

## A study of the radioprotection effect of guarana (Paullinia cupana) on the fetuses of ICR mice

### THE RADIATION PROTECTION EFFECTS OF GUARANA

Yeunhwa Gu, Takeo Hasegawa, Ikukatsu Suzuki\*, Youichi  
Yamamoto, Yeogbyung Yoon<sup>†</sup>, Soo-Yong Rhee<sup>‡</sup>

Department of Radiological Technology, Suzuka University of Medical Science,

<sup>\*</sup>Department of Clinical Nutrition, Suzuka University of Medical Science,

<sup>†</sup>Department of Radiology, Shinheung Junior College, 117,

<sup>‡</sup>Department of Physics, College of Science, Hanyang University,

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**Abstract** - Guarana, a tropical plant is found in powdered form in health food and is very popular soft drink in Brazil as an energy booster with its high caffeine contents. We examined its radioprotection effects during organogenesis stages of ICR mice by malformations rate and cellular lead 8 the embryo by radiation and analyzed the mechanism of the radioprotection effects in the fetus of ICR mice. The results of this study showed that Guarana reduced clearly the embryonic death rate and teratogenesis rate by radiation. Its radioprotection effect might be related with its antioxidant effect or free radical scavenger. We need to exposure the Guarana as a potential radioprotection agent. Therefore, we investigated about radiation effects by Guarana using to mice experiments in this paper.

*Key words: Radioprotection effects, Organogenesis, Malformation, Guarana*

### INTRODUCTION

The aims of the radioprotection for are a human and its function on environmental concern. Radiation has many harmful points as well as positive ones for human life. First, nuclear the power plant industry has been contributing greatly to the use to the radiological peace. And, as for present clinical medicine, the medical techniques that have been developed as a result of radiation cannot be disputed. But, there are many problems that a radioprotection plans at the nuclear power plant that haven't been solved. Moreover, the study of fetal effects by radiation for medical research is not only the concern of medical science but also has broader social implications as well. The mission of the nuclear power plant makes it critical to make radioprotection toward the people concerned engaged in the nuclear power

plant and the periphery inhabitants a perfect thing. It is the aim of which radioprotection from the internal exposure of the human and the external exposure is the most benefit. The fetus is the most sensitive issue when the issue of radiation exposure to humans is talked about(1), 2), 3), 4), 5), 6), 7). Therefore, if it is grasped precisely and protection criterion and protection resource are established, children and adults won't have a problem from the viewpoint of radioprotection. The aims of the radioprotection are a human and environmental safety concern. The malformation that much research does in the animals, and relations between the fetal death and the radiation were now being made distinct. There is even stage when expectant mother doesn't notice pregnancy until the organogenesis, the stage when a malformation is induced. Because it is the decisive stage which rational symptom is not like this, the

potential that an effect is taken from the radiation is high. In this determination stage, the malformation toward the radiation was observed by using the ICR mice. These mice have often been used in Japan for this type of the research. We examined the fetal effect toward the radiation. Previous research clearly shows TMG to be similar to the mechanism of the vitamin E and to have the protecting effect that faces free radical by the simulation of *In vitro* [8]. So, the reasons it is as a radioprotective agent of the effect on the fetus toward the radiation was examined by using the *Paullinia cupana* in this research. Therefore, it is a purpose to obtain information as a medicament of the radioprotection.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS AND MATING PROCEDURE

A closed colony of ICR (Crj: CD-1) mice were purchased from Charles River Japan, Inc. They were housed in a room at a temperature of 22-23 degree and a relative humidity of 60 to 70% with a 12-hour light-dark cycle (the light phases starting at 6:00 and 18:00). The mice were given free access to food (CA-1, CLEA Japan, Inc.) and tap water. One or two female mice, which are 10 to 18 weeks old, were placed together with one male mouse of the same age range in the same cage for only three hours from 6:00 to 9:00 for mating. The female mice in which vaginal plugs were found were assumed to have become pregnant at 8:00, at which time day 0 of gestation was, designated [9, 10]. As for the medication method of the *Paullinia cupana*, internal use of 500mg/kg for no less than two weeks was administered to the pregnant mice [11].

