Drug Interactions between Phenytoin and Rosuvastatin on Lipid Profile, Liver Functions and Oxidative Stress Biomarkers in Irradiated Rats

Dina M. Lotfy*, Seham H.M. Hassan, Mostafa E. El Sayed, Thanaa M. Fahim.
National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.
*Corresponding author. Tel: +201141383375. E-mail address: pharmadinaa@yahoo.com (D.M. Lotfy).

Abstract

Background: phenytoin is one of the most commonly used anticonvulsants for treating generalized tonic-clonic seizures and status epileptics. Rosuvastatin is a new generation HMG-CoA reductase inhibitor. This enzyme converts HMG-CoA to mevalonic acid in the cholesterol biosynthetic pathway which is the rate limiting step in cholesterol synthesis.

Aim: This study was aimed to investigate the possible interactions between phenytoin and rosuvastatin when used together in irradiated rats. **Methods:** The experiments were carried out to investigate the acute effect of each drug individually and in combination with radiation on lipid profile [Total cholesterol, Triacylglycerols, High density lipoproteins, Low density lipoproteins and Very low density lipoproteins, Risk factor, Atherogenic Index], liver function tests (AST & ALT) and oxidative stress biomarkers (MDA, NO & SOD).

Results: Data revealed that, phenytoin in irradiated rats significantly increased serum total cholesterol compared to normal control. Rosuvastatin significantly decreased serum total cholesterol compared to irradiated control. Combination of two drugs significantly increased serum total cholesterol; triacylglycerols and serum VLDL-c levels compared to normal and irradiated rats and significantly increased Atherogenic Index and Risk factor compared to normal control. Phenytoin significantly increased serum ALT level compared to normal and irradiated rats and significantly increased serum MDA and serum NO levels compared to normal rats. But phenytoin significantly decreased MDA & NO levels and significantly increased SOD activity compared to irradiated rats. Rosuvastatin significantly increased serum ALT level compared to normal control but it significantly decreased MDA and significantly increased SOD activity compared to irradiated rats. Combination phenytoin and rosuvastatin in irradiated rats significantly increased serum ALT level compared to normal and irradiated rats and it significantly increased MDA, NO levels but it significantly decreased SOD activity compared to normal control.

It could be concluded that administration of phenytoin concurrently with rosuvastatin not recommended in patients receiving radiotherapy as dangerous side effects may be occurred.

Keywords: Irradiation; Drug interactions; Lipid profile; Liver functions; Oxidative stress biomarkers.

Introduction

Ionizing radiation has become an integral part of modern medicine and is used in diagnostic as well as therapeutic purposes. In some cases, radiation is the single best treatment in some cases such as cancer. [1] Ionizing radiation has been known to induce oxidative stress through generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cell which is suggested to culminate in cell death.[2] Free radicals caused lipid peroxidation in the membrane of cells reflected in altered physico-chemical properties in lipid bilayer and eventually to cellular toxicity.[3]

Drug interaction is the modification of the effect of one drug (object drug) by the prior or concomitant administration of another drug (precipitant drug). It may either enhance or diminish the intended effect of one or both drugs and may modify the diagnostic, preventive or therapeutic activity of either drug. [4]

Epilepsy is a chronic neurological disorder characterized by spontaneous, recurrent, paroxysmal cerebral discharges collectively referred to as seizures.[5] Among the potential causes of seizures are congenital defects, metabolic abnormalities, stroke, lack of oxygen to the brain, brain tumors and infections such as abscess.[6]

Dyslipidaemia is a major risk factor for cardiovascular diseases, obesity and mortality.[7] Hyperlipidaemia decreases the strength of the oxidative defence system.[8] Statins are the treatment of choice for management of hypercholesterolemia, act by inhibition of HMG-CoA reductase enzyme that enzyme is

 DOI: 10.12816/0009355

the rate limiting enzyme in *de novo* synthesis of cholesterol.

Many patients suffer from epilepsy and hypercholesterolemia and are receiving antiepileptic such as phenytoin and hypercholesterolemia drugs such as rosuvastatin may be exposed to irradiation and possible dangerous effects may developed. It becomes of importance to study the interactions between the two types of drugs and irradiation.

