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Study of the Radioprotective Efficiency of Administration of Rutin and Cysteine in Irradiated Pregnant Rats and Their Fetuses

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Abstract

The present study was conducted to investigate the potentially protective effect of rutin and cysteine on pregnant rats who had received whole-body gamma irradiation and the development of their fetuses. In the present work sixty female albino rats were arranged into six equal groups. Control (G1); 2nd group: will receive saline then irradiation (2Gy) and served as irradiated control (G2); 3rd group: will receive rutin (1.064 mmol/kg, oral) daily for two weeks (G3); 4th group will receive rutin (1.064 mmol/kg, oral) daily for two weeks and then irradiated (2 Gy) (G4); 5th group: will receive \ cysteine (30 mg/kg,i.p) (G5); 6th group: will receive cysteine (30 mg/kg,i.p) 30 min. before irradiation (G6). The rats exposed to 2Gy gamma-irradiation increased the MDA, E2, FSH and LH levels significantly as compared to control group. In contrast; a significant decrease in the levels of SOD, CAT, progesterone, prolactin, AMH, IgM and IgG were observed in irradiated group as compared to control. The oral supplementation of rutin and cysteine has significantly attenuated the severity of irradiation. Also, the whole body γ-irradiation (2 Gy single dose) caused embryonic death and embryos replaced by residual bodies. The fetal wight, fetal body length, fetal head length and the fetal head width were significantly decreased in irradiated group when compared to control group. While, the treatment with rutin or cysteine in G4 and G6 before the whole body γ-irradiation caused a nonsignificant decrease in these mentioned measurements, as compared with the control..

Keywords: γ-irradiation, Pregnant rats, Rutin, Cysteine; Radioprotection

1. Introduction

The growing use of nuclear and radiation technologies across various fields has led to an increased risk of radiation exposure [1]. The harmful effects of ionizing radiation on biological systems are primarily due to the production of reactive oxygen species (ROS) in cells through the radiolysis of water. ROS and oxidative stress are known to cause metabolic and morphological changes in both humans and animals [2]. Unregulated ROS production can lead to lipid modifications, which play a key role in the development of cardiovascular and neurodegenerative diseases. This issue is particularly concerning during pregnancy, as developing fetuses are highly sensitive to radiation, which can result in congenital malformations, growth retardation, or even fetal death [3]. Maternal exposure to gamma radiation at a dose of 3 Gy on the 6th and 12th days of gestation has been shown to cause pre-implantation death, increased intrauterine death, reduced growth rates, and uterine retardation [4]. The teratogenic effects of embryonic and fetal irradiation are significant as biological indicators of radiation impact. Developing mammalian embryos are more sensitive to ionizing radiation than adults, with numerous experimental studies indicating that radiation-induced abnormalities in mammals are closely related to the developmental stage at which radiation occurs [5, 6].

Administering molecules with strong antioxidant, anti-inflammatory, and immunomodulatory properties before radiation exposure is considered a key strategy for developing radioprotective agents. Several synthetic compounds, such as amino thiols (Amifostine), nitroxides (Tempol), and DNA-binding agents (Hoechst 33342), have shown a significant increase in post-irradiation survival in mice [7, 8]. While some of these compounds have advanced through various clinical trial stages, many failed for several reasons [9]. Among these, Amifostine is the only compound approved by the United States Food and Drug Administration for limited clinical use [10].

In addition, numerous natural resources, including plants, minerals, vitamins, and antioxidants, have been evaluated for their radioprotective properties in recent years. Herbs, known for their richness in antioxidants, immunostimulants, anti-inflammatory, and antimicrobial agents, often have minimal or negligible toxicity. These multifaceted properties and low toxicity make phytocompounds more advantageous than synthetic compounds [11]. Rutin, a natural flavonoid derivative

commonly found in plants, is recognized for its anti-inflammatory and vasoactive properties [12]. It scavenges free radicals, reduces inflammation, and enhances the body's defense systems, potentially reducing radiation-induced cellular damage. Rutin has been reported to prevent gastric mucosal ulceration in animal models [13] and has also been identified as a strong scavenger of hydroxyl and superoxide radicals [14].

Cysteine, a sulfur-containing amino acid, plays a vital role in cellular defense against oxidative stress. It is a precursor to glutathione (GSH), one of the body's most important antioxidants, which helps neutralize ROS and maintain cellular redox balance [15]. N-Acetyl-l-cysteine (NAC) is a potent agent that mitigates the effects of radiation by scavenging radiation-induced radicals. NAC and other radical scavengers have been shown to reduce radiation-induced DNA damage, chromosomal abnormalities, cell death, tissue damage, and individual lethality [16]. However, in some cell types, the protective effects on cell survival were not observed [17]. This may be due to the reduction of radicals by scavengers and the simultaneous interaction of NAC with cellular proteins, leading to altered protein activities and cellular radiosensitivity [18]. The aim of the current study was to investigate the potentially protective effect of rutin and cysteine on pregnant rats who had received whole-body gamma irradiation and the development of their fetuses.

2. Experimental

2.1. Animals
Adult female albino rats, weighing 150-200g, were obtained from the National Research Center (Giza, Egypt). The animals were kept under suitable laboratory conditions throughout the period of investigation. They were allowed free access to food consisting of standard pellets and water was also provided ad libitum. Adult female albino rats received Research ethics Committee in National Center for Radiation Research and Technology - Egyptian Atomic Energy Authority (NCRRT-REC) approved the study protocol (Approval number: F/20A/24).

2.2 Chemicals
Rutin (Byron Chemical Company, USA) was freshly dissolved in distilled water and orally administered daily for two weeks before irradiation in a dose of 1.064 mmol/kg.

