The study of the radiation protection of propolis to the radiation effects in mice

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INTRODUCTION

The radiation has even harmful parameter for the human besides one case though the benefit which a radiation brought to the man is very great. And, the fetus malformation which against radiation by before by much research is being made clear (1,2,3,4). However the individiual of which sensibivity is high is a fetus against the radiation, and it is paid attention to socially (2). Therefore, if even an effects to this fetus is grasped precisely and protection is done, there is no problem from the viewpoint of the protection of radiation as well in the protection of radiation surface. It was examined about the radidiation protecting effect of propolis to the radiation of fetal effects by using the ICR mice in this study. In propolis and Greek, the meaning of "an invader from the externus, prevention protective wall", staphylococcus salivarius is mixed with the sap which honey bee collected from the trees, make. It is the material which contains the beneficial effect componentum taken out. It is made the reactivation of the nest box, aseptic condition in the nest box, and the part of the emergency food is played except for the protecting effect to the exogenous material which enters from the externus in propolis in the case of the food crisis. And, a temperature inside the mold cavity and air humidity are adjusted, too (5,6).

There are saccharides, amino acid, flavonoid, a mineral, delfenoid, caffeine, aldepirin C, a vitamin group, and so on as a componentum of propolis (7). It is being reported when there are antibacterial action, anti-inflammatory function, pain relief, anesthetic action, immunization, anti-oxidation in the physiology function to the human body of propolis (8). It is much physiology function of propolis, or it pays attention to immunisatio and anti-oxidation for this study. Macrophage and N-K cell are activated as an immunization, and it is made to control neoplasm, the immunocompetence accelerans function due to the increase in the antibody forming cell. And, activity of macrophage and immunisatio hyperactivity by the influence of the cell-mediated immunity are announced the thing that it is involved in the antitumor action (10). It is announced that there are anti-neoplasm effectus that it against ill, and active oxygen function (11). And, when anti-cancer drug is administered, it is said that febricula, anorectic, adverse drug reactions such as blood cell count decrement Sumptomatic appear. The thing which water soluble propolis was used with together controls leukocyte count and the decrement of the platelet count by the simulation of the adverse drug reactions of the anti-cancer drug in some thought. It is made both hypoleukocytemia and thrombocytopenia to be recovered in some thought. It has fructification with the multiplier effect of the anti-cancerosus function which activation function was fitted to to activate neoplasm catastaltic. And, it is announced that it has the effectus which makes propolis reduce the adverse drug reactions of the anti-cancerosus function (5,12). Therefore, the anti-oxidation of this propolis and immunization were used, and the research of the radiologic protecting effect was done by this research.

MATERIALS AND METHODS

Experimenta lanimals

A closed colony of ICR(Crj:CD-1) mice was purchased from Charles River Japan Inc. They were housed in a room at a temperature of 21-23 and a relative humidity of 50 to 70% with a 12-hour light-dark cycle (the light phase ; 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 10 to 18 weeks old and one male mouse of the same age range were mated for only three hours from 6:00 to 9:00. The female mice in which vaginal plugs were detected were assumed to have become pregnant at 8:00 (2).

Irradiation with X-rays

The pregnant mice were placed in special cages made of plastic or paper for exposure. They were treated with single whole-body X-rays at 1.5 Gy with dose rates of 0.2 Gy/min. We used X-irradiation equipment(225 kV) of philips. The system belonging to the Suzuka University of Medical Science was used. An exposure group is two groups of the 1.5 Gy exposure group, the propolis pluse1.5 Gy radiation exposure group. It isn't radiated in the control group and only propolis administration group

The exhaustion procedure of propolis

And, three put original lump 200 g of propolis and aqua distillata 2000 ml in the flask, and it was stirred for two hours in 50 temperture. After that, it was put on the centrifugal separation for 20 minutes 5000 with ppm, and supernatant was filtered. The same maneuver was done the precipitate, and congelation dried (the second exhaustion) filtrate

The procedure of the propolis administration

Abdominal cavity administered propolis 100mg / 10 ml / g to the Female mice after the spare breeding termination in the every other day interval. And, before it was made to become pregnant, propolis did administration two weeks in the same way at least by the childbearing 15 days

