Middle East Journal of Applied Sciences 4(2): 365-372, 2014
ISSN 2077-4613

Rhoifolin Alleviates Gamma-Irradiation Induced Toxicityin Whole Body Irradiated Swiss Albino Mice (Biochemical and haematological studies)

Omama E. ELShawy

Department of Clinical Health Radiation Research.National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA). Cairo, Egypt.P.O.Box 29, Nasr City, Cairo, Egypt

ABSTRACT

Natural radio protective compounds have been used to mitigate the toxicity induced by ionizing radiation exposure during radiotherapy planning. This study has been undertaken to explore the radio protective potency of rhoifolin against whole body gamma irradiation induced haematological and hepatic alterations in Swiss albino mice. Twenty-four male mice (25-30 g) were randomly assigned into 4 groups (6 mice / group). i: Normal control mice received (saline + 0.05% DMSO). ii:mice received rhoifolin (36 mg/Kg body weight) orally for seven consecutive days. iii: mice were exposed to a single irradiation dose (10 Gy). iv: Experimental group: animals administered rhoifolin as in (G 2), thirty minutes after the last rhoifolin administrated dose; mice were exposed to gamma rays as in (G3). All animal groups sacrificed 24 hrs after irradiation exposure. Blood samples and liver tissues were taken for the various haematological and biochemical analysis. Results indicated that Irradiation provokes a significant reduction in Hb content, Ht% as well as RBCs and WBCs counts, associated with marked alterations in biochemical parameters in plasma and homogenate tissue of treated mice. Rhoifolin treatment pre-irradiation exposure ameliorated the haematological and biochemical changes in mice. In conclusion**,** rhoifolin showed a marked improvement and renders protection against whole body irradiation, which might be due to its ability to repair the damages, reduce the LPO and augmentation of the endogenous antioxidants associated with an improvement of the other investigated biochemical parameters and the blood indexes.

Key words**:** Rhoifolin, radiation protection, liver, biochemical analysis, haematological indices, albino mice.

Introduction

With increasing use of radiation for the medical diagnostic and treatment purposes, it is essential to protect humans against deleterious effects of radiation, in addition to its utility in cancer treatment (Chaudhary *et al.*,2008).Ionizing radiation inflicts its adverse effects through the generation of oxidative stress that unleash large-scale destruction or damage of various biomolecules (Moritake *et al.,* 2003; Yusuf *et al.,* 2011). Free radicals generating react with body tissues caused lipid peroxidation, DNA lesions and enzyme inactivation, all of which are mediators of radiation damage. Molecules with the ability to scavenge free radicals can therefore serve as radio protective molecules in preventing radiological damage (Tiwari *et al.,* 2010; Alcaraz *et al.,* 2009). It has been considered that radiotherapy for cancer patients could be improved by the use of radio protectors to protect normal tissue. But synthetic protectors, so for tested are found to be toxic at their effective dose levels, which limit their clinical application (Jindal *et al.,* 2010).

Recent studies have shown that commonly used medicinal plants and herbs are good sources of radioprotectors in experimental models and in patients receiving radiotherapy (Adaramoye *et al.,* 2008). Many investigators demonstrated that flavones are commonly abundant in medicinal plants and herbs (Wojdyło *et al.,* 2007) are nontoxic and non-mutagenic (Patel *et al.,* 2007). Rhoifolin found to have various biological activities. It exhibits beneficial effect for diabetic complications (Rao *et al.,* 2011), anti-inflammatory (Eldahshan and Azab, 2012) and anti proliferative effects (Eldahshan, 2013).Therefore, the present study is an attempt for the first time to explore the efficacy of rhoifolin in modulating the radiation induced haematological and biochemical alterations in the liver of Swiss albino mouse and the mechanisms underlying these effects.

Materials And Methods

Animals:

All animal experiments were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. Twenty four male albino mice weighing 20-25g were used in this study. They were maintained under controlled conditions of temperature and light (14 and 10 hrs of light & dark, respectively).The animals were acclimated to these conditions for one week.

Corresponding Author: Omama E. ELShawy, Department of Clinical Health Radiation Research.National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA). Cairo, Egypt.P.O.Box 29, Nasr City, Cairo, Egypt
E-mail: dromaymaelshawi@hotmail.com.

All reagents were of the highest purity available. Chemicals for measurement of antioxidants,lipid peroxidation purchased from Sigma Chemical Co. (St. Louis, MO). All kits used for investigation of Plasma biochemical parameters were supplied by Bio-diagnostic Company, Cairo, Egypt.

Irradiation:

The animals were exposed to whole body gamma radiation by the use of a Canadian Gamma Cell-40 (137Cs) at the National Centre for Radiation Research and Technology (NCRRT) Cairo, Egypt. The dose rate was 0.670 Gy /min as calibrated during the experimental periods.