In the present study we will use phenytoin (Antiepileptic drug) and rosuvastatin (Hypocholesterolaemic drug) to examine the possible drug interactions between the individual drug as well as their combination in irradiated rats via estimation of lipid profile, liver function and oxidative stress biomarkers.

1. Material and Methods

1.1. Experimental animals:

Male albino Westar rats weighing (120-150 g) were obtained from the breeding unit of The Nuclear Research Centre, Atomic Energy Authority Egypt. Animals were maintained on standard pellet chow diet, free access to water and kept under good ventilation conditions. The animal's treatment protocol has been approved by the animal care committee of the National centre for Radiation Research and Technology, Cairo, Egypt.

1.2. Radiation process:

Irradiation was performed by Cesium-137 biological irradiator (Gamma cell-40) belonging to The National Centre For Radiation and Research Technology, Nasr City, Cairo, Egypt. The dose rate was 0.758 rad/ sec as calibrated at the time of irradiation. Rats were exposed to 6 Gy delivered as fractionated dose (2Gy/day for 3 days).

1.3. Drugs and Reagents:

Phenytoin was purchased from Sigma Company (Egypt), dissolved in saline and administrated in a dose of 60mg/kg i.p. [9]

Rosuvastatin was purchased from AstraZeneca Company (Egypt), dissolved in saline and administrated in a dose of 1.25 mg/kg i.p. [10]

ALT, AST, Total cholesterol, Triglycerides and HDL-C kits were obtained from Bio diagnostic Company (Egypt).

1.4. Experimental Design:

Male albino Westar rats were divided into 5 groups each of 8 rats:

Group 1: kept as normal control (received saline).

Group 2: irradiated rats (6Gy delivered as fractionated dose 2Gy/day for 3 days).

Group 3: Rats were exposed to radiation (2Gy/day for 3 days) and then treated with acute dose of phenytoin (60mg/kg i.p.).

Group 4: Rats were exposed to radiation (2Gy/day for 3 days) and then treated with acute dose of rosuvastatin (1.25 mg/kg i.p.).

Group 5: Rats were exposed to radiation (2Gy/day for 3 days) then treated with combination of phenytoin (60mg/kg i.p.) and rosuvastatin (1.25 mg/kg i.p.) concurrently.

1.5. Collection of blood and serum separation:

Rats were irradiated for 3 consecutive days and then, the drugs and their combination were administrated one day after irradiation process, rats were sacrificed 3hrs after drug administration. Blood samples were collected in non-heparinized and heparinized tubes by heart puncture for biochemical assay.

1.6. Biochemical assays:

Serum total cholesterol (TC) was measured colorimetric ally using a test reagent kit according to the method of ***Richmond.***[11] **Serum Triacylglycerols** were measured colorimetric ally using a test reagent kit according to the method of ***Fossati and Prencipe.***[12] **Serum HDL-cholesterol** was measured colorimetric ally using a test reagent kit according to the method of ***Lopes et al.***[13] **Serum LDL-cholesterol** was calculated according to **Friedwalds**[14] formula: LDL-cholesterol (mg/dl) =Total Cholesterol – (Triglycerides/5 + HDL-c). Serum VLDL-cholesterol was calculated according to **Friedwalds**[14] formula: [VLDL-cholesterol (mg/dl) = Triglycerides/5]. Atherogenic Index (A.I.) was calculated according to ***Odamaki et al.***[15] [A.I.= (T.C.- HDL-c) /HDL-c]. Risk factor (R.F.) was calculated by the following equation [R.F=T.C./HDL-cholesterol].**Serum AST and ALT** activities were determined colorimetric ally according to the methods of ***Reitman and Frankel.***[16] **Serum MDA** level was determined according to ***Yoshioka et***

***al.*[17] Serum NO level** was determined according to ***Geng and Hassan.*[18] Blood SOD activity** was determined according to the method of ***Minami and Yoshikaw.*[19]**

1.7. Statistical analysis:

Data are expressed as mean ± S.E. of the mean. Statistical comparisons between different groups were done by using one way analysis of variance (ANOVA), followed by Turkey- Kramer for multiple comparisons test to judge the difference between different groups. Significance was accepted at $P<0.05$.