### IRRADIATION WITH X- RAYS

The pregnant mice were placed in plastic cages for irradiation, and were treated with a single whole body X-radiation at 2 Gy with dose rates of 0.35 Gy/min. The pregnant mouse of 192 hpc (hours post conception): (organogenesis stage) was made within the irradiation gauge

after the fertilized, Phillips X-ray irradiation device (225kV) was used. The time of embryo exposure was 196hpc. The total number of irradiated dams observed in this study was 40, a total of 38 non-irradiated control and sham control dams was also prepared, and 659 non-irradiated live fetuses served as controls:

### OBSERVATION OF EXTERNAL MALFORMATION AND OTHER EFFECTS

After irradiation, the pregnant mice were sacrificed by cervical dislocation on day 18 of gestation and the total number of corpus luteum in the ovaries, implantation sites, and live and dead embryos and fetuses were counted. The live fetuses were removed from the uterus and examined for external malformations using a dissecting microscope. The body weight and sex of each fetus were also measured.

### STATISTICAL ANALYSIS

For teratological effects, it is not appropriate to consider the fetus and embryo to be an experimental unit. The litters (pregnant mice) were taken into account during the statistical analysis of the experimental data. In the per litter analysis, an average fetal response within a litter was calculated. For statistical tests, we used non-parametric tests that are the Wilcoxon tests [12].

And, as for fetal body weight, a t-test was done.

## RESULTS

### Preimplantation death

The result of the preimplantation death rate is shown in Fig.1. Statistical significant difference was recognized between the preimplantation death rate of the 2.0Gy group and the control group ( $P < 0.05$ ). Statistical significant difference wasn't recognized in the other groups toward the control group. There is no this causal association directly because preimplantation death is different from the radiation exposure time of this study. Moreover, it is absorbed by the metabolism of which implantation site is early,

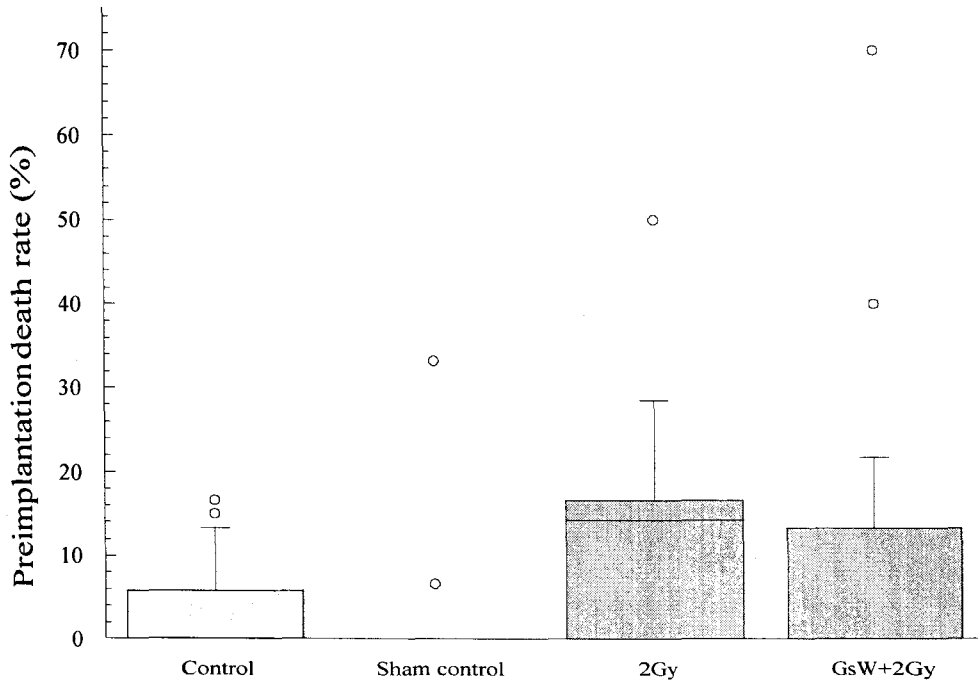


Fig.1. Implantation death rate of ICR mice irradiated at Organogenesis period. 2.0Gy and GsW+2.0Gy group was detected with statistical significance( $P<0.05$ ) in comparison with control groups by Wilcoxon-test.