Cysteine (Sigma-Aldrich, USA) was freshly dissolved in distilled water and administered i.p 30 min. before irradiation in a single dose of 25 mg/kg.

2.3 Experimental design
Six groups of animals each of 10 rats were used in the present study.

1st group: was received saline and served as normal control.

2nd group: was received saline then was irradiated at a single dose of 2 Gy gamma rays and served as irradiated control.

3rd group: was received rutin (1.064 mmol/kg) orally by stomach tube daily for two weeks.

4th group: was received rutin (1.064 mmol/kg) orally by stomach tube daily for two weeks and then was irradiated (2 Gy).

5th group: was received cysteine (30 mg/kg,i.p) single dose.

6th group: was received cysteine (30 mg/kg,i.p) 30 min. single dose before irradiation.

2.4 Irradiation of animals
Pregnant rats were whole body irradiated at a single dose of 2 Gy gamma rays in the 17th day of gestation and sacrificed on day 20 of gestation (1-day prior delivery) [19]. Irradiation was delivered at a dose rate of 0.48 Gy/min. The radiation source was ^{137}Cs Gamma Cell-40 biological irradiator, belonging to the National Center for Radiation Research and Technology, Cairo, Egypt.

At the end of the experimental period, rats were fasted overnight; euthanized with intraperitoneal injection with urethane and subjected to a complete necropsy. serum was collected without anticoagulant then centrifuged at 3000 rpm for 15 minutes to obtain serum for evaluate biochemical parameters.

2.5 Measured parameters:
Enzyme linked immunosorbent assay (ELISA) was used to determine the levels of IgM and IgG in serum according to the reported method [20]. F.S.H and L.H in blood were determined according to the method described [21]. Super oxide dismutase (SOD) and catalase (CAT) activity were determined in blood according to [22]. Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method previously described [23]. Progesterone and estradiol were determined in blood according to the method [22]. Prolactin and anti-mullerian hormone (AMH) were determined in blood according to the method [24].

2.6. Morphological studies:
For embryological study, the pregnant rats were all cut open on the 20th gestational day (1 day prior to parturition) and their uteri were removed and pictured instantly. The fetuses were removed from the uteri and examined to determine the total number of live fetuses, number of malformed fetuses, fetal Wight (g), fetal body length (cm), fetal head length (cm) and fetal head width (cm) [25]. The abnormalities in the uterine horns were studied. The weight of fetuses was recorded using rough Metller Balance.

2.7. Statistical analysis
The obtained data were presented as means ± SD. One-way analysis of variance (ANOVA) was carried out. The statistical comparisons among the groups were performed with Duncan's test, using a statistical package program (COSTAT), Program

3,03,198. Significant differences were considered at P<0.05. The formula which is used to calculate percentage change is (Final Value – Initial Value) / | Initial Value | × 100.

3. Results

3.1. Effect of rutin and cysteine on variations in lipid peroxidation and antioxidant indicators in pregnant rats exposed to 2 Gy irradiation.

The values of the table 1 represent the mean ± SD significant at (p< 0.05). Whole body irradiation of rats provoked oxidative stress demonstrated by a significant increase of MDA level associated with a significant decrease of SOD and CAT activity with percent of changes at 238.3. -45.36 and -47.92 respectively, as compared to the control group during the experimental intervals (Tables 1 and Figure 1). The supplementation of rutin and cysteine to rats during 14 successive days before irradiation via gavages has significantly attenuated the severity of radiation-induced oxidative stress. Through their significant decrease occurred in the oxidative biomarkers' levels and significant increase of antioxidants levels was observed, compared to their respective values of the irradiated rats.

Table 1. Effect of rutin and cysteine on serum SOD, CAT, and MDA in pregnant rats exposed to gamma irradiation.

Groups Parameters	G1 (Control)	G2 (Radiation)	G3 (Rutin)	G4 (Rad.+Rutin)	G5 (Cysteine)	G6 (Rad.+ Cysteine)
SOD (U/mg)	195.5±5.64[c]	106.83±13.94[e]	261.17±10.30[a]	186.0±12.28[c]	224.33±14.61[b]	163.67±10.33[d]
CAT (U/min/mg)	191.67±7.40[b]	99.83±1.70[d]	320.33±8.98[a]	165.67±49.3[bc]	280.0±59.33[a]	145.30±5.99[c]
MDA (nmol/mg)	145.33±13.44[d]	491.67±72.51[a]	134.33±18.26[d]	276.0±23.66[c]	141.67±9.61[d]	320.0±74.83[b]

Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).

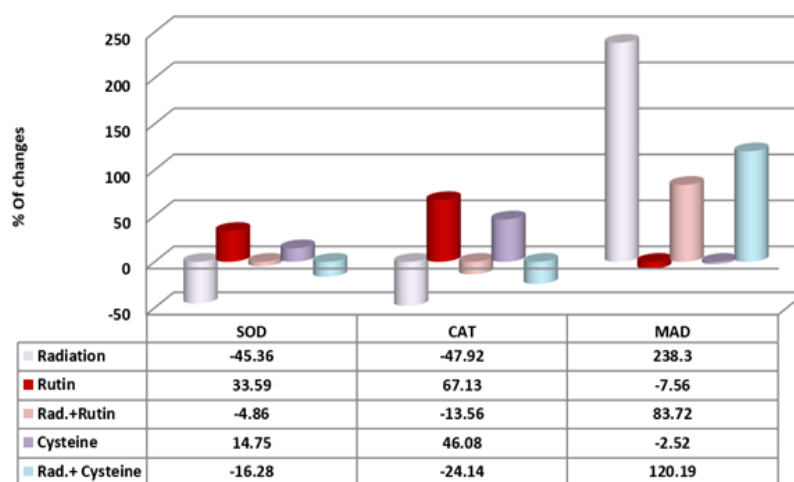|	SOD	CAT	MAD
Radiation	-45.36	-47.92	238.3
Rutin	33.59	67.13	-7.56
Rad.+Rutin	-4.86	-13.56	83.72
Cysteine	14.75	46.08	-2.52
Rad.+ Cysteine	-16.28	-24.14	120.19

Figure 1. The change in percentages of superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) in all the tested groups in comparison) to the corresponding control group.

