform light regime (light from 5.00 AM until 7.00 PM). Room temperature was 22 ± 2°C, and they were fed with standard food chaw and watered ad libitum.

The first group was not irradiated (*control group*, n=15). In the second group (n=15) the rat’s heads were irradiated, but their bodies were shielded and thus protected. This group is described as the *irradiated group*. The rats in the third group (n=15) had not only their bodies protected, but the region of the pituitary gland as well. This group is described further in the paper as the *protected group*. During the experiment, a number of
rats died, making the final number of animals 30. Thus, 10 animals in each group were finally studied.

_Irradiation_

The heads were irradiated with a Philips roentgen, usually used for radiotherapy. The conditions were as follows: 240 kV; 7.5 mA; filter 10 mm Al; speed 18.321 Gy/min and time 19 min 2 sec. The field width was 14 cm and the distance of the focus from the skin was 87.5 cm.

The overall irradiation dose of 27.92 Gy was divided into eight single doses of 3.49 Gy each. The animals were treated twice a week (Friday and Tuesday), every alternate week. The rats were treated for the first time at the age of eight days. Thereon they were treated at 12th, 22nd, 26th, 36th, 40th, 50th and 54th days of age. During each session, eight rats were irradiated and the room temperature in which the irradiation treatment was carried out was 18°C. Only the heads were irradiated, as a 6mm thick lead plate of fitting dimensions protected the rest of the bodies. In protected group, pituitary gland was also protected with 6 mm thick lead plate.

The rats were set in a specially constructed wooden stand. In order to stop frantic movements, the extremities were fixed by rubber tubing. The head was fixed in such a way that a thin wire was placed on one side behind the incisors, and on the other side in order to immobilize them in such a fashion as not to hurt the soft tissues.

_Measurements of the tibia_

The right tibia was prepared\textsuperscript{36} and its weight was determined on an automatic Mettler scale. The length was measured with a precise measuring instrument with a precision of a tenth of a millimeter.

_Measurements of the skull_

The heads and the jaws were cleaned of all soft tissues\textsuperscript{36}, with an orthodontic measuring instrument Dentaurum, with a precision of a tenth of a millimeter, the following parameters were taken:

1. Skull length – from the tip of the nasal bone to the most distal point of the occipital bone (Figure 1);
2. Skull width – the distance between the most prominent points of parietal bones (Figure 1);
3. Skull front height – from the mid coronary suture between the frontal and parietal bones up to the middle of the suture between the sphenoid bone and the sphenoid base (Figure 2);
4. Skull hind height – from the midsuture between the intraparietal and occipital bones down to the midline of the lower Foramen magnum edge (Figure 2);
5. Lenght of maxilla – the distance between anterior and posterior nasal spines (Figure 3);
6. Lenght of mandible – from the posterior edge of the angulus to the apex of the alveolar calyx of the incisor on the vestibular side (Figure 4);
7. Height of mandible – from the point between ramus and corpus on the upper edge of mandibule to the most convex point of incisura premaseterica on the lower edge of corpus. (Figure 4);
8. Lenght of ramus – from the tip of the condylar extension to the lower edge of the angulus (Figure 4).
9. Width between the condyles – distance between most lateral points of condylar extensions (Figure 5).

RESULTS AND DISCUSSION

In the literature available on this topic, we have not encountered any works that have separately examined the effects of irradiated pituitary gland and the corollaries of direct effect of x-rays on the head bones’ growth, therefore some of the results obtained could not be compared or discussed with other authors. These results can be considered to be our original contribution to the study of the effects of pituitary hypofunction on rat head bones’ growth; at the same time, it represents a baseline study in research of the effects of radiation-induced pituitary hypofunction on the growth of facial and jaw bones in patients irradiated in this region during the adolescence.

Irradiation of rat’s head with multiple doses of x-rays results in impaired growth. Protection of the pituitary during irradiation decreases the negative effects of radiation on the growth of rats, and on the growth of rat’s jaws and teeth. The first few doses in the protected group even showed a stimulative effect on overall growth.
Body length and mass growth, as well as pituitary weight in the completely irradiated group was significantly impaired (p<0.001)\textsuperscript{37,38}. 