Preparation of rhoifolin:

Rhoifolin (apigenin 7 neohesperidoside) was isolated from *Chorisiacrispiflora* leaves (Eldahshan and Azab, 2012) in the form of yellowish powder. Rhoifolin was dissolved in (saline + 0.05% disulfidesulfoxide (DMSO), and was immediately prepared before oral administration*(po).*

Animal groups and experimentals:

Animals were divided into four groups. i) Control group: mice were orally administered 1ml(saline + DMSO)/ time, for 7 consecutive days. ii) Rhoifolin treated group: mice received the optimum dose (36 mg/kg b.wt./day) of rhoifolin in solution (*po*) for 7 consecutive days. The optimum dose was estimated previously (Elshawi and Eldahshan, 2014) (iii) Irradiated group: mice subjected to single irradiation dose (10Gy), andvi) Experimental group: animals treated as in group 2, thirty minutes post administration of the last dose of rhoifolin, mice exposed to single irradiation dose (10Gy). At the end of the experiment, animals were kept overnight fastened and allowed free access to water only and were sacrificed 24 hours post irradiation exposure under diethyl ether anaesthesia. Blood samples and liver tissues were taken for haematological and biochemical analysis.

Preparation of samples:

Whole blood was collected by heart puncture into heparinized test tubes. A portion was separated into vials for the entire blood counts. The total numbers of erythrocytes (RBCs) and leukocytes (WBCs), haemoglobin (Hb) concentration, hematocrite (Ht) % were estimated by an automatic counter (Coulter Model T-450; Contronics, UK). Plasma was separated from the other portion of the blood samples for the other biochemical assays. Whole liver was quickly excised, rinsed in ice cold saline, blotted and dry. The liver tissue was accurately weighed and homogenized in ice cold phosphate buffer (pH 7.4) to prepare 10% (W/V) tissue homogenate. Liver homogenate and a portion of blood samples were centrifuged at 3000g for 15 min at 4^0C, the supernatant and plasma were collected and stored at - 20°c till biochemical analysis.

Biochemical analysis and measurements for each parameter was carried out according to its specific procedure for estimation. Lipid peroxidation product measured as Malondialdehyde (MDA) (Ohkawa *et al.,* 1979). The level of nitric oxide (NO)(Montgomery and Dymock, 1961), and GSH (Beutler *et al.,* 1963), and the activities of SOD (Minami and Yoshikawa, 1979), CAT(Johansson and Borg, 1988) and lactate dehydrogenase (LDH)(Young, 2001) were also estimated. The content of DNA (Ceriotti, 1952), RNA (Ceriotti, 1955), and total cholesterol (Allain *et al.,* 1974) all were assayed in liver homogenate. Plasma biochemical parameters were measured on the basis of the following methods: total protein (Burtis, and Ashwood, 1994), alkaline phosphatase (ALP)(Belfield and Goldberg, 1971), γ-glutamyltransferase (GGT) (Szasz *et al.,* 1974), albumin (Burtis, and Ashwood, 1994) and total globulin (Luxton 1999).

Statistical Analysis:

The data are expressed as mean ± standard error of the mean (M±SE). The significant differences among groups were determined by one-way analysis of variances using SPSS package program, version 15. The results were considered significant if the values of p were <0.05.

Results:

The results in table 1 demonstrated that treatment of mice with rhoifolin (36 mg/kg b.wt/day) for 7 consecutive days didn't induce any significant change in the haematological parameters of mice compared to control. Unlike, irradiated animals (10 Gy) showed a significant decrease in RBCs and WBCs counts as well as in Hb concentration and % Ht by -25, -51, -22, -26%, respectively compared to those of the controls. Otherwise,

the severity of alterations in these parameters in the experimental group (G4) was decreased by 13, 35, 18, 13%, respectively compared to the values of the irradiated group.

The results obtained in the current investigation demonstrated a significant decrease in the content of DNA, RNA and total cholesterol by -49%, -39%, -48% respectively in liver of irradiated mice as compared to the normal values. Furthermore, rhoifolin treatment pre-irradiation in the experimental group (G4) reduced the reduction of these parameters significantly ($p \leq 0.05$)by 47%, 28%, and 25%respectively compared to the corresponding values of the irradiated mice (table 2).

On its own, rhoifolin did not induce any significant change in the estimated parameters presented in Table 3. Meanwhile, a considerable rise was occurred in MDA and NO as well as in LDH level by 41% and 49% and -20% respectively, accompanied by a significant decrease in GSH content, SOD, CAT activitiesby-35%, -27%, and -37% respectively in irradiated mice compared to the controls. Unlike, administration of rhoifolinin the experimental group significantly lowered the elevated MDA level (-25%), NO concentration (-37%) and the activity of LDH (-20%) in the liver homogenates of experimental group as compared to the corresponding values in the irradiated group. Otherwise, GSH concentration, as well as SOD and CAT activities were significantly higher by 52%, 21%, 21% respectively compared to the irradiated group (table 3).