2. Results

2.1. Lipid Profile:

2.1.1. Effect of phenytoin, rosuvastatin and their combination on serum total cholesterol and serum triacylglycerols levels of whole body fractionated irradiated rats.

The results are given in Table (1) illustrated that; fractionated irradiation of rats at a total dose of 6 Gy delivered as 2Gy/day for 3days significantly increased serum total cholesterol level to 95.01 mg/dl of normal control value, but it didn't significantly change serum triacylglycerols levels.

Combination of Irradiation (2Gy/day for 3days) and Phenytoin (60mg/kg) didn't significantly change serum total cholesterol and triacylglycerols levels compared with irradiated control value. Thus, phenytoin doesn't protect against hypercholesterolemia caused by radiation treatment.

Combined treatment with radiation (2Gy/day for 3days) and rosuvastatin (1.25mg/kg) significantly decreased serum total cholesterol level to 80.20% of irradiated rats, but it didn't affect serum triacylglycerols levels. Rosuvastatin protects against the radiation effect on serum total cholesterol level, but doesn't protect against the radiation effect on serum triacylglycerols levels.

Combined treatment with radiation (2Gy/day for 3days), phenytoin (60mg/kg) and rosuvastatin (1.25mg/kg) significantly increased serum total cholesterol and triacylglycerols levels to 121.88% and 139.36% of irradiated control value respectively. Therefore, the tested combination made the condition more badly than the use of either drug with radiation.

2.1.2. Effect of phenytoin, rosuvastatin and their combination on serum HDL-C, serum LDL-C, serum VLDL-C levels of whole body fractionated irradiated rats.

The results are given in Table (2) showed that; fractionated irradiation of rats at a total dose of 6 Gy delivered as (2 Gy /day for 3 days) didn't significantly change serum HDL-C, LDL-C and VLDL-C levels compared to normal control value.

Double treatment with Irradiation (2Gy/ day for 3days) and phenytoin (60mg/kg) didn't significantly change serum HDL-C, LDL-C and serum VLDL-C levels compared to irradiated control values.

Double treatment with Irradiation (2Gy/ day for 3days) and rosuvastatin (1.25mg/kg) didn't significantly change serum HDL-C, LDL-C and serum VLDL-C levels compared to irradiated control values.

Combined treatment with irradiation (2Gy/ day for 3days), phenytoin (60mg/kg i.p.) and rosuvastatin (1.25mg/kg i.p.) significantly increased serum VLDL-C level to 139.46 % of irradiated control value. Thus, the combination made the condition more badly than the use of either drug with irradiation.

2.1.3. Effect of phenytoin, rosuvastatin and their combination on risk factor and Atherogenic index of whole body fractionated irradiated rats.

The results are given in Table (3) presented that; fractionated irradiation of rats at a dose of 6 Gy delivered as 2Gy/day for 3 days didn't significantly change serum risk factor and Atherogenic index compared to normal control values.

Double treatment with irradiation (2Gy/day for 3days) and phenytoin (60mg/kg i.p) didn't significantly change serum risk factor and serum Atherogenic index compared to irradiated control values.

Double treatment irradiation (2Gy/day for 3days) and rosuvastatin (1.25mg/kg) didn't significantly change risk factor and Atherogenic index compared to irradiated control values.

Combined treatment with irradiation (2Gy/day for 3days), phenytoin (60mg/kg i.p) and rosuvastatin (1.25mg/kg i.p) significantly increased risk factor and Atherogenic index to 2.11 and 1.14 of normal control value respectively, but it didn't

significantly change serum risk factor and serum Atherogenic index compared to irradiated control values.

2.2. Liver Function Tests:

Effect of phenytoin, rosuvastatin and their combination on serum AST and serum ALT levels of whole body fractionated irradiated rats.

The results are given in Table (4) presented that; fractionated irradiation of rats at a dose of 6 Gy delivered as 2Gy/day for 3 days didn't significantly change serum AST and serum ALT levels compared to normal control values.

Double treatment with Irradiation (2Gy/day for 3days) and phenytoin (60mg/kg i.p) significantly increased serum ALT level to 192.67% of irradiated control value, but it didn't significantly change serum AST level compared to irradiated control value. Radiation doesn't protect against liver toxicity caused by phenytoin therapy.