The observation of the skeletal malformation by the bone tinction

The cutaneous of the fetus who fixed it with the ethanol and organicus were removed, and three days and more anhydration were done with the ethanol and the acetone. After that, arzalinred of the 0.4 per cent and the Alcian blue of the 0.1 per cent were made up respectively, and acid alcohol (with the hydrochloric acid and the ethanol, preparation) was dealt to it, and it dyed for 6 hours in 37 temperture . After that, it put into 1% for one hour and for 13 hours in the ethanol in the KOH solution, and maceration of the soft tissue was measured. It proceeded in the next stage by the stage that skeleton looked beautiful. 80% put into 40% for 24 hours and for 48 hours and for 24 hours at least in each of the glycerin, and preparation was finally kept 100% of the glycerin, and 20% observated skeletal malformation.

Observation in the cellularis level

It began to take pregnancy the eighth days embryo, and slice of cellularis. The slice of cellularis which had it was put in the preparation, and the following function was done. The procedure of the observation isoantibody tinction of apoptosis by the isoantibody tinction. Or 90% of put 70% of 100% of the object glass on each 30% methyl alcohol for 5 minutes and for 3 minutes with. After that, it was put in the phosphate buffered saline, and object glass was put in the phosphate buffered saline solution of Proteinase (20ug/ ml) after three minutes. After it was put in each of the aqua distillata for 5 minutes 4 times after 15 minutes, it was put into 2.0% for 5 minutes in each of the H_2O_2 / phosphate buffered saline solution, the phosphate buffered saline solution. The thing that TdT and reaction buffer solution were made up was paid in the cellularis after equilibration buffer solution was dropped in the cellularis and covered by the cover slip and it was put for 15 minutes in the room temperature. After that, it was moved in the concentration reaction insertion ablution buffer solution for the occupation which was put 37 temperature for 1 hour and which was warmed in a 37 degrees after 1 hour, and made to put a slide in and out respectively once in every 10 minutes, and put for 30 minutes. Next, anti-digoxigenin, Peroxides is dropped in the cellularis, and puts it on the phosphate buffered saline solution for five minutes three times for three minutes phosphate buffered saline solution. DAB solution was dropped in the cellularis, and left for five minutes. After this, a tinction check was done, and it was put into for one minute three times and for five minutes with the microscope in the aqua distillata in the aqua distillata. It was put into it for five minutes in the Hematoxillin solution, and 2 irrigated three times with the aqua distillata, and it was put on the warm water for five minutes. It was put into it for 5 minutes finally in 30%, 50%, and 90% 100% methyl alcohol, and put into, cover glass for 1 minute 2 times in Xylene after that, and die sequente cellularis was operated. Apoptosis of the cellularis that HE stains depends, micronuclei and the observation of the chromatic agglutination. Made object glass was put in 100%, 85% 70% methyl alcohol for 30 seconds in the same way as the above, and 2 was irrigated 3 times with the phosphate buffered saline. It was put into it in the ammonia water, and it was put for one minute after object glass was irrigated for five minutes with was put into, the aqua distillate in the Hematoxillin tinction liquid. After that, it was put into it for 3 minutes in eosin tinction liquid, and it was irrigated with the aqua distillata, and anhydration was done with 70%, 85% 100% methyl alcohol. After that, was put on Xylene for three minutes twice, and entrance was lowered to the slide, and cover glass was put and operated in the die sequent.

Observation of external malformation and other effects

The pregnant mice were euthanized by cervical dislocation on day 18 of gestation and the total numbers of corpora lutea in the ovaries, of implantation sites and of live and dead embryos/fetuses were counted. The live fetuses were removed from the uterus and examined for external gross malformations under a dissecting microscope. The body weight and sex of each live fetus were also determined.

Statistical methods

For teratological effects, it is not appropriate to consider the fetus/embryo as an experimental unit (13). The litter (pregnant mouse) was taken into account as an experimental unit in the statistical analysis of the

experimental data. Then, in the per litter analysis, the average fetal response within a litter was calculated. For statistical tests, we used nonparametric methods. Kruskal-Wallis tests for comparisons among dose groups or Wilcoxon tests for comparisons between two groups (14).