3.2. Effect of rutin and cysteine on variations in sex hormones in pregnant rats exposed to 2 Gy irradiation.

The statistical results cleared that rat exposed to 2Gy gamma-irradiation significantly increased the E2, FSH and LH levels at P< 0.05 with change percentages of 243.72, 207,33 and142.16%, respectively, as compared to control group. On the other hand, the levels of progesterone, prolactin and AMH of irradiated rats decreased by -77.11, -87.07 and -69.17 respectively, as compared to control group. These alternations were almost nearly reverted to normalcy in rats supplemented with rutin and cysteine in group 4 and in group 6. Interestingly, the rutin displayed a more amelioration in sex hormones than cysteine (Table 2 and Figure 2).

Table 2. Effect of rutin and cysteine on serum progesterone, E2, FSH, LH, prolactin and AMH in pregnant rats exposed to gamma irradiation.

Groups Parameters	G1 (Control)	G2 (Radiation)	G3 (Rutin)	G4 (Rad.+Rutin)	G5 (Cysteine)	G6 (Rad.+ Cysteine)
Progesterone(ng/ml)	13.98±2.9[a]	3.2±0.81[d]	14.26±1.17[a]	10.1±0.85[b]	13.3±0.96[a]	7.30±0.68[c]
E2 (pg/ml)	61.0±10.1 [d]	209.67±10.32[a]	58.66±7.78[d]	88.16±6.99[c]	55.83±7.9[d]	104.5±13.76[b]
FSH (mlu/ml.)	5.32±0.72[c]	16.35±1.24[a]	5.65±0.68[c]	7.67±0.53[bc]	5.95±1.03[c]	8.18±0.73[b]
LH (mlu/ml)	7.02±0.28[d]	17.00±1.19[a]	7.40±0.63[d]	8.83±0.30[c]	6.58±0.59[d]	9.67±0.54[b]
Prolactin (ng/ml)	13.30±0.82[b]	1.72±0.38[d]	16.33±0.82[a]	9.37±1.45[c]	16.15±1.23[a]	8.33±1.54[c]
AMH (ng/ml)	3.73±0.69[a]	1.15±0.21[c]	3.88±0.32[a]	2.12±0.55 [b]	3.93±0.61[a]	1.68±0.40b[c]

Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).
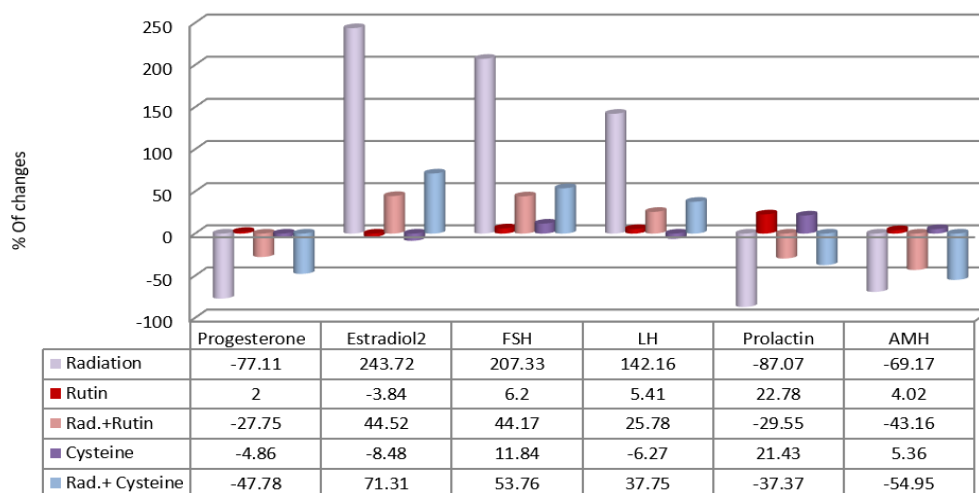
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Figure 2. The change in percentages of progesterone, estradiol 2, FSH, LH, prolactin, AMH hormones in all the tested groups in comparison to the corresponding control group.

3.3. Effect of rutin and cysteine on variations in IgM and IgG in pregnant rats exposed to 2 Gy irradiation.

The results, in Table (3) and Figure 3, show that there was significant decline in the level of IgM and IgG of gamma-irradiated group with percent of changes at -59.52 and -55,51 respectively, compared to control groups. Rats protected with rutin and cysteine before exposed to γ-irradiated showed significant increase in the level of IgM and IgG relative to γ-irradiated group.

Table 3. Effect of rutin and cysteine on serum IgM and IgG in pregnant rats exposed to gamma irradiation.