During the experiment, some changes that could be attributed to the effects of radiation were also recorded. However, further studies of these changes were not planned, but we did consider it of interest to make a few comments on them. Epilation of the hair on the head started at the age of 25–29 days (after the fourth irradiation treatment) and was evident all the time during the experiment. In the protected group, hair loss was obvious after the fourth treatment only on the exposed parts of the head. The protected areas had a normal fur coat. Some of the irradiated rats by the 60th days of age had inflamed eyes with concurrent bleeding, apathy, weakness (rats were somnolent and were gathered at the bottom of the cage). Water consumption increased and feed consumption decreased. There were no cases of spontaneous teeth fallout.

At the age of 60 days, rats reach sexual maturity and secondary sexual characteristics are evident. The most prominent sexual feature is large testicles, which are lacking fur and are easily seen. Irradiated rats at the age of 60 days had testicles, which were atrophic and furry. When decapitated, the bleeding was very scarce and the blood was dark and dense.

*Length and weight of the tibia*

In our experiment, tibia was measured in order to determine the effect of pituitary irradiation on the growth of long bones, which were not in the radiation field.

The average weight of the tibia was the highest in the control group (403.37 mg), slightly lower in the protected group and the lowest in the irradiated group. Analysis of variance and a student’s test showed a statistical significance (p<0.001) compared to the control and the protected group. There was no significant difference between the protected and the control group (Table 1).

The results have shown that the tibiae were the longest in the control group and the shortest in the irradiated group. The difference was statistically significant between the control and the irradiated group. The length of the tibia was shown to be the most variable within the irradiated group.

Table 1
When irradiating the extremities with doses of 5, 7, and 17.5 Gy, some authors reported slow bone growth, but to a lesser extent than in our irradiated group when pituitary irradiation was performed\textsuperscript{24,25,26}, a dose of 20 Gy caused severe damage to osteogenesis and growth\textsuperscript{27}.

Demajo\textsuperscript{32} performed measuring tibial length as an indicator of systemic effects of head irradiation. In the control group, the values agree with our finding, while the values of tibia length of the animals irradiated with 9.6 Gy are greater than the values of the irradiated animals from our study. Regardless of fractionation, the dose of 27.92 Gy is much higher than that used by Demajo, so the values are lower.

Within the control and the protected group, there is no significant correlation between the weight and the length of the tibia. Within the irradiated group of rats, the correlation is negative; however, this is not up to a significant degree. The negative value of correlation in the irradiated group of animals is the result of a more intensive tibia weight loss compared to the length.

\textit{Skull length}

The longest average length was measured in the control group of animals. This value was significantly lower in the protected group (p<0.05). The lowest results (p<0.001) were recorded within the irradiated group (Table 2).

\textit{Skull width}

Same as for skull length, the largest average values were recorded in the control group. In the protected group, the values were significantly lower (p<0.05). The lowest values for skull width were in the irradiated group of rats. The values for the irradiated group were significantly lower compared to the control group (p<0.001) (Table 2).

There was no significant correlation between the measured parameters in all observed groups.

\textbf{Table 2}

A greater delay in width than in the length of the skull in the protected group compared to the control group is probably due to the protection of the pituitary gland by the lead, which also included the bones of the roof of the skull. The protection did not cover
the parietal bones where the width of the skull was measured, so the irradiation had a greater effect on the transverse than on the sagittal dimension.

**Frontal and hind skull height**

In the control group, the frontal skull average height is significantly higher (p<0.05) compared to the protected group. In the irradiated group this difference is even more impressive (p<0.001). Variations of the measured values are moderate (Table 3).

From the obtained readings for the hind height it can be clearly seen that the average readings were significantly lower in the protected group (p<0.05) and the irradiated group (p<0.001) (Table 3).

In the control and the protected group, there is no significant correlation between skull front and hind height. The correlation values are almost identical as in the irradiated group the lack of frontal and hind skull height is comparable. Within the irradiated group, the correlation between the measured parameters is high. Based on this finding, it can be concluded that in the irradiated group the radiation resulted in a significant inhibition of growth of both the frontal and hind portions of the skull.

**Table 3**

The significantly low values for skull length and width in the irradiated group are the result of the joined effects of radiation and pituitary hypofunction on the growth of the skull.