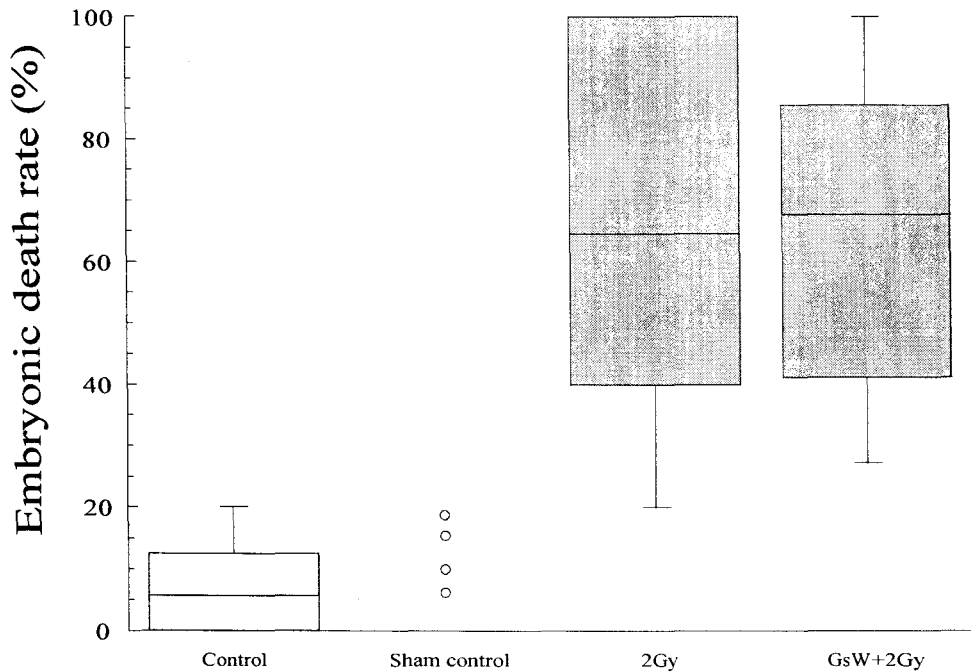


Fig.2. Embryonic death rate of ICR mice irradiated at Organogenesis period. 2.0Gy and GsW+2.0Gy group was detected with statistical significance( $P<0.001$ ) in comparison with control groups by Wilcoxon test.

and it is thought that it appeared with the preimplantation death.

### Embryonic death

Implantation site, placental remnant and absorption embryo were considered to be embryonic death. Embryonic death is the death which induced from 4.5 hpc (days post conception) to 13.5 hpc [1]. The result of the embryonic death rate is shown in Fig.2. Statistical significant difference was recognized between the embryonic death rate of the 2.0Gy group and the GsW + 2.0Gy (Guarana extracts of water solution) administrated group toward the embryonic death rate of control group and sham control group ( $P < 0.001$ ). But, when comparing the embryonic death rate of the 2.0Gy group and the GsW + 2.0Gy group, the embryonic death rate of the GsW + 2.0Gy group was reduced. If Guarana (paullinia cupana) wasn't administered, the embryo beyond the haploid number that did implantation was found

out in the exposure beyond 2.0Gy.

### Fetal death

A maceration fetus observed 18 dpc (days post conception) was considered to be fetal death. Fetal death is defined as death that induced 14 dpc. The result of the fetal death rate is shown in Fig.3. As for the fetal death rate of the GsW + 2.0Gy group, statistical significant difference wasn't recognized as the control group in comparison with the sham control group with the 2.0Gy group. Therefore, though guarana was used, it was found differences weren't significant toward the control group in the 2.0Gy exposure. Because the incidence of the embryonic death was very high, it isn't thought that the difference for the fetal death to be significant.

