In vivo apoptosis of fibroblast pulp cells by ionizing radiation from radiotherapy of the head and neck area has not yet been demonstrated. The study aimed to show in vivo the effect of a single dose of ionizing radiation on apoptosis of fibroblast pulp cells. The sample group consisted of 24 healthy male Wistar rats that were 3–4 months old and 150–200 g in weight. The rats were divided into 4 groups of 6 rats that were subjected to Cobalt 60 radiation to the head at the levels of 0, 100, 200 or 400 rad. The rats were sacrificed 24 hours after radiation exposure, and the lower incisors were taken for histopathological processing. Apoptosis was detected by using the TUNEL Assay method. The apoptotic fibroblast pulp cells were counted under light microscope by multiple observers using the blind test approach. The fraction of apoptotic cells was counted as mean of labial and palatal sides of the teeth below odontogenic and free-cell zone. The data were statistically analyzed using one-way anova. The results showed the percentage of apoptotic fibroblast pulp cells was 6.4, 23.7, 34.5 and 17.8% after 0, 100, 200 and 400 rad doses, respectively. There were significant differences in the apoptotic percentages between the four groups (p < 0.05). In conclusion, the highest fraction of apoptotic fibroblast pulp cells was found after a single 200 rad dose, and this fraction decreased after a single dose of 400 rad.