Double treatment with Irradiation (2Gy/day for 3days) and rosuvastatin (1.25mg/kg i.p) didn't significantly change serum AST and serum ALT levels compared to irradiated control values.

Combined treatment with Irradiation (2Gy/day for 3days), phenytoin (60mg/kg i.p) and rosuvastatin (1.25mg/kg i.p) significantly increased serum ALT level to 215.52% of irradiated control value, but it didn't significantly change serum AST level.

2.3. Oxidative stress biomarkers:

The results are given in Table (5) showed that; Double treatment with irradiation (2Gy/day for 3days) and phenytoin (60mg/kg i.p) significantly decreased serum MDA & serum NO levels to 70.55%and 51.12% of irradiated control values respectively, but it significantly increased blood SOD activity to 173.47% compared to irradiated control value.

Double treatment with irradiation (2Gy/day for 3days) and rosuvastatin (1.25mg/kg i.p) significantly increased blood SOD activity to 177.87% of irradiated control value, but it significantly decreased serum MDA & serum NO levels to 62.41% and 59.51% compared to irradiated control values respectively

Combined treatment with Irradiation (2Gy/day for 3days), phenytoin (60mg/kg i.p) and rosuvastatin (1.25mg/kg i.p) significantly increased serum MDA & serum NO levels to 61.98 nmol/ml and 117.50 u/ml compared to normal control values respectively, but the combination significantly decreased blood SOD activity to 14.94 u/ml compared to normal control value.

Combined treatment with Irradiation (2Gy/day for 3days), phenytoin (60mg/kg i.p) and rosuvastatin (1.25mg/kg i.p) didn't significantly change serum MDA, serum NO levels and blood SOD activity compared to irradiated control values.

4. Discussion

Cholesterol is the main lipid found in the blood, bile and brain tissues. Liver metabolizes the cholesterol and it is transported to the blood stream by lipoproteins. Decreased levels are found in mal-absorption, malnutrition, hyperthyroidism, anemia and liver diseases .[20] Triacylglycerols are simple lipids formed in the liver by glycerol and fatty acids. They are transported by very low density lipoprotein (VLDL) and low density lipoprotein (LDL). They constitute about 90% of fat, stored as source of energy in the tissue and plasma.[21]

In the present study, marked significant elevation in serum total cholesterol (TC), increased in serum triacylglycerols, serum LDL-c, serum VLDL-c levels, Risk factor (RF) and Atherogenic Index(AI) were observed after exposure to 6Gy whole body fractionated gamma radiation (2Gy/day for 3 days). These results might be due to an increase in plasma lipids levels in rats post irradiation. [22] The hypercholesterolemia might be attributed to the stimulation of cholesterol synthesis in the liver after gamma irradiation. Hypercholesterolemia might be \due to the increase in the activation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme, the key regulatory enzyme in the reduction of the overall process of cholesterol synthesis.[23]

In the current study results of lipid profile obtained from administration of phenytoin after whole body fractionated irradiation were approximately equal to those of irradiated rats. This may be due to increase in cholesterol synthesis in liver after gamma irradiation (Induction of hypercholesterolemia). [22]As well as phenytoin treatment may cause elevation in lipid profile due to liver toxicity.[24]

Administration of rosuvastatin after fractionated gamma irradiation significantly decreased serum total cholesterol in comparing to irradiated rats. This result may be due to rosuvastatin block the conversion of HMG-CoA to mevalonte in hepatic cholesterol biosynthesis pathway.[25]

In the present study, the double treatment with phenytoin and rosuvastatin after whole body fractionated irradiation showed highly significant increase in lipid profile including total cholesterol, triglycerides and very low density lipoprotein. This action may be due to the stimulation of cholesterol synthesis in the liver after gamma irradiation.[22] Moreover; hypercholesterolemia after gamma irradiation may be due to activation of HMG-CoA reductase enzyme which is the key enzyme in cholesterol biosynthesis. [23]