### Incidence of malformation

The number of the incidences of the malformation is shown in Fig.4. The modality of the malformation that it induced in each exposure group is exencephaly, cleft palate,

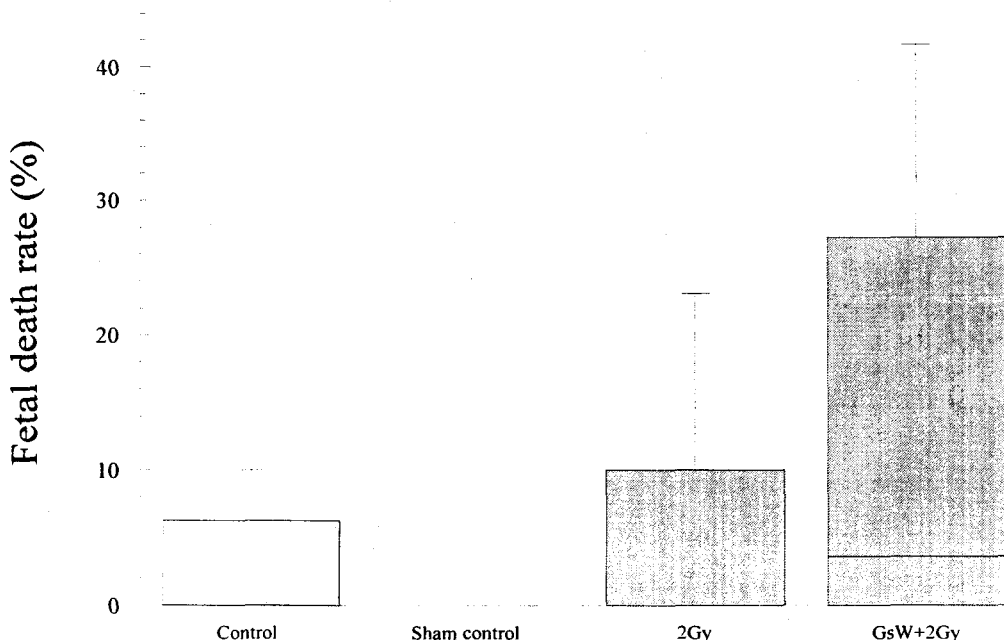


Fig. 3. Fetal death rate of ICR mice irradiated at Organogenesis period. 2.0Gy and GsW+2.0Gy group was detected with statistical significance ( $P < 0.05$ ) in comparison with control groups by Wilcoxon test.

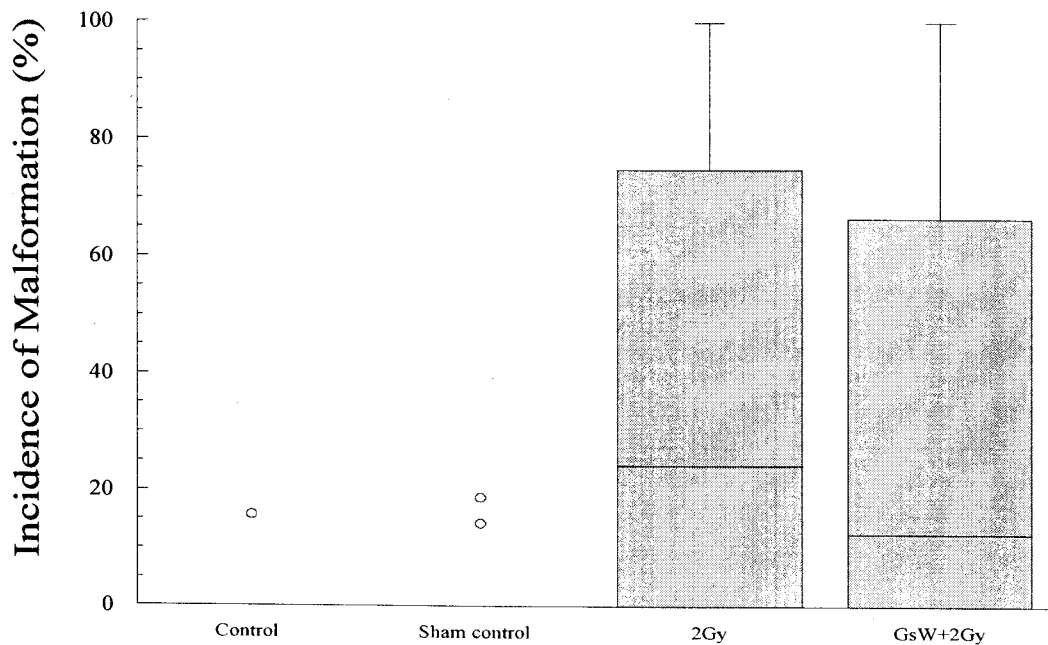


Fig. 4. Malformation rate of ICR mice irradiated at Organogenesis period. 2.0Gy and GsW+2.0Gy group was detected with statistical significance ( $P < 0.001$ ) in comparison with control groups by Wilcoxon test.