Groups Parameters	G1 (Control)	G2 (Radiation)	G3 (Rutin)	G4 (Rad.+Rutin)	G5 (Cysteine)	G6 (Rad.+ Cysteine)
IgM(g/l)	2.88 ± 0.89^a	0.97 ± 0.13^c	2.70 ± 0.93^a	1.61 ± 0.29^b	2.58 ± 0.85^a	1.23 ± 0.39^b
IgG (g/l)	18.24 ± 1.53^a	8.18 ± 0.46^c	19.44 ± 1.68^a	12.82 ± 1.93^b	17.84 ± 2.29^a	11.44 ± 0.93^b

Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).
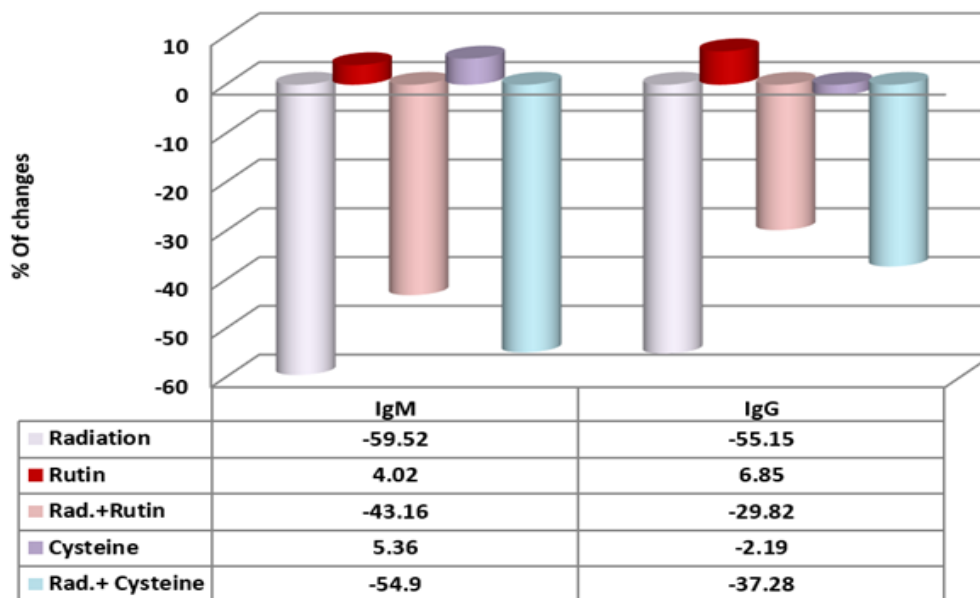

Figure 3. The change in percentages of IgM and IgG in all the tested groups in comparison to the corresponding control group.

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3.4. Protective effect of rutin and cysteine on morphology of uterus of pregnant rats and their fetus exposed to 2 Gy irradiation.

The obtained data revealed that whole body γ-irradiation (2 Gy single dose) caused embryonic death and embryos replaced by residual bodies. The fetal weight, fetal body length, fetal head length and the fetal head width were significantly decreased in irradiated group at percent of changes -28.87, -29.88, -9.09 and -12.64 respectively, when compared to control group. While, the treatment with rutin and cysteine in G4 and G6 before the whole body γ-irradiation caused a nonsignificant decrease in these mentioned measurements, as compared with the control (Table 4 and Figure 4).

Table 4. The total number of live fetuses, Number of malformed fetuses, Fetal Wight (g), fetal body length (cm), fetal head length (cm) and fetal head width (cm) in different groups.

Groups Parameters	G1 (Control)	G2 (Radiation)	G3 (Rutin)	G4 (Rad.+Rutin)	G5 (Cysteine)	G6 (Rad.+ Cysteine)
The total number of live fetuses	94	23	95	43	96	41
Number of malformed fetuses	4	16	3	10	2	9
Fetal weight (g)	1.42 ± 0.061^a	1.01 ± 0.13^c	1.48 ± 0.04^a	1.25 ± 0.05^b	1.51 ± 0.05^a	1.22 ± 0.10^b
Fetal body length (cm)	2.51 ± 0.04^a	1.76 ± 0.22^c	2.51 ± 0.03^a	2.26 ± 0.15^b	2.51 ± 0.04^a	2.27 ± 0.21^b
Fetal head length (cm)	0.99 ± 0.06^b	0.90 ± 0.05^d	1.12 ± 0.08^a	0.93 ± 0.01^{bc}	1.09 ± 0.10^a	0.95 ± 0.02^{bc}
Fetal head width (cm)	0.87 ± 0.01^a	0.76 ± 0.02^d	0.86 ± 0.04^{ab}	0.83 ± 0.01^c	0.87 ± 0.01^a	0.84 ± 0.01^{bc}

Means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$).

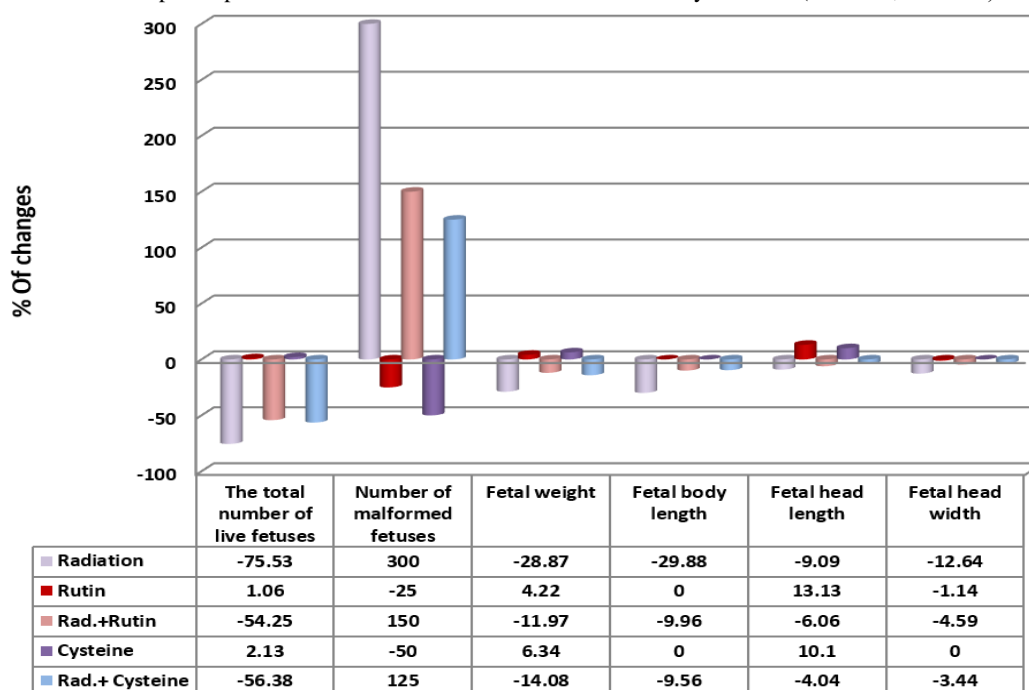|	The total number of live fetuses	Number of malformed fetuses	Fetal weight	Fetal body length	Fetal head length	Fetal head width
Radiation	-75.53	300	-28.87	-29.88	-9.09	-12.64
Rutin	1.06	-25	4.22	0	13.13	-1.14
Rad.+Rutin	-54.25	150	-11.97	-9.96	-6.06	-4.59
Cysteine	2.13	-50	6.34	0	10.1	0
Rad.+ Cysteine	-56.38	125	-14.08	-9.56	-4.04	-3.44