The current study revealed that whole body fractionated gamma radiation (2Gy/day for 3 days), caused an elevation in serum AST and serum ALT levels compared to normal control. This elevation in liver enzymes might be due to drastic physiological effect caused by irradiation, either directly by interaction of cellular membranes with gamma ray or through the action of free radicals produced by radiation. [26] Liver damage may increase the cell membrane permeability due to extensive breakdown of liver parenchyma accompanied with rise in the transaminases enzymes from their subcellular sites of production to extracellular process and consequently to blood circulation**.** Also, ionizing radiation enhanced lipid peroxidation in cell membrane which contains fatty acids and excessive production of free radicals. This in turn increases the cytoplasmic membrane permeability to organic substances and causes leakage of cytosolic enzymes such as AST and ALT.[27]

Administration of phenytoin after exposure to gamma irradiation showed highly significant increase in serum ALT level and slight increase in serum AST level from irradiated control. This may be due to the effect of phenytoin on both mitochondrial and cytosolic enzyme activity.[24]

In the present study rosuvastatin treatment after gamma irradiation ameliorated the increase in liver enzymes caused by gamma irradiation in comparison with irradiated rats.

Our investigation showed that, administration of combined therapy of phenytoin with rosuvastatin after fractionated irradiation showed highly significant increase in serum ALT level in comparison with irradiated control. These results may be due to either by direct interaction of cellular membrane with gamma irradiation or through the action of free radicals produced by radiation increasing the permeability and leakage of cytoplasmic enzymes such as AST and ALT.[27] As well as phenytoin causes induction of liver enzymes [mitochondrial (ALT) and cytosolic (AST) enzymes].[24]

In the present study whole body fractionated gamma radiation (2Gy/day for 3 days) showed highly significant increase in serum malondialdehyde (MDA) level. These results are in agreement with ***Ping et al.***[28] The generation of lipid peroxide and its appearance in the rat's liver and kidney which accompanied with exposure to gamma radiation may be a resultant of a chain of reactions or may be initiated by an indirect mechanism that enables the escape from anti-oxidation. [29] The marked increase in MDA level is likely to be a result of the inactivation of the scavenger enzymes induced by reactive oxygen species (ROS).[30]

Results of this study showed significant increase in serum nitric oxide (NO) level in irradiated rats. The increase in serum NO level might be due to the entry of Ca^{2+} ions into the membrane as well as the cystol of NO producing cells though irradiation induced membrane lesions.[31]

Our study indicated that whole body fractionated gamma radiation (2Gy/day for 3 days) caused a decrease in blood SOD activity. This result is in agreement with those of ***Ping et al.***[28] Gamma radiation increased the formation of reactive oxygen species (ROS) which result in decrease of SOD activity.[32]

According to the results of this investigation, administration of phenytoin after fractionated gamma irradiation showed a significant amelioration in serum MDA level when compared with irradiated control. It significantly decreased serum NO level when

compared with irradiated control. This result may occur as antiepileptic drugs diazepam and phenytoin produce results similar to that obtained by ***Kenshi et al.*** [33] with the NOS (Nitric oxide synthase) inhibitors.[33] As well as, phenytoin after gamma irradiation exposure caused significant increase in blood SOD activity and slightly ameliorated lipid peroxidation caused by gamma irradiation.

In our study, rosuvastatin showed amelioration in oxidative stress biomarkers after whole body fractionated gamma irradiation. It significantly decreased serum MDA level and significantly increased blood SOD activity when compared to irradiated control. This effect may be due to that, rosuvastatin prevents the formation of oxygen free radicals and has antioxidant property. [34] On the other hand administration of rosuvastatin after gamma irradiation showed significant decrease in serum NO level when compared to irradiated control. This result indicates that excessive production of NO after gamma irradiation by cell membrane lesions.[35] [while] rosuvastatin protects the vascular endothelium from the inflammatory process and cell lesions.[36]

The present investigation showed that, the combined treatment of phenytoin with rosuvastatin after whole body fractionated gamma irradiation didn't ameliorate the disturbance in oxidative stress biomarkers caused by irradiation. So combination not recommended to be used after radiation therapy. These results may be due to that, phenytoin hepatotoxicity which resulted from arene oxide metabolites of phenytoin.

5. Conclusion

Administration of antiepileptic drug such as phenytoin concurrently with hypocholesterolaemic drug such as rosuvastatin not recommended in patients receiving radiation therapy as dangerous side effects on liver functions and lipid profile may occur.