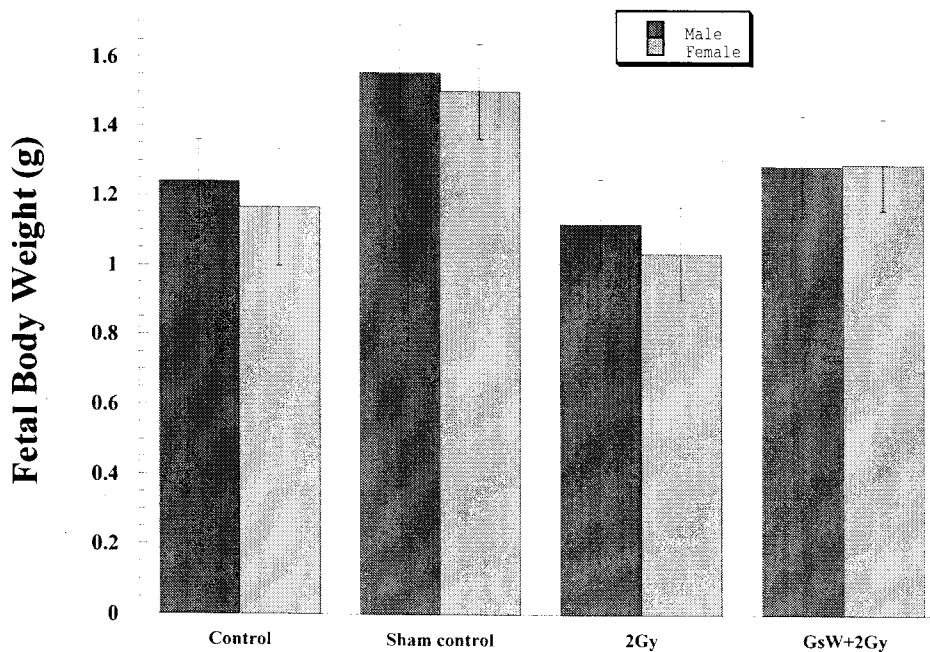


Fig. 5. Fetal body weight of the ICR mice irradiated at Organogenesis period. 2.0Gy and GsW+2.0Gy group was detected with statistical significance ( $p < 0.05$ ) in comparison with control groups by T-test.

open eye, gastroschisis, and anomalies of tail. A statistical significant difference was recognized as the development frequency of the malformation of the 2.0Gy group in comparison with the induction frequency of the control group ( $P < 0.001$ ). A statistical significant difference was recognized as the teratogenesis rate of the GaW+2.0Gy group as well in comparison with it of the control group. As for the malformation, statistical significant differences were recognized as the control group from the above result toward the sham control group in all the exposure groups. But, though it was the highest when it was seen from the teratogenic rate, a decrease was recognized with the 2.0Gy group and the GsW + 2.0Gy group by the GsW+2Gy group of less than 2/3. Because there are many subjects that died because it had a malformation in the embryonic death, the effect of the malformation and the effect of each death, are separate issues and are not relevant to this research

#### **Fetal body weight**

After one weighed fetal body weight respectively and the average of every mother was taken, the fetus' average weight of each exposure group was a mean by the male female of each exposure group. The fetal body weight of each exposure group is shown in Fig.5. Both significant difference of the fetal body weight decrease were recognized in the 2.0Gy group in embryonic age 18-day fetal body weight toward the body weight of the control group and the sham control group of the male and female ( $P < 0.05$ ). But, some thought differences weren't recognized in it of the GsW+2.0Gy group in the fetal body weight decrement toward the body weight of the control group and the sham control group. And, when compared with a male and female ratio of body weight, there was little fetal body weight difference. The female weighed about the circa 0.05g more as for each group as well.