The levels of ALT, AST, γ-GT as well as ALP, total globulin and albumin in plasma, as well as total protein (in plasma and hepatic tissue) were illustrated in Table 4. No significant change in these parameters induced byrhoifolin treatment alone. Unlike, a significant elevation was observed in the activity of ALT, AST,γ-GT and ALP by 295.6, 141.4, 142.2, 108.4% respectively comparable with significant decrease in total protein in plasma and tissue as well as the protein fractions (total globulin and albumin) by -18.8, -35.8, -44.18, -34.02 % respectively compared to the corresponding normal values. More so, rhoifolin treatment prior to irradiation exposure in experimental group markedly ameliorated the changes in these parameters compared to the values recorded in the irradiated group ($p \leq 0.05$).

Table 1: Changes in blood indexes of mice post exposure to gamma irradiation whether mice supplemented with rhoifolin or not.

Parameters	Animal Groups			
	Control	Rhoifolin	Radiation	Rhoifolin + Radiation
RBCs (10^6/mm^3)	10.25±0.43	10.28±0.53^{NS} *0.29	7.68±0.29^{a} *-25.07	8.69±0.61^{a,b} *-15.21 $^{\#}$13.15
WBCs (10^3/mm^3)	13.13±0.74	12.94±0.77^{NS} *-1.44	6.43±0.27^{a} *-51.03	8.68±0.39^{a,b} *-33.89 $^{\#}$34.99
Hb(g/dl)	11.11±0.44	11.08±0.59^{NS} *-0.27	8.61±0.52^{a} *-22.50	10.17±0.45^{b} *-8.46 $^{\#}$18.11
Ht(%)	38.84±0.67	38.85±0.39^{NS} *0.03	28.76±0.83^{a} *-25.95	32.56±1.85^{a,b} *-16.16 $^{\#}$13.21

*Values are presented as means ± S.E (n = 5 variables). *: represent the % of change (loss or increase) from the normal values.#: represent the % of change (loss or increase) from values of the radiated group. a: significances (control vs radiation, control vsrhoifolin + Radiation at $p \leq 5$, b: significance Radiation vsrhoifolin+ Radiation at $p \leq 5$.NS =non significant.*

Table 2: DNA, RNA, total protein and total cholesterol contents in theliver of irradiated mice whether mice supplemented with rhoifolin or not.

Parameters	Animal Groups			
	Control	Roifolin	Radiation	Rhoifolin + Radiation
DNA mg/g tissue	24.56±1.96	25.52±0.86 *3.91	12.56±0.75^{a} *-49	18.52±1.12^{a,b} *-24.59 #47
RNA mg/g tissue	10.21±0.83	10.30±1.13 *0.88	6.17±0.54^{a} *-39.56	7.88±0.28^{a,b} *-22.82 #28
Total Cholesterol mg/g tissue	5.42±0.42	5.27±0.34 *-2.76	2.79±0.23^{a} *-48.52	3.43±0.22^{a,b} *-36.71 #25

*Values are presented as means ± S.E (n = 5 variables). *: represent the % of change (loss or increase) from the normal values.#: represent the % of change (loss or increase) from values of the radiation group. a: significance (control vs Radiation, Control vsRhoifolin + Radiation) at $p \leq 5$. b: significance Radiation vsRhoifolin + Radiation at $p \leq 5$.*

Table 3: Malondialdehyde (MDA), Nitric oxide (NO), Antioxidants and Lactate dehydrogenase (LDH) activities in the liver of irradiated mice whether mice supplemented with Rhoifolin or not.

Parameters	Animal Groups			
	Control	Rhoifolin	Radiation	Rhoifolin + Radiation
MDA (nmol/g)	2.17±0.18	1.95±0.34 *-4.21	4.63±0.37 [a] *113.36	3.46±0.31[a, b] *59.44 #-25.26
NO (µmol/g)	2.19±0.29	2.20±0.27 *1.004	3.27±0.22 [a] *49.31	2.06±0.38 [b] *-5.93 #-37
GSH (µg/g)	3.25±0.31	3.30±0.54 *-1.51	2.09±0.33 [a] *-35	3.18±0.20 [b] *-2.15 #52
SOD (µmol/g)	43.07±2.17	44.86±2.82 *4.15	31.06±2.36 [a] *-27.88	37.64±1.66[a,b] *-12.6 #21
CAT (µmol/g)	64.54±3.31	62.71±3.15 *-2.83	40.61±2.89 [a] *-37	49.38±2.97[a,b] *-23.5 #21.5
LDH(U/g)	5.10±0.21	4.87±0.23 *-4.5	8.51±0.32 [a] *66.86	6.07±0.36[a,b] *19 #-20.44

*Values are presented as means ± S.E (n = 5 variables). *: represent the % of change (loss or increase) from the normal values.#: represent the % of change (loss or increase) from values of the radiation group. a: significances(Control vs Radiation, Control vsRhoifolin + Radiation) at p ≤ 5. b: significance (Radiation vsRhoifolin + Radiation) at p ≤ 5.*

Table 4: Variations in biochemical parameters in liver of irradiated mice whether mice supplemented with rhoifolin or not.