Therefore we used Kruskal-Wallis tests or Wilcoxon tests for preimplantation, embryonic and fetal death and malformations. We used the T-test for statistical analysis of the fetal body weight.

RESULTS

preimplantation death rate

When an implantation rate was calculated, the implantation rate of each one pregnancy mice was calculated in consideration of the individual difference (Litter effects) of the mother beast. Then, it calculated a place for the implantation rate of the whole of Group on the value. The implantation rate when radiation exposure is done after the conception eight days is shown to Table1. The implantation rate of all the manipulation groups and a significant difference official approval with the implantation rate of control group and the sham control group were done in the Wilcoxon official approval. Some thought decrement wasn't recognized as the increase in the radiation dose with in the statistical significant as a result by which exposure group (P=0.4).

Embryonic death rate

Implantation sites, placental remnants and resorptions embryo were handled as an embryonic death after the implantation by the radiation exposure as an embryonic death. Embryonic death is estimated to be the death which enduced from the conception posticus 4.5 days by 13 days. Embryonic death due to the exposure after the conception eight days is shown to Table1 and Fig.2. The embryo death rate of control mice is 5.86%, and the embryo death rate of the sham control group is 1.20%. Statistical significant difference was recognized in as a result which went to the embryonic death rate of the 1.5Gy exposure group (27.4%), the embryonic death rate of control and the space of the sham control group in the Wilcoxon official approval ($P<0.001$). And, as for the propolis administration 1.5Gy exposure group (15.56%) as well, the statistical significant difference was recognized in as a result of the Wilcoxon official approval ($P<0.001$). But, the decrement of the circa 1/2 was recognized more than the 1.5Gy exposure group independent exposure group from the death rate and the propolis administration 1.5Gy exposure group.

Fetal death rate

The fructification of the fetal death rate is shown to Table1 and Fig.3.

The fetal death rate of control mice is 1.63%, and the fetal death rate of the sham control group is 0% in the same way as the fetal death rate from Fig.3 as well to be clear. The statistical significant difference was recognized in the result, which went between 1.5Gy exposure groups (7.14%) fetal death rate of control and sham control group space in the Wilcoxon official approval ($P<0.001$). And, as for the propolis administration 1.5Gy exposure group (3.89%), statistical significant difference was recognized in the result of the Wilcoxon official approval ($P<0.05$). But, the decrement of the circa 1/2 was recognized more than the 1.5Gy exposure group independent exposure group as for the fetal death rate as well the propolis administration 1.5Gy exposure group.

External malformations

The number of the development of the external malformations when radiation exposure was done after the conception eight days is shown to Table2. The modality of the malformation that it is induced for the radiation group is exencephaly, cleft palate, chest hernia, open eye, gastroschisis, anomalies of tail, and so on. The modality of the malformation which faces though it is only cleft palate and that even an exposure group varies was recognized as the malformation which occurred in control group and the sham control group. The control group and a sham control group were compared with the malformation incidence, and statistical significant difference was recognized by the 1.5Gy exposure group ($P<0.001$). And, as for the propolis administration 1.5Gy exposure group as well, statistical significant difference was recognized as a result of the Wilcoxon official approval ($P<0.001$). There was no difference about the malformation between the propolis administration 1.5Gy exposure group and the 1.5Gy exposure group independent exposure group.

Fetal body weight

Fecundation eight-day postoperative irradiation group fetal body weight is shown to Table1. A 1.5 Gy exposure group and propolis were administered in comparison with the control group and the sham control group, and the decrement of the body weight was recognized as group ($p < 0.001$). But, as for the fetal body weight, a propolis administration 1.5Gy exposure group was a little higher than the 1.5Gy exposure group independent exposure group.

It faced a male and female ratio and a minimum fetus as other influences, and there was no each group in the significant difference sham.