Figure 4. The change in percentages of the total number of live fetuses, Number of malformed fetuses, Fetal Wight (g), fetal body length (cm), fetal head length (cm) and fetal head width (cm)in all the tested groups in comparison to the corresponding control group.

Morphological observations of the control uterus obtained from pregnant rats on day 20 of gestation showed normal distribution of the implanted fetuses between the two horns. The uterus and fetuses of the pregnant rats were whole body irradiated at a single dose of 2 Gy gamma rays in the 17th day of gestation showed reduced number of implanted sites and high incidence of prenatal mortality (Figure 5). Furthermore, the fetuses of this group revealed severe malformations as exencephaly, bending of the body (protrusion), micromelia and spina bifida of total. Embryos of mother rats treated with rutin or cysteine before irradiation illustrating normal development. The embryos have normal morphology and normal length (Figure 6)
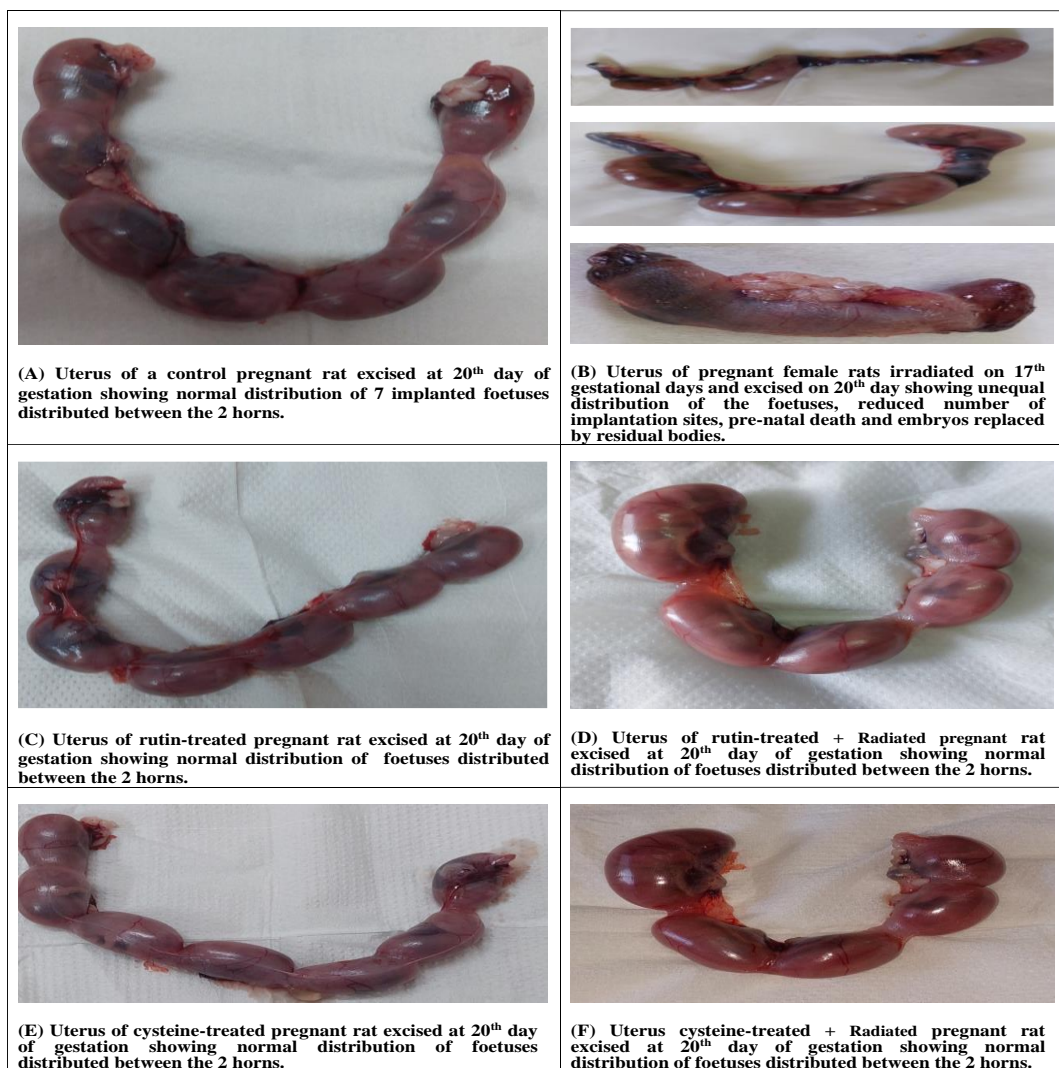
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(A) Uterus of a control pregnant rat excised at 20th day of gestation showing normal distribution of 7 implanted foetuses distributed between the 2 horns.