Parameters	Animal Groups			
	Control	Rhoifolin	Radiation	Rhoifolin + Radiation
ALT (U/L)	22.8±1.93	23.2±2.13 *1.75	90.2±5.50[a] *295.61	66.8±2.78[a,b] *192.98 #-25.94
AST (U/L)	30.4±2.08	27.4±1.36 *-2.63	73.4±4.58 [a] *141.44	52.8±3.18[a,b] 73.68 #-28.07
□-GT (U/L)	42.2±2.37	41.2±1.49 *-2.36	102.2±3.36 [a] *142.18	84±5.58[a,b] *99.05 #-17.81
ALP (U/L)	52.6±2.9	52.0±5.14 *-1.14	109.6±4.64 [a] *108.36	88.8±4.34[a,b] *68.82 #-18.97
Total protein (g/dl)	10.6±0.59	11.1±0.79 *1.04	5.24±0.43 [a] *-81.99	7.76±0.49[a,b] *-26.79 #48.09
Total Protein mg/g tissue	31.52±2.2	33.84±1.2 *1.07	20.22±0.98 [a] *-35.85	24.52±0.89[a,b] *-22.20 #2126
Total globulin(g/dl)	4.3±0.56	4.1±0.44 *-4.6	2.4±0.16 [a] *-44.18	3.5±0.16[a,b] *-18.60 #45.83
Albumin (g/dl)	4.82±0.26	4.92±0.15 *7.58	3.18±0.21 a *-34.02	4.00±0.23a,b *-17.01 #25.78

*Values are presented as means ± S.E (n = 5 variables). *: represent the % of change (loss or increase) from the normal values.#: represent the % of change (loss or increase) from values of the radiation group. a: significance (Control vs Radiation, Control vsRhoifolin + Radiation) at p ≤ 5. b: significance (Radiation vsRhoifolin + Radiation) at p ≤ 5.*

Discussion:

Exposure to ionizing radiation generates reactive oxygen species (such as superoxide radicals, hydroxyl radicals, iron oxygen complexes, hydrogen peroxide and lipid peroxides) resulting in oxidative damage to cell membranes, disturbance in metabolism as well as in structure and function of body organs (Kanshala,2004, Mantena *et al.*,2008). Different medicinal plants have been evaluated for their potential effects as a radioprotective agent. To our knowledge, no studies have been done to investigate the protective role of rhoifolin against gamma radiation (10Gy) induced liver damage and haematological disorders.

In the present study, the results showed that whole body gamma irradiation of mice(10 Gy) produced acute haematological disorders. This was evident from the significant decrease in RBCs and total WBCs count, haemoglobin level, and Ht percentage. The decline in RBC count may be attributed to haemorrhage and lesions

in blood vessels (Verma *et al.,* 2008). The marked depletion in haemoglobin concentration may be due to depletion in the synthesis of haemoglobin after radiation exposure (Nunia *et al.,* 2004). The depression of haematocrit value could be result from cell depletion in peripheral blood and disturbances of the steady state mechanisms in blood forming organs as well as an increase in plasma volume after irradiation (Singh *et al.,* 2006). The fall in total leukocyte counts might beattributed to a fast decline of radiosensitive lymphocytes (Samarth *et al.,*2003). The blood cells count in the experimental group (radiation + rhoifolin) was higher than that of the corresponding irradiated group values, suggesting that rhoifolin could boost the recovery of haematopoietic function and brought about a subsequent rise in haematological constituents in peripheral blood by reducing cells injure after whole body irradiation of mice.