In the protected group, these parameters are lower compared to the control group, but significantly higher compared to the irradiated group. Based upon statistical analysis, the difference between the protected group and the control group is smaller than between the irradiated group and the protected group. This data enlightens the fact that the greater negative effect on the sagittal and transversal growth of the skull was due to the irradiated pituitary hypofunction, compared to the direct effects of radiation. The decreased growth of the skull width compared to skull length in the protected group compared to the control group was probably due to the protection of the pituitary by a lead plate that covered the bones of the skull. However, the plate did not cover the parietal bones, thus radiation affected more the transversal dimension than the sagittal.
In the irradiated group, the sagittal dimension was more affected as more bones and sutures are present in this direction compared to the transversal. Hence, it is more probable that the lack of the growth hormone affects more the sagittal dimension. In the protected group, the height of the skull is smaller compared to the control group, but significantly higher compared to the irradiated group. This difference is the direct consequence of the effects of radiation. Within the irradiated group, there was a simultaneous effect of radiation on the bones and insufficient growth hormone synthesis due to the radiation damage of the pituitary gland. The results in the protected group could have been affected by the lead protection as the plates covered also the hind portions of the skull. Thus, differences for frontal skull height are a more reliable indicator for the studied groups.

The anterior skull heights were measured at the same points by Haralabakis and Dagalakis\textsuperscript{18}. In the 60-day-old control group, the value is about 11 mm, which is slightly higher than in the animals in our control group.

\textit{Maxillary length}

The control group is significantly bigger than both the protected and the irradiated group, but the level of significance is different. In regards to the protected group, it amounts $p<0.05$ and in regards to the irradiated it is $p<0.001$. Such a large backlog of the irradiated group compared to the control group is a result of the summarized effect of radiation and pituitary hypofunction on the growth of the upper jaw. In the protected group, only the effect of the radiation was manifested, which is why it is smaller than the control group, but also significantly bigger than the irradiated group. Based on these observations, it can be said that the maxillary growth in the sagittal is more influenced by the hypofunction of the irradiated pituitary gland than by radiation itself (Table 4).

The animals from the same farm where we conducted the research were measured by Demajo\textsuperscript{31,32}. In the control group, the maxilla length is $21.82 \pm 0.31$ mm, which is entirely in accordance with our results in the control group. In the group where the heads of rats aged eight days were irradiated with 9.6 Gy, the length of the maxilla on 57th day is lower than the value recorded in our irradiated group. Although the rats in our study had received almost three times higher dose, their maxillae were less delayed in sagittal growth. Total dose fractionation and the time between irradiation applications enabled the activation of reparative processes, which prevented more drastic growth retardation.
Mandibular length

The control group of animals has longer mandibles than the other two groups. The level of difference in length compared to the control group in the protected group was at the significance level $p < 0.05$ and in the irradiated group the level was $p < 0.001$. In the protected group, the length of the lower jaw is longer than in the irradiated group, the significance of the difference is at the $p < 0.001$ level. All this suggests that pituitary hypofunction with consequent growth hormone deficiency caused a greater delay in sagittal growth of the mandible than direct radiation exposure. A similar finding is ascertained in the analysis of sagittal parameters of the maxilla, so that it can be said that the irradiation of jaws causes a smaller growth delay of the sagittal dimension of the head and jaws compared to the lack of growth hormone (Table 4).

In animals whose heads were irradiated with 9.6 Gy when they were eight days old, Sedlecki\textsuperscript{31} recorded a significantly shorter mandible length on day 57 compared to the control group. In 57 days old animals, Demajo\textsuperscript{32} measured higher values than ours ($26.03 \pm 0.21$ mm), and in the group where the heads were irradiated with 9.6 Gy when the animals were 8 days old, the values of the sagittal dimension of the mandible were also significantly higher than in our irradiated group ($23.87 \pm 0.38$ mm).

Duterloo and Vilmann\textsuperscript{17} measured a mandible length of 18.02 mm in 30 days old rats and found that the mandible had grown 2.17 mm from the 14th day. In our control group, the rats are twice as old and the difference in length is slightly more than 2 mm. As the growth rate is twice as fast in the first month than in the second month of life, our results are in line with the findings of these authors.

Mandibular height and Ramus length

Mandibular height in the protected group is significantly lower than in the control group, but also significantly higher compared to the irradiated group. Based on the level of significance, it can be seen that there is a greater backlog of the irradiated group compared to the protected group than to the protected group compared to the control group. (Table 5).