CONCLUSION

Physiology function to the human of propolis is being made clear by much research. But, as for the excuse of the radiation protecting effect, each function of propolis isn't made clear yet. Therefore, the anti-oxidation of propolis, immunization, and so on were used, and this research did the research of the radiation protecting effect. Kujumgievs did the examination of the antibacterial action of propolis in 1999. Propolis faced Staphylococcus aureus and Bacillus coil, and the thing that obviously activity was made clear by the fructification. It says that flavonoid and ester have immunization potentiation about the effects (15). Moreover, Mirzoevas used propolis, and they were investigated by using subtilis, the Bacillus coil, R sphaeroides. propolis was very active efficient with the bactericidal effects. It was recognized as the effects that cinnamic of Propolis and flavonoid component were to remove energy transducing cytoplasm membrane and then to control bacterial mobility (16). Scheller is being reported when obviously an immunoactvation increases, the immunoactvity of the mouse spleen cell is examined by using propolis (17). Krol was recognized prominent multiplier effect to ampicillin and the streptomycin when the multiplier effect of the antibiotic to propolis was examined (18). And, Pascual was examined propolis effects to the oxygen electrode free radical. propolis was made clear by the fructification to get rid of peroxide (19). And, the free radical ablation function of facing propolis by Schellers was proved (20). Kimoto was controlled, and made the air trap of the apoptosis and the tumor growth by artepillin C of Brazilian propolis clear (21). It thinks by this research with the thing that the probability that the fetus who has malformation from the incidence of the embryonic death and the fetal death being high before the childbirth dies is high as a result of the radiation exposure. But, it has the effects which reduces embryonic death and fetal death by administering propolis. And, because probability of survival is high, a teratogenesis rate isn't thought high become, either. After propolis of three weeks is injected into anterior at least, the decrease of the free radical by the radiation is thought about as for these. When propolis was administered to the post irradiation operation 3 days ago, it is thought that it is effects due to the immunological enhancement and the anti-inflammatory function (20,22,23,24). Therefore, it is thought that PLD (potential lethal death) by the radiation and SLD (sub lethal death) were decreased (25). propolis was administered about the teratogenesis rate, and a 1.5 Gy exposure group was lower than the 1.5Gy radiation exposure group. It may be able to say whether it lacks function as a radioprotective agent more than this thing about propolis. But, generally propolis faces radiation more than therefore administering propolis about the embryonic death rate and the fetal death rate in the 1.5 Gy radiation independent exposure group and 1.5 Gy radiation exposure group's being lower, and it isn't said easily that protection isn't done. Because, probability of survival is high from administering propolis and death rate's being poor, and the fraction of the 1.5 Gy radiation exposure group that the fetus who caused malformation is born is high. As for the 1.5Gy exposure group, it is thought whether it was thought about by the malformation fetus that the potential that it is selected by the embryo or the fetal stage was high and such fructification couldn't get it by an anamorphosis child. Therefore, the decision of the radiation protecting effect of propolis is thought teratogenesis rate, embryonic death rate and fetal death rate all synthetically must judge. A teratogenesis rate of1.5 Gy group and propolis plus 1.5 Gy group, and or it is thought equally by a that the latter is shorter. There isn't thought radiation protection have activity be propolis from this thing. Therefore, propolis is thought though radiation damage is repaired. Volpert was reported when a NADPH oxidize activities degree is clear with the leukocyte myeloperoxidase though the leukocyte which faces propolis, and the drug interaction of the leukocyte

fermentative were researched (26). Moreover, Schellers did |/FONT>-ray radiotherapy to the mice which administered propolis, and observed the protecting effect. As for the radiation exposure group after the propolis administration, obviously probability of survival was high in comparison with the 6Gy exposure group. And, leukocyte was returned to the normalize, too. It follows, and propolis proposes that free radical is decreased as an anti-oxidizing agent and it has radiation protection effect in the same way as this research (17). And, Ghazalys did research by using propolis of the water exhaustion against the damage by the radiation. Then, it was shown that it had anti-inflammatory radioactivity. 5m l / kg internal use did as simulation procedure, and radiation protection effect was recognized in the same way as this research (7). And, usual radioprotective agent had the anxiety of the adverse drug reactions with the medicine. But, it paid attention to it by this research as a radioprotective agent. propolis is paid attention to as a health food. It isn't so harmful to the human body, and the anxiety of the adverse drug reactions is the thing of what be not here, and it is thought that it should be examined to the future realization, too. But, because the development of the malformation could be controlled, it isn't thought on this condition that the examination which an administration concentration condition is to is necessary.

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