The data have shown that whole body gamma irradiation of mice (10 Gy) provoked a significant decrease in liver DNA and RNA contents compared to normal group. This is in conformity with the previous studies (Arya and Sharma, 2010, Sharma and Sisodia, 2009). The DNA damage could be due to direct hits from ionizing rays, or as secondary damage from radicals generated by radiation. In addition, the oxidative stress produced after irradiation exposure can also cause DNA damage owing to the damages of the bases and the sugar-phosphates, as well as single-or double-strand breaks within DNA. The decrease in DNA contents could be also attributed to the inhibition of DNA synthesis (Johari *et al.,* 2011). Also Abou-Sief *et al.* (2003) attributed the decrease in RNA content to the generation of reactive oxygen species (ROS) within the organism. Although these ROS are being produced continuously during normal respiration as well as through other biological pathways but exposure to ionizing radiations raises the generation of ROS in biological systems and are regarded as the major causative factor responsible for molecular damage. The marked decrease liver cholesterol in irradiated mice in our study is in a good harmony with others (Dhanraj *et al.,* 2007). They attributed this to the stress response caused by irradiation, which stimulates the synthesis of steroid hormones via hypothalamic-pituitary system. Moreover, our results clearly demonstrated that rhoifolin pre-irradiation treatment (G4) caused an increase in DNA and RNA contents as well as hepatic total cholesterol. This suggests the protective effect of rhoifolin against the radiation induced the depletion of aforementioned parameters as compared to the values of the irradiated group.

The present study revealed that whole body gamma irradiation (10 Gy) augmented the lipid peroxidation in the liver as evidenced by the increase in MDA level. The result is in concurrent with others (Geng *et al.,* 2012). This is probably owing to the increase in production of the free radical that initiates lipid peroxidation (Gaura and Bhatia, 2009)as a result of the interaction of $^{\bullet}OH$ resulting as a bi-product of water radiolysis upon exposure to ionizing radiation with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes(Spitz *et al.,* 2004). The perpetuation of cellular membrane integrity depends on protection or repair mechanism capable of neutralizing oxidative reactions. As presented in Table 3, treatment of mice with rhoifolin pre-irradiation (G4) significantly reduced the lipid peroxidation (LPO) in comparison with irradiated group as reflected by inhibition in MDA level, which testifies to our belief that one of the possible mechanisms of radiation protection by rhoifolin may be due to its scavenging capacity of the free radicals generated due to irradiation exposure and prevents the formation of endo-peroxidation. On the other hand, the significant increase in liver NO level of the irradiated mice comes in harmony with the findings of earlier investigators (Voevodskaya and Vanin, 1992, Nakagawa *et al.,* 201). They demonstrated that gamma irradiation enhanced NO production in liver, lung, kidney, intestine, brain, heart and bone marrow. This influence could be due to up-regulation of NOS by gamma irradiation with further increase in NO production in mammalian cells (Lestaevel *et al.,* 2003, Gisone, *et al.,* 2004). GSH is one of the major components of cellular antioxidant system. It is the principal non-protein thiol functioning as an antioxidant and as a cofactor for enzymes involved in detoxification of xenobiotics (Singh, *et al.,* 2006).It acts as a radical scavenger due to redox sulphydryl group directly reacting with oxidant and transforms itself into oxidized glutathione. The significant decline in hepatic GSH content that was noticed in irradiated group could be owing to its enhanced utilization as an attempt to detoxify the free radicals generated by radiation. The increase in hepatic GSH content in the experimental group, as well as the decrease in MDA (LPO) in the same group as mentioned abovemay contribute to some extent to radioprotective activity of rhoifolin. Superoxide dismutase (SOD) and catalase (CAT) are of the primary antioxidant enzymes in the mammalian cells that are thought to be necessary for life in all oxygen metabolizing cells. SOD convert superoxide radical into hydrogen peroxide and molecular oxygen (O_2), while the catalase convert hydrogen peroxide into oxygen and water, and thereby protected against radiation-induced sickness and mortality(Anscher, *et al.,* 2005).The decrease in the activity of these antioxidant enzymes could be due to their interaction with ROS, which cause their denaturation and partial inactivation (Kregel and Zhang, 2007).LDH is very important measure to check for tissue damage. As the cells die, their LDH is released and find its way into the blood. Consistent with previous studies (Arya and Sharma, 2011),the significant elevation in the level of LDH observed in the present investigation could be attributed to the alteration of cell membrane permeability and disintegration. In the present study, it was observed that rhoifolin treatment pre-irradiation limited the alteration in the LDH enzyme. The results are in accordance with Arya and Sharma, (2011).They

reported that the results may be contribute to that the natural plant products could provide protection against irradiation damages.