Sedlecki\textsuperscript{31} measured the height of the mandible at the first molars, unlike our measurement at the third molars; they also found that irradiation of the head of 8-day-old rats caused a significant retardation in growth, recorded at day 57.
In 57 days old rats, Demajo\textsuperscript{32} measured a mandible height that was lower than in animals from our control group. In animals, whose head was irradiated with 9.6 Gy on day 8, the height of the mandible on day 57 was also lower than the value in our irradiated group.

Ramus length in the protected group is significantly lower than in the control group, but also significantly higher compared to the irradiated group. Based on the level of significance, it can be noticed that there is a greater backlog of the irradiated group when compared to the protected group than to the protected group compared to the control group. This ratio is conditioned by the greater influence of the lack of growth hormone on the growth of the mandible in the vertical than the negative effect exerted by radiation. Since the same happens with the length of the mandible, it can be said that the total growth of the body of the mandible is more damaged by the pituitary hypofunction than by radiation. The mandible growth center is in the condyle, so any adverse effects on the wrist have an impact on the entire jaw. The x-ray direction was such that they passed through the skull before the condyle, so they probably lost their strength and could not fully affect the joint (Table 5).

**Table 5**

Compared to the control group, a significant delay in the ramus length of the 57th day of age was found in the group of the rats whose heads were irradiated with 9.6 Gy on the eighth day of age\textsuperscript{32}, which is consistent with our findings.

The growth hormone deficiency had a somewhat greater effect on ramus length compared to the effect of x-rays, but it can be summarized that, compared to the control group, there is an equal delay in the growth of the vertical dimension of the mandible in both experimental groups.

*Bicondylar distance*

As with the other parameters of the mandible, the values of the bicondylar distance are the lowest in the irradiated group. The protected group is lagging behind the control group; however, but there is a greater backlog of irradiated group compared to the protected group. Irradiation had less effect on the transverse growth of the mandible than the hypofunction of the irradiated pituitary (Table 6).
In 30-day-old males\textsuperscript{17}, the measured bicondylar width was slightly lower than the values measured in twice as old animals in our irradiated group, so it can be said that the x-ray radiation with pituitary hypofunction caused a growth of the mandible in the transverse direction that is twice as slow.

**Conclusion**

In animals with locally irradiated head, the measurements of the craniofacial system showed statistically significantly lower values compared to measurements of the animals in the control group, as well as in the group where their pituitary glands were protected, which is a consequence of the combined effect of the x-ray radiation and pituitary hypofunction.

The growth of the craniofacial system in rats in the sagittal direction is most sensitive to radiation and growth hormone deficiency.

Measurements of growth of numerous parameters of the stomatognathic system in rats with entire head locally irradiated showed a statistically significant delays compared to both not irradiated control group and to the group where the pituitary gland was protected.

In the group where the pituitary gland during the irradiation of the head was protected, there is a statistically significant delay in the growth of the stomatognathic system compared to the (not irradiated) control group.

Damage to the stomatognathic system is greater due to pituitary hypofunction than due to direct exposure to x-rays.

Radiation-induced pituitary hypofunction and direct irradiation caused greater growth delay on the lower than on the upper jaw.

Studies on the effects of electromagnetic irradiation on biological systems are always useful as irradiation is a standard procedure not only in medicine and the dangers related to it are ever growing.
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**Figures**

**Figure 1.**

![Figure 1](image1)

**Figure 2**

![Figure 2](image2)

**Figure 3.**

![Figure 3](image3)
Figure 1. Skull length (Rh–Oc), skull width (Pa–Pa).
Figure 2. Frontal (Cs–Sp) and hind (Pi–Ba) skull height.
Figure 3. Length of maxilla (Rh-Ps).
Figure 4. Length (Id-D) and height (mh-Ipm) of mandible and length of ramus (Cd-Go).
Figure 5. Width between the condyles (Cd-Cd)

### Tables

#### Table 1. Tibia length and weight

|                      | Tibia length mm |                       | Tibia weight g |                       |
|----------------------|-----------------|-----------------------|----------------|-----------------------|
|                      | X               | SD                    | CV(%)          | X                     | SD                    | CV(%)          |
| Control              | 31.74           | 0.2503                | 0.7886         | 403.37                | 2.8898                | 0.7164         |
| Protected            | 31.52           | 0.1549                | 0.4914         | 400.78                | 0.8917                | 0.2225         |
| Irradiated           | 26.74           | 1.2176                | 4.5535         | 366.12                | 8.1033                | 2.2133         |

|                      | Tibia length mm |                       | Tibia weight g |                       |
|----------------------|-----------------|-----------------------|----------------|-----------------------|
|                      | X               | SD                    | CV(%)          | X                     | SD                    | CV(%)          |
| T–test               | Protected       | Irradiated            | Protected      | Irradiated            |
| Control              | n.s.            | p<0.001               | n.s.           | p<0.001               |
| Protected            | p<0.001         | p<0.001               |                |                       |