(B) Uterus of pregnant female rats irradiated on 17th gestational days and excised on 20th day showing unequal distribution of the foetuses, reduced number of implantation sites, pre-natal death and embryos replaced by residual bodies.

(C) Uterus of rutin-treated pregnant rat excised at 20th day of gestation showing normal distribution of foetuses distributed between the 2 horns.

(D) Uterus of rutin-treated + Radiated pregnant rat excised at 20th day of gestation showing normal distribution of foetuses distributed between the 2 horns.

(E) Uterus of cysteine-treated pregnant rat excised at 20th day of gestation showing normal distribution of foetuses distributed between the 2 horns.

(F) Uterus cysteine-treated + Radiated pregnant rat excised at 20th day of gestation showing normal distribution of foetuses distributed between the 2 horns.

Figure 5. Photomicrograph of uteri on day 20 of gestation in different groups [(A): control pregnant rat, (B): Radiated pregnant rats, (C): rutin-treated pregnant rat, (D): rutin-treated + Radiated pregnant rat, (E): cysteine-treated pregnant rat and (F): cysteine-treated pregnant rat].

4. Discussion

The results of the current research showed that whole-body gamma irradiation of pregnant rats at a dose of 2 Gy on the 17th day of gestation caused a major biochemical disturbance, uterine injury, and damage to the fetuses of the rats. Supplementing with antioxidants has beneficial effects via multiple routes, including as direct scavenging of reactive oxygen species (ROS) and repair of damage. Moreover, antioxidants improved clinical pregnancy rates and live birth weight. Study characteristics showed a considerable improvement when mother rats were given either cysteine or rutin as a protective measure prior to radiation.

When exposed to ionizing radiation, the body produces too many reactive oxygen species (ROS). This is because the radiation causes the water in cell structures to radiolyze, which in turn increases the creation of ROS like hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and superoxide anion (O2) [26]. ROS, like OH, pose a serious risk to bio-membranes because they interact with essential molecules, changing and even destroying them. This is linked to an increase in lipid peroxidation and a decrease in the body's antioxidant enzyme activity [27]. The results of this study showed that, when compared to normal controls, irradiated rats (2 Gy, single dose) produced ROS that caused cell damage through an adverse effect on antioxidant defense mechanisms, as evidenced by a decline in SOD and CAT concentrations and a significant elevation in lipid peroxidation products (MDA) concentrations.
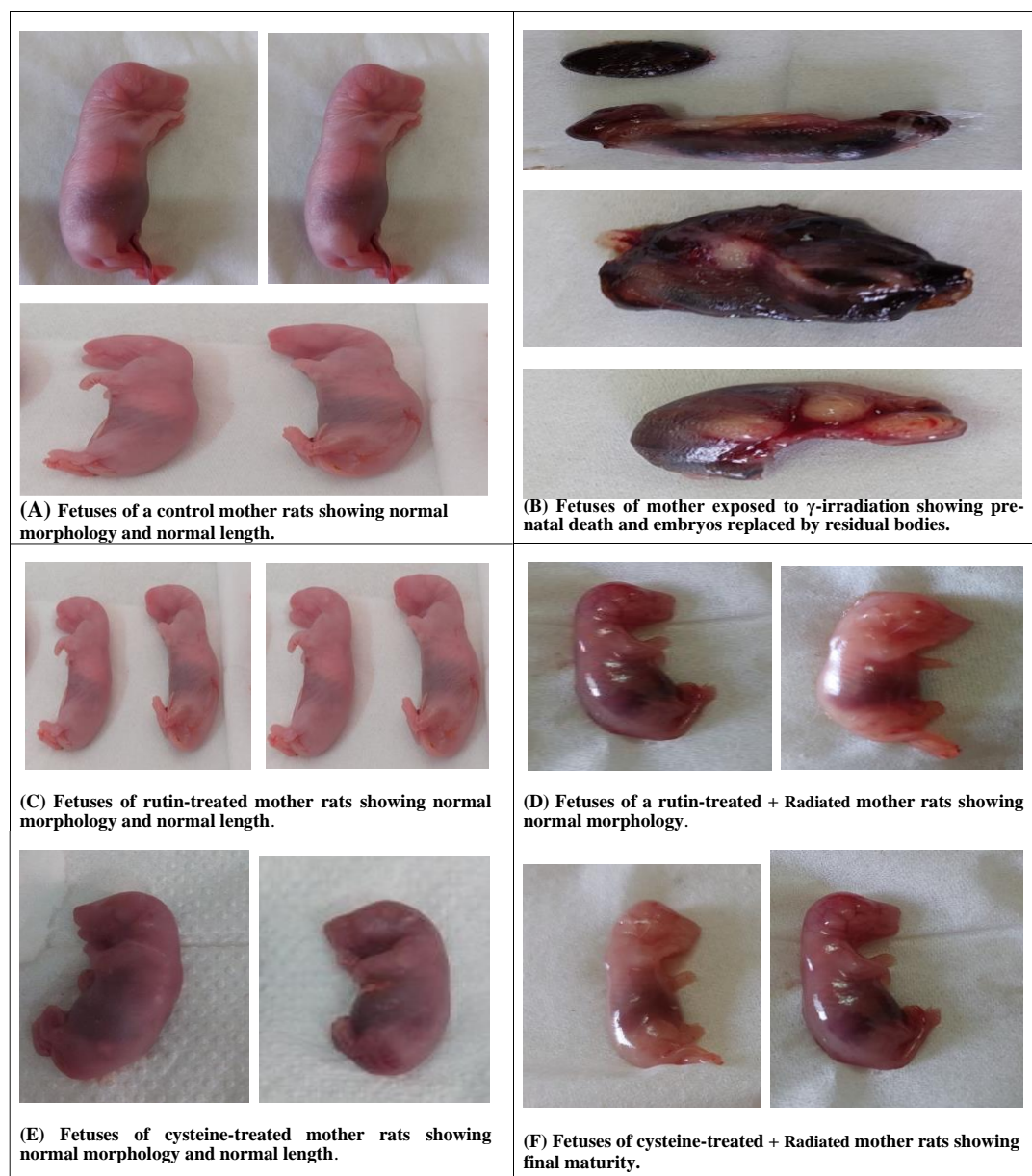
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(A) Fetuses of a control mother rats showing normal morphology and normal length.