Exposure to gamma radiation (10 Gy) induced hepatotoxicity as manifested by increase in serum ALT, AST, GGT and ALP. The marked elevation in the levels of ALT and AST enzymes could be a result of exposure of mice to irradiation and might be due to their release from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (Kregel and Zhang, 2007).The significant increase in serum GGT and ALP activities was noted in this study is in agreement with other reports (Kilciksiz, *et al.,* 2008, Arya and Sharma, 2011). Also, Emdin *et al*. (2005)stated that GGT is known as a key enzyme in the catabolism of GSH, a process induces production of ROS, pointed to that GGT plays a pro-oxidant role (Eghdami and Sadeghi, 2010). Therefore, the elevation in GGT level observed in the irradiated mice could be attributed to the oxidative stress induced by the whole body irradiation that inversely associated with antioxidants (Emdin *et al.,* 2005).On the other hand the elevation of alkaline phosphatase activity could be explained as a result of whole body irradiation that causes disruption of many powerful hydrolytic enzymes such as phosphatase and nucleases which cause damage upon release(Kilciksiz, *et al.,* 2008The amelioration of the aforementioned enzymes in the experimental group that received rhoifolin pre-irradiation exposure compared to irradiated group suggesting that rhoifolin has hepatoprotective effect and might reduce plasma GGT levels to exert its antioxidant effect and helps in stabilizing the cell membrane.

In agreement with Sisodia *et al.* (2004), our results demonstrated a marked decrease in tissue and plasma total protein accompanied by marked alterations in serum protein fractions (albumin and globulin) post irradiation exposure. The reduction in protein content may be due to its lyses or inhibition of release of synthesized polypeptides from polysomes (Lee, *et al*., 2004).It was observed that irradiation caused proteinuria associated with low serum albumin and total protein, and could be related in part to hepatic dysfunction and decreased protein synthesis (Moulder *et al.,* 2004). Moreover, a marked improvement was noted in protein content and its fractions in the experimental (G4) as compared to the corresponding values of the irradiated group. This view is in agreement with Bhatia *et al.* (2008) who demonstrated an increase of protein concentrations in animals supplemented with various medicinal plant extract. They attributed this to the antioxidant property of the medicinal plants enables to quench the free radicals generated wing to irradiation exposure, so that DNA and RNA can be protected from free radical attacks and enhancement of protein synthesis.

In conclusion, and in the view of our results, it could be concluded that rhoifolin exhibit a radioprotective effect and has the efficacy to mitigate the hematopoietic and hepatic injuries induced by exposure to lethal dose of gamma radiation. The radioprotective mechanisms of rhoifolin in the current study could mainly attribute to the inhibition of lipid peroxidation and modulation of GSH level, SOD and CAT activities in liver. The efficacy of rhoifolin may also due to its ability to stimulate or to protect haematopoiesis in bone marrow and a subsequent increase of haematological constituents in the peripheral blood and ameliorating the other hepatic enzymes of this animal model. Further investigations are required to add further information about the clinical applicability of rhoifolin in radiation protection.

References

Abou-Seif, M.A., M.M El-Naggar, M. El-Far, M. Ramadan, N. Salah, 2003. Amelioration of radiation-induced oxidative stress and biochemical alteration by SOD modelcompounds in pre-treated γ-irradiated rats. Clinica Chimica Acta, 337: 23-33.

Adaramoye, O., B. Ogungbenro, O. Anyaegbu, M. Fafunso, 2008. Protective effects of extracts of Vernoniaamygdalina, Hibiscus sabdariffa and vitamin C against radiation-induced liver damage in rats. J Radiat Res., 49(2): 123-31.

Alcaraz, M., C. Acevedo, J. Castillo, O. Benavente-Garcia, D. Armero, V. Vicente, M. Canteras, 2009. Liposoluble antioxidants provide an effective radioprotective barrier. *Br. J Radiol*. 82(979): 605-609.

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond, P.C. Fu, 1974. Enzymatic determination of total serum cholesterol.Clin. Chem., 20(4): 470-475.

Anscher, M.S., L. Chen, Z. Rabbani, S. Kang, N. Larrier, H. Huang, T.V. Samulski, M.W. Dewhirst, D.M. Brizel, R.J. Folz and Z. Vujaskovic, 2005. Recent progress in defining mechanisms and potential targets for prevention of normal tissue injury after radiation therapy. Int. J. Radiat. Oncol. Biol. Phys, 62(1): 255-259.

Arya, S. and J. Sharma, 2010. Modulation of radiation induced changes in nucleic acid content of liver of *Swiss albino mouse* by Tinosporacordifolia (Miers)." Iran. J. Radiat. Res., 8(3): 179-185.

Arya, S. and J. Sharma, 2011. Radioprotective Effect of Unripe Stone Fruit Pulp Extract on some Liver Enzymes of Swiss Albino Mouse. Pharmacologyonline, 1: 1067-1082.

Belfield, A., D.M. Goldberg, 1971. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine-Enzyme., 12: 561-573.

Beutler, E., O. Duron, B.M. Kelly, 1963. Improved method of the determination of blood glutathione, *J Lab Clin Med*. 61: 882-888.

Bhatia, A.L., R. Kamal, G. Verma and K.V. Sharma, S. Vats and M. Jain, 2008. Radioprotective role of Gymnemic acid on mice: study on hepatic biochemical alterations. Asian. J. Exp. Sci., 22(3): 427-432.