## **CONCLUSION**

Active oxygen (free radical) appears in the organism, and oxidization target injury lesion is given to lipid, a protein, and the nuclei by radiation exposure. It follows that various histronic damages are occurred [13-15]. There is little clinical or epidemiological observation though much observation of it in vitro and in vivo with the vitamin E as on the radiation. It is chisels for medical use with the clinical observation used experimentally a little because of the therapeutic side effects prophylactics toward the bone marrow transfusion and the brain tumor radiological damage [16]. Actually, it has been shown that the lipid hyperoxidation when X-rays and gamma rays were radiated, and DNA damage occurred in the catastatic by a natural agent [1, 8, 17]. But, this observation lacks radioprotection effect in the natural agents in one case, too [18-20]. Recently, oxidization affection by the active oxygen, the free radicals are being made clear in various sicknesses and in retrogradation [21]. Moreover, attention to, and research about the natural anti-oxide substantial, the synthesis anti-oxide substantial is done very much radioprotection by the anti-oxide [22]. Fonseca has shown that the extracts of guarana were genotoxic as assessed by lysogenic induction in Escherichia coil and they were also able to induce mutagenesis in Salmonella typhimurium. The genotoxic activity in the extracts was related to the presence of a molecular complex formed by caffeine and a flavonoid (catechin or epicatechin) in the presence of potassium [23]. Therefore, as for this research as well, it is thought by guarana that its part as a radical scavenger that erases the active oxygen that occurred in the physical case is played, too. Fonseca et al shows the flavonoid has radioprotective effects [23]. The exposure group of the organogenesis of the sensibility against the embryonic death is the highest [24]. When an exposure group increased more in comparison with control group as for the embryonic death, it has by using F1 of the male CDA/MK mice and the female dd/MK mice as a result of doing simulation. But, a

Table 1. Dead and live embryos / fetuses in each experimental group.

Dose	No. of Dams	No. of Preimplantation deaths (%)	Total No. of implantation	No. of Embryonic deaths(%) (mean±SD)	No. of Fetal deaths(%) (mean±SD)	No. of Live Fetuses (%) (mean±SD)	Litter size mean±SD	Fetal body Weight (g)a mean±SD
Control	20	13 (4.0)	328	18 (5.5) (5.8±6.5)	5 (1.5) (1.8±3.3)	292 (89.0) (92.1±7.7)	14.7±2.8	M:1.241±0.122 F:1.167±0.168
Guarana (Sham control)	18	6 (2.4)	251	7 (2.8) (2.8±5.9)	0 (0.0) (0.0±0.0)	238 (94.8) (97.2±5.9)	13.2±2.9	M:1.558±0.137 F:1.503±0.137
2.0Gy	20	29 (11.9) a	244	134 (54.9) b (66.4±26.6)	11 (4.5) (5.2±7.4) a	70 (28.7) b (28.3±26.0)	3.5±3.5 a	M:1.122±0.128 a F:1.037±0.133 a
Guarana + 2.0Gy	20	22 (8.8) a	249	146 (58.6) b (66.9±24.9)	24 (9.6) (10.8±14.0) a	57 (22.9) b (21.5±21.4)	2.9±2.8 a	M:1.289±0.146 F:1.294±0.132

We used Wilcoxon tests for preimplantation, embryonic and fetal death to the between each treatment groups and significantly different from control group. We used the t-test for statistical analysis of the fetal body weight to the between each treatment groups and significantly different from control group.

M : Male, F : Female

a Significantly different from control group P < 0.05

b Significantly different from control group P < 0.01

Table 2. Number of fetuses with external malformations.