(B) Fetuses of mother exposed to γ-irradiation showing pre-natal death and embryos replaced by residual bodies.

(C) Fetuses of rutin-treated mother rats showing normal morphology and normal length.

(D) Fetuses of a rutin-treated + Radiated mother rats showing normal morphology.

(E) Fetuses of cysteine-treated mother rats showing normal morphology and normal length.

(F) Fetuses of cysteine-treated + Radiated mother rats showing final maturity.

Figure 6. Photomicrograph of fetuses in different groups. Extruded fetuses from [(A): control mother rat, (B): Radiated mother rats, (C): rutin-treated mother rat, (D): rutin-treated + Radiated mother rat, (E): cysteine-treated mother rat and (F): cysteine-treated mother rat].

The most prevalent enzymatic antioxidant, SOD, which changes superoxide to H_2O_2 in the mitochondria and cytoplasm, thus exposed subjects to radiation is connected to the decline in serum SOD concentrations [28]. Other enzymes, like CAT, that are used to scavenge free radicals and neutralize them in the body, can then convert H_2O_2 to H_2O. These enzymes (SOD & CAT) play a critical role in regulating the delicate equilibrium between superoxide and H_2O_2, as an excess of the latter may react with endogenous transition metals by Fenton mechanics, producing hydroxyl ions [29]. Moreover, the highly reactive (OH•) assault primarily targets the polyunsaturated fatty acids found in phospholipids found in cell membranes [30]. According to Ye et al. [31], radiation caused damage to the subcellular organelles' membranes, which may have resulted in the peroxidation of their lipid components and an increase in the concentration of MAD. Hussein and Abd Rabu [19] noted that pregnant mice subjected to 2 Gy of ϒ-radiation at the 14th day of gestation showed a substantial rise in MDA and a decrease in GSH.

In the current investigation, we discovered that at day 17 of gestation, 2 Gy of gamma irradiation causes a large decline in prolactin and anti-mullerian hormone concentrations along with a considerable increase in estradiol, Follicle stimulating

hormone and luteotropic hormone in contrast to the control group. FSH, LH, prolactin, estradiol, and progesterone all had the following variation tendencies during pregnancy: FSH and LH significantly decreased because of the inhibition effect of the hypothalamus-pituitary negative feedback, which was caused by a large increase in estrogen and progestin secreted by the corpus luteum in the early stages of pregnancy and the placenta in the later stages of pregnancy [32]. According to previous study, radiation exposure during pregnancy may have caused a considerable fall in progesterone levels, which may have resulted in fetal mortality and resorption, ending the gestation period [33]. It was found that the placental tissue damage by gamma irradiation included trophoderm cell enlargement or death as well as a decrease in the quantity of blood vessels in the placenta tissue [34]. This condition inhibited the secretion of progesterone, preventing the pregnancy from effectively rejecting the toxic components caused by placental dysfunction [35]. In addition, the elevated levels of E2 in irradiated group may be due to decrease in progesterone levels where, McCormack and Greenwald [36] found that when progesterone levels in the blood decrease, estrogen (such as estradiol) levels rise gradually. According to certain research, progesterone reduces oestrogen receptor expression to reduce estrogen response, while estrogen enhances progesterone receptor expression in the uterus [37].

Furthermore, the hyper-secretion of FSH and LH seen following whole body gamma irradiation may be the consequence of either a positive feedback mechanism in reaction to the decreased blood progesterone levels or the activation of the hypothalamic-pituitary axis. Moreover, radiation caused central nervous system damage that might have an impact on hormone secretion [38]. Moreover, exposure to gamma radiation also resulted in a reduction in the AMH level. According to previous report, AMH is regarded as a sensitive biomarker of the longitudinal loss of ovarian reserve since it is produced in the granulosa cells of late prenatal and small antral follicles [39]. An elevated FSH level coupled with a low AMH value significantly increases the risk of ovarian failure [40]. These results matching with previous study where 5Gy X-ray irradiation induce ovarian damage in female rats this indicating by decline in P, E2 and AMH as well as significant increase in FSH and LH [41]. Additionally, ovarian failure in female mice exposed to X radiation (4 Gy) was significantly impacted by significantly greater concentrations of FSH and LH and lower levels of progesterone and AMH [42].

Major immunoglobulins like IgG and IgM are essential for neutralizing bacteria, viruses, and poisons as well as for complement activation and opsonization [43]. The notable reduction in serum IgG and IgM in 17 gestation rats exposed to Ɣ-radiation clarified the effect of Ɣ-radiation on B and T cell activities; this is indicating a suppression of the humoral immune response and a heightened vulnerability to bacterial and viral infections. Oxidative stress brought on by Ɣ-radiation can affect the immune system by impeding the ability of hematopoietic stem cells to develop into various immune cells, including macrophages, dendritic cells, lymphocytes, and so on [44]. It was reported that gamma irradiation induces immunosuppression through decline IgG and IgM [45, 46]. Furthermore, oxidative stress usually causes inflammation and reduces immune system function, which leads to growth-retarded fetuses [41].