Burtis, C. and E. Ashwood, 1994. TIETZ Text Book of clinical chemistry. 2nd-Ed. W.B. Saunders Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. Chapter 28.

Ceriotti, G., 1955. Determination of nucleic acids in animal tissues.J Biol Chem., 214(1): 59-70.

Ceriotti, G.A., 1952. microchemical determination of desoxyribonucleic acid.J Biol Chem.,198(1): 297-303.

Chaudhary, R., S. Jahan, U. Gupta and P.K. Goyal, 2008. Radioprotective Potential of *TrigonellaFoenumGraecum*Seeds Extract. *Pharmacologyonline*, 2: 14-26.

Dhanraj, S., J. Archana, S. Inder and P.K. Goyal, 2007. Modulation of radiation-induced biochemical alterations in mice by rosemary (Rosemarinusofficinalis) extract. International Journal of Phytotherapy & Phytopharmacology., 14(10): 701-705.

Eghdami, A., and F. Sadeghi, 2010. Determination of Total Phenolic and Flavonoids Contents in Methanolic and Aqueous Extract of AchilleaMillefolium. Org. Chem. J, 2: 81-84.

Eldahshan, O.A. and S.S. Azab, 2012. Anti-inflammatory Effect of Apigenin-7-eohesperidoside (Rhoifolin) in Carrageenin-Induced Rat Oedema Model. Journal of Applied Pharmaceutical Science, 02(08): 74-79.

Eldahshan, O.A., 2013. Rhoifolin; a Potent Antiproliferative Effect on Cancer Cell Lines.British Journal of Pharmaceutical Research, 3(1): 46-53.

Elshawi, O.E. and O.A. Eldahshan, 2014. Protective Effect of Rhoifolin on Gamma Irradiation Induced Cardiac Dysfunctions in Albino Mice. Arab Journal of Nuclear Sciences and Applications , 47(1): 198-207.

Emdin, M., A. Pompella and A. Paolicchi, 2005. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation, 112: 2078-2080.

Gaura, A. and A.L. Bhatia, 2009. Effects of Radiation on the Protein and DNA Content in Genistein Treated Swiss Albino Mice. Iranian Journal of Pharmaceutical Sciences, 5(4): 239-248.

Geng, Y., X. Du, X. Cao, Y. Chen, H. Zhang, H. Liu, Z. Chen and X. Zeng, 2012. The therapeutic effects of *Zingiber officinale*extract on mice irradiated by 60Co γ-ray. Journal of Medicinal Plants Research, 6(13): 2590-2600.

Gisone, P., D. Dubner, M.R. Pérez, S. Michelin and S. Puntarulo, 2004. Role of Nitric Oxide in the Radiation-induced Effects in the Developing Brain.*in vivo*, 18: 281-292.

Jindal, A., A. Agrawal, S. Jahan, P. Verma, P.K. Goya, 2010. Protective Efficacy of *Rosemarius Officinalis* Extract against Radiation Induced Biochemical Alterations in Mice.Pharmacologyonline. 2: 194-203.

Johansson, L.H. and L.A.H. Borg, 1988. A spectrophotometric method for determination of catalase activity in small tissue sample. *Anal Biochem*.174: 331-336.

Johari, P., M. Kumar, A. Kumar, 2011. OLTIPRAZ: DNA and RNA Protection. *Pharmacologyonline,* 1: 651-659.

Kanshala, P., 2004. Cell membrane oxidative damage induced by γ-radiation and apoptotic sensitivity. *J. Environ Pathol Toxicol Oncol.* 23: 1-7.

Kilciksiz, S., C. Demirel, N. Erdal, S. Gürgül, L. Tamer, L. Ayaz and Y. Ors, 2008. The effect of N-acetylcysteine on biomarkers for radiation-induced oxidative damage in a rat model.Acta Med Okayama, 62(6): 403-9.

Kregel, K.C. and H.J. Zhang, 2007. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J PhysiolRegulIntegr Comp Physiol, 292: 18-36.

Lee, D.H., R. Blomhoff and D.R. Jacobs, 2004. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radical Res., 38(6): 535-539.

Lestaevel, P., D. Clarençon, A. Gharib, A. Peinnequin, R. Cespuglio, P. Gourmelon, A. Alonso, J.D. Laval and E. Multon, 2003. Nitric oxide voltammetric measurements in the rat brain after gamma irradiation, *Rad. Res.*, 160: 631-636.

Luxton, R., 1999. "Clinical Biochemistry": Biomedical Sciences explanined, C.J Pallister Company Butter worth, Heinemann Oxford New Delhi; Oxford, Auhland Boston. PP. Chapter., 12: 155.