Type of malformation	Control	Guarana	2.0Gy	Guarana + 2.0Gy
Exencephaly	0	0	9 b	8 b
Cleft palate	0	1	2	4 c
Malrotated limb	0	0	0	0
Open eye	0	0	0	1
Anophthalmia	0	0	2	2
Hydrocephaly	0	0	3 c	0
Anomalies of tail	3	0	22 a	7 c
Abdominal hernia	0	0	1	0
Polydactly	0	0	1	0
Other	0	3 c	0	4 c
Total number of malformations	3	4	40	26
Incidence of malformations(%±SD)	0.8±3.5	1.8±5.4 c	36.9±40.2 a	28.9±36.0
Total number of dams	20	18	20	20
Total number of live fetuses	292	238	70	57

We used Wilcoxon tests for malformations to the between each treatment groups and significantly different from control group.

a Significantly different from control group P < 0.001

b Significantly different from control group P < 0.01

c Significantly different from control group P < 0.05

significant difference wasn't recognized, it is being reported for the preimplantation stage [25]. Murakami and Kameyama showed irradiated 0.5Gy to the 8 dpc (days post conception) of pregnant mice, and it observes embryonic death. And, an embryonic death of 20-30% was recognized (being reported as for the exposure of 1Gy [26]. Jacobsen shows that irradiated 0.05Gy-1Gy to the 7 hpc of pregnant mice, and the dose effect of the embryonic death was examined, and made dose response clear [27]. A statistical significant difference was recognized as the control group and the sham control group in comparison with the 2Gy group ( $P < 0.05$ ). But, the GsW+2Gy group did n't recognize a statistical significant difference with the control group in comparison with the Sham control group. Therefore, radioprotection effect by the guarana administration was clearly shown toward the embryonic death rate. The radioprotective action by the administration of the guarana is radical scavenger prevention function due to the radiological chemical action and oxidation prevention function, and it is here as for this research as well [28-30]. The oxidization effects of that organism component are thought a possible protection by anti-oxide substantial. Actually, it has been observed that the lipid hyperoxidation when X-rays and gamma rays were radiated, and DNA damage occurred in the catastatic by the manipulation of the guarana [23]. It could get the same result as for this research as well to the embryos. Therefore, it is thought that guarana has a radioprotection effect. Brent recommends all permanent defects that a living being isn't prepared to repair be defined as malformation [31]. Ohzus shows that irradiated 0.05 - 0.25Gy in 10 hpc and 34 hpc, and observed exencephaly and hyperdactyly [25]. And, the effect of the malformation of the organogenesis by the radiation has stage differences in developmental disorders [32]. It was made clear that sensibility to the malformation induction was the highest between the viviparity 7.5-10.5 days of the mice that radiological depends by preceding research [32]. Jacobsen shows that

irradiate 0.05 in the viviparity 7.5 days, and the malformation induction of the cephalic, the jaw, and the aperture were observed [10]. Muramamis shows that irradiate 2Gy in the viviparity the ninth day and a malformation such as exencephaly and cleft palates are recognized [33]. The threshold dose of the malformation (exencephaly) in the case of the radiation exposure is between 0.5Gy-1.5Gy, and the threshold dose of the embryonic death is between 1.5Gy-2.5Gy [32]. A difference in the threshold dose is a difference in the sensibility of the radiation dosage that affects a malformation and embryonic death. Perhaps the number of the critical cellularis which causes teratogenesis, a difference in the number of the critical cellularis in the case of the embryonic death, and so on effects it [24]. Radioprotection effect by the guarana administration was made clear to the malformation incidence of this research. 2Gy were irradiated to the mice after the body weights 50mg/kg of flavonoid administration, and Divis shows the made radioprotection effect clear [34]. As for the fetal body weight, it is pointed out that it is critical as a character to grasp an effect such as a radiation by many researchers. Decrease of weight was recognized as the effect of the fetal body weight decrement by this research in the only 2Gy-exposure group. Decrease of fetal body weight was recognized in the organogenesis by the preceding research in the exposure group beyond 1.5Gy [35]. It isn't histrionic with the specific organizes against the decrement of the fetal body weight against the exposure group of the organogenesis, and histrionic development delayed type hypersensitivity effects the organizes of the body as a whole. Moreover, it is in proportion to the histrionic with the normal organizes, and it is thought that loss of weight happened [36]. The effect of radiation of the fetal body weight decrement in the organogenesis is thought about that effects by not only the direct effect but also the uterus environment or blastogenesis stage didn't function normally, either. On the other hand, Konermanns was radiated by 0.4 to the mice of



the preimplantation stage (0-5 days) and the decrement of the fetal body weight wasn't recognized [37]. But, as for the fetal body weight as well, radioprotection effect by the guarana was made clear in the same way as the effect of the embryonic death and the malformation.

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