These biochemical changes mirrored the morphological alternation in the mother uterus and fetus where we found that single dose of 2 Gy gamma rays in the 17th day of gestation showed reduced number of implanted sites and high incidence of prenatal mortality. Furthermore, the fetuses of this group revealed reduce in weight, body length, head length and the head width, and severe malformations as exencephaly, bending of the body (protrusion), micromelia and spina bifida of total. This may be attributed to direct effect of radiation on DNA of fetus (includes loss or damage to the bases and single or double strand breaks of DNA) or indirect effect by increase ROS specially hydroxyl ions (•OH) generated close to DNA attack the base pair and may cause mutation [47]. Moreover, the present research observed that increase in ROS through increase MDA as well as decrease in CAT and SOD that makes suppression immunity and disturbance in sex hormones which may leads to severe malformations in the fetuses. It was reported that increased DNA damage consequently affects the embryonic development and could increase the chances of an abnormal embryo or may also result in resorption [48]. It was reported that degenerated cells of placenta taken from irradiation exposed pregnant rats may be responsible for reduced size of fetuses and may be due to the disturbance in transport of nutrients to the fetuses and resulted in fetal disorders [49]. It was stated that irradiation caused fetal growth and embryo lethality due to inhibition of protein synthesis or placental dysfunction [50]. Moreover, it was found that exposed pregnant rats to gamma irradiation showed an increase in the incidence of intrauterine fetal death, as well as induced uterine growth retardation [51]. It was found a complete embryonic resorptrions on day 9 and complete and partial resorption on day 11 of gestation after in utero exposure to 2 Gy gamma rays in mice [52]. There was a notable fetal intrauterine death along with impaired growth of the live ones in the studied uteri of the pregnant rats excised three days after whole body 4 Gy gamma irradiation (day 20 of gestation) [53].

Two factors that might cause tissues to malfunction and ultimately destroy the target tissue are oxidative stress and inflammation. Thus, lowering oxidative stress and reducing inflammation by antioxidants are the two most crucial strategies for mitigating such negative impacts induced by radiation. Therefore, the biochemical changes in mother rats and their fetus abnormalities encountered in the irradiated groups were very much remodeled by the protection by rutin or cysteine treatments. Rutin (polyphenol) and cysteine (rich by SH group) are examples of bioactive compounds with antioxidant, anticancer, anti-inflammatory, and antibacterial properties [54]. In addition to reducing the number of dead fetuses, the supplementation of cysteine and rutin polyphenols as a protective measure before irradiation at day 17 of gestation improved female antioxidant status, sex hormones, and immunity. This may be the result of rutin's or cysteine's enhanced antioxidant capacity, which scavenging of free radicals and other oxidizing intermediates thus helps to ameliorate progesterone and estradiol levels and improve IgM and IgG content in pregnant rats.

Rutin as flavonoids have antioxidant activity include hydrogen donors, chelation of iron or copper ions, inhibition of oxidases, and scavenging of lipid and protein-derived radicals [55]. Cysteine has been shown to be effective at scavenging free radicals, decomposing peroxide, catalyzing the exchange of sulfhydryl disulfide, hydrogen atom donation by –SH groups, and perhaps aiding in the restoration of damaged areas [56]. It was report that rutin has an antioxidant action that includes enhancing the activity of enzymes such as SOD, GST, GGT, CAT, and GPx GR as well as activating the Nrf2/HO 1 pathway [57]. Rutin suppresses well-known proinflammatory molecules and mediators, such as TGF-β1, COX-2, iNOS, TLR4, and XO, which is

how it gets its anti-inflammatory effects. Moreover, it was discovered that in rats with polycystic ovarian syndrome, rutin was crucial in lowering oxidative stress, increasing antioxidant status, and perhaps lowering ROS and lipid peroxidation [58]. Rutin treatment significantly reduced the ROS in reproductive system induced by Cisplatin through decreasing MDA levels and increasing GSH-Px, SOD and CAT activities and GSH levels [59]. It was reported that rutin significantly protected against inflammation, oxidative stress, and DNA damage induced by acrylamide in reproductive system of female rats, likely due to its antioxidant properties [60]. Furthermore, it was reported that rutin possesses sufficient potential for increasing immune activity by cellular and humoral mediated mechanisms [61]. It was also reported that the e potent antioxidant nature of rutin mitigate the oxidative stress induced by gamma radiation and thus protect the mice from hemopoietic damage [62].

By raising glutathione and SOD and lowering MDA, rutin or cysteine have been shown to have a possible protective effect against radiation [63]. It was reported that treatment of rats with N-acetylcysteine that mitigated radiation-induced myocardial damage through enhancement antioxidant [64]. In addition, N-acetyl cysteine showed beneficial effects against damage induced on islets of Langerhans cells in rats with X-ray ionizing-radiation [65]. Cysteine enhanced antioxidant status irradiated rats by decreasing MDA and increase GSH in small intestine [66]. Numerous mechanisms have been suggested to clarify the radioprotective effects of sulfhydryl compounds including free radical scavenging, hydrogen atom donation by –SH groups and –COOH groups), repair of free radicals in target molecules, target stabilization through DNA binding, mixed disulfide formation, and overall improved protection against oxidative stress [67, 68].

5. Conclusions

Exposure pregnant rats to ionizing radiation at 17th day of gestation induce excessive production of ROS that leads to adverse effect on antioxidant defense mechanisms, disturbance in sex hormones, immunosuppression, uterine injury, and damage to the fetuses of the rats. It's interesting to note that taking rutin or cysteine supplements during pregnancy improves antioxidant capacity and immunological function against radiation damage.

6. Conflicts of interest

There are no conflicts to declare".

7. Acknowledgments

8. References

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