Mantena, S.K., M.K. Unnikrishnan, R. Joshi, 2008. In vivo radioprotection by 5-aminosalicylic acid. *Mutat Res*., 650: 63-79.

Minami, M., H. Yoshikawa, 1979. A simplified assay method of superoxide dismutase activity for clinical use", *Clin Chim Acta*., 92: 337-342.

Montgomery, H.A.C., J.F. Dymock, 1961. The determination of nitrite in water. Analyst., 86: 414-416.

Moritake, T., K. Tsuboi, K. Anzai, T. Ozawa, K. Ando, T. Nose, 2003. ESR spin trapping of hydroxyl radicals in aqueous solution irradiated with high-LET carbon-ion beams. Radiat Res., 159: 670-675.

Moulder, J.E., B.L. Fish and E.P. Cohen, 2004. Impact of angiotensin II Type 2 receptor blockade on experimental radiation nephropathy. Radiat Res, 161: 312–7.

Nakagawa, H., N. Ikota, T. Ozawa and Y. Kotake, 2001. Dose- and time-dependence of radiation-induced nitric oxide formation in mice as quantified with electron paramagnetic resonance.Nitric Oxide BiolChem, 5: 47-52.

Nunia, V., P.K. Goyal, 2004. Prevention of Gamma Radiation Induced Anemia in Mice by Diltiazem, J. Radiat. Res., 45(1): 11-17.

Ohkawa, H., N. Ohishi, K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem, 95: 351-358.

Patel, D., S. Shukla and S. Gupta, 2007. Apigenin and cancer chemoprevention, progress, potential and promise (review). Int J Oncol., 30: 233-45.

Rao, Y.K., M.J. Lee, K. Chen, Y.C. Lee, W.S. Wu, Y.M. Tzeng, 2011. Insulin-Mimetic action of Rhoifolin and Comoiin Isolated From Citrus Grandis (L.) Osbeck Leaves: Enhanced Adiponectin Secretion and Insulin Receptor Phosphorylation in 3T3-L1 Cells. Evidence-Based Complementary and Alternative Medicine. Vol 2011, Article ID 624357:9 Pages.

Samarth, R.M., A. Kumar, 2003. Radioprotection of Swiss albino mice by plant extract Menthapiperita (Linn).J. Radiat. Res., 44: 101-109.

Sharma, K.V. and R. Sisodia, 2009 .Evaluation of free radical scavenging activity and radioprotective efficacy of *Grewiaasiatica* fruit. J Radiol Prot, 29: 429-443.

Singh, I., D. Soyal, P.K. Goyal, 2006. *Emblica officinalis*(Linn.) fruit extract provides protection against radiation-induced hematological and bio chemical alterations in mice. J. Environ. Pathol. Toxicol. Oncol., 25(4): 643-54.

Singh, I., Dhanraj, P.K. Goyal, 2006. *Emblica officinalis*(Linn.) fruit extract provides protection against radiation-induced hematological and bio chemical alterations in mice. J. Environ. Pathol. Toxicol. Oncol., 25(4): 643-54.

Sisodia. R., R.K. Yadav, K.V. Sharma and A.L. Bhatia, 2008. *Spinacia oleracea* Modulates Radiation-Induced Biochemical Changes in Mice Testis. Indian J Pharm Sci., 70(3): 320-326.

Spitz, D.R., E.I. Azzam, J.J. Li, D. Gius, 2004. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. Cancer and Metastasis Reviews, 23: 311-322.

Szasz, G., J.P. Persihn, 1974. Alklin. Chem. Klin. Biochem, 12: 228, C. F. Pointe. Scientific Inc., Lincoin Park, Muchigon,.USA. 44.

Tiwari, P., A. Kumar, M. Ali, K.P. Mishra, 2010. Radioprotection of plasmid and cellular DNA and Swiss mice by silibinin. Mutat Res., 695(1-2): 55-60.

Verma, P., P. Sharma, J. Parmar, P. Sharma, A. Agrawal, P.K. Goyal, 2008. Amelioration of radiation-induced hematological and biochemical alterations in Swiss albino mice by *Panax ginseng* extract. *Integr Cancer Ther.,* 10(1): 77-84.

Voevodskaya, N.V., A.F. Vanin, 1992. Gamma-irradiation potentiates L-arginine-dependent nitric oxide formation in mice.Biochem Biophys Res Commun, 186: 1423-1428.

Wojdyło, A., J. Oszmian´ski, R. Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry, 105: 940–949.

Young, D.S., 2001. Effects of disease on Clinical LaboratoryTests.4th ed. AACC Press, Washington.

Yusuf, S.W., S. Sami, I.N. Daher, 2011. Radiation-induced heart disease: a clinical update. Cardiol Res Pract., 317659.