#### Table 2. Skull length and width

|                      | Skull width mm | Skull length mm |                      | Skull width mm | Skull length mm |                      |
|----------------------|----------------|-----------------|----------------------|----------------|-----------------|----------------------|
|                      | X              | SD              | CV(%)                | X              | SD              | CV(%)                |
| Control              | 15.48          | 0.1033          | 0.6673               | 39.81          | 0.9837          | 2.4710               |
| Protected            | 15.34          | 0.1174          | 0.7653               | 38.88          | 0.2898          | 0.7454               |
| Irradiated           | 14.15          | 0.3951          | 2.7922               | 33.78          | 1.6281          | 4.8197               |

| T–test               | Protected      | Irradiated      | Protected           | Irradiated     |
| Control              | p<0.05         | p<0.001         | p<0.05              | p<0.001        |
| Protected            | p<0.001        | p<0.001         |                      |                |

Frontal skull high mm | Hind skull height mm
### Table 3.

**Frontal and hind skull height**

|       | X     | SD    | CV(%)  |       | X     | SD    | CV(%)  |
|-------|-------|-------|--------|-------|-------|-------|--------|
| Control | 10.96 | 0.3718 | 3.3923 | 9.79  | 0.2961 | 3.0245 |
| Protected | 10.60 | 0.1944 | 1.8340 | 9.53  | 0.1160 | 1.2172 |
| Irradiated | 9.03  | 0.3335 | 3.6932 | 8.32  | 0.3706 | 3.6971 |

|       | T–test | Protected | Irradiated | Protected | Irradiated |
|-------|--------|-----------|------------|-----------|------------|
| Control | p<0.05 | p<0.001   | p<0.05     | p<0.001   |
| Protected |       | p<0.001   |            | p<0.001   |

### Table 4.

**Length of maxilla and mandible**

| Maxillary length mm | X | SD | CV(%) | Mandibular length mm | X | SD | CV(%) |
|---------------------|---|----|-------|-----------------------|---|----|-------|
|                     | X | SD | CV(%) |                       | X | SD | CV(%) |
| Control             | 21.73 | 0.7543 | 3.4712 | 20.68 | 0.6286 | 3.3965 |
| Protected           | 20.71 | 0.4175 | 2.0159 | 19.95 | 0.5148 | 2.5805 |
| Irradiated          | 18.43 | 0.6201 | 3.3646 | 17.96 | 0.6059 | 3.3736 |

| T–test | Protected | Irradiated | Protected | Irradiated |
|--------|-----------|------------|-----------|------------|
| Control | p<0.05    | p<0.001    | p<0.05    | p<0.001    |
| Protected | p<0.001   |            | p<0.001   |            |
### Table 5.

**Height of mandible and ramus**

|                        | Mandibular height mm | Ramus height mm |
|------------------------|-----------------------|-----------------|
|                        | X         | SD       | CV(%) | X         | SD       | CV(%) |
| Control                | 6.18      | 0.1619   | 2.6197| 10.02     | 0.5266   | 5.2555 |
| Protected              | 5.88      | 0.1814   | 3.0850| 9.48      | 0.0789   | 0.8323 |
| Irradiated             | 5.47      | 0.2359   | 4.3126| 8.55      | 0.2173   | 2.5415 |
| **T–test**             |           |          |       |           |          |       |
| Control                | p<0,05    |          |       | p<0,01    |          |       |
| Protected              |           |          |       | p<0,05    |          |       |
| Irradiated             |           |          |       | p<0,001   |          |       |

### Table 6.

**Bicondylar distance**

|                      | Bicondylar distance mm |
|----------------------|------------------------|
|                      | X         | SD       | CV(%) |
| Control              | 19.68     | 0.3120   | 1.5845|
| Protected            | 19.09     | 0.4122   | 2.1592|
| Irradiated           | 15.40     | 0.2449   | 1.5903|
| **T–test**           |           |          |       |
| Control              | p<0,05    |          |       |
| Protected            |           | p<0,001  |       |

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