6. Conflict of interest: None.

References

1.Pandey BN and Mishra KP (1999). Radiation induced oxidative damage modification by cholesterol in liposomal membrane. Radiat. Phys. Chem., **54**: 481- 89.

2.Aoshima H,Satoh T, Sakai N, Yamada M, EnokidoY, Ikeuchi T, Hatanaka H (1997).Generation of free radicals during lipid hydroperoxide-triggered apoptosis in PC12h cells. Biochem. Biophys. Acta.,**1345**(1): 35-42.

3.Pandey BN, Mishra KP(2002). Effect of radiation induced lipid peroxidation on diphenylhexantriene fluorescence in egg phospholipid liposomal membrane. J Biochem. Mol. Bio. Biophys., **6**: 267-72.

4. Kohler GI(2004). Elements of Clinical Pharmacy. J. BS. Shah., **1**: 135- 48.

5. Bishop M and Allen C (2007). Chronic illness and disability theoretical empirical and clinical aspects. Springer Publishing, **1**: 241–66.

6. Deb S, Sohanpal SK, Soni R, Lenôtre L, Unwin G (2007).The effectiveness of antipsychotic medication in the management of behaviour problems in adults with intellectual disabilities. J. Intellect Disabil. Res., **51**:766– 77

7- Rizvi F, Iftikhar M , George JP(2003). Beneficial effects of fish liver preparations of sea bass (Lates calcarifer) versus gemfibrozil in high fat dietinduced lipid-intolerant rats. J Med. Food., **6**:123.

Through Javed A Ansari, Uma Bhandari, Pillali K K, Haque SE (2012). Effect of rosuvastatin on obesity-induced cardiac oxidative stress in wistar rats- A preliminary study. Indian J. Exp. Biol.,**50**: 216- 22.

8. Liou W, Chang L Y, Geuze H J, Strous G J, Crapo J D, Slot J W (1993). Distribution of CuZu superoxide dismutase in rat liver. Free Radic. Biol. Med.,**14**:201.

Through

Javed A Ansari, Uma Bhandari, Pillali K. K. and Haque S.E (2012). Effects of rosuvastatin on obesity-induced cardiac oxidative stress in Wister rats- A preliminary study. Indian J. Exp. Biol.,**50**: 216- 22.

9. Minaiyan M, Ghafghazi T, Majdzadeh-Ardakani M (2008). Study of pharmacokinetic interaction of ascorbic acid and phenytoin in rats. DARU.,**16**(2):70-75.

10. Timothy J Stalker, Allan M Lefer, Rosario A Scalia (2001). A new HMG-CoA reductase inhibitor, rosuvastatin, exerts anti-inflammatory effects on the microvascular endothelium: the role of mevalonic acid. Br. J Pharmacol.,**133**: 406- 12.

11. Richmond W(1973). Preparation and properties of a cholesterol oxidase from No cardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem.,**19**(12):1350- 56.

12. Fossati P and Prencipe L (1982). Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem.,**28**(10): 2077- 80.

13.Lopes-virella MF, Stone P,Ellis S, Colwell JA (1977).Cholesterol determination in high-density

lipoproteins separated by three different methods. Clin. Chem.,**13**(4): 285- 91.
14. Friedewald WT, Levy RI, and Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem.,**18**: 499 –502.
15. Odamaki M, Furinya R, Ohkaw S, Yoneyamar T, Nishikino M, Hishd A, and Kamagai H (1999). Altered abdominal fat distribution and its association with serum lipid profile in non- diabetic haemolysis patients. Nephrology Dialysis Transplantation, 14: 2427-2433.
16. Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. J Clin. Pathol.,**28**(1):56-63.
17.Yoshioka T, Kawada K, Shimada T, Mori M (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. J Obstet. Gynecol., **135**(3):372- 76.
18.Geng J T, Wu Q,Hansson K (1994). Protein kinase activation inhibits cytokine induced nitric oxide synthesis in vascular smooth muscle cells. Biochem. Biophys. Acta., **223**:125-32.
19. Minami M and Yoshikawa H (1979). A simplified assay method of superoxide dismutase activity for clinical use. Clin. Chem. Acta., **92**(3):337-42.
20. Amin A, Saud A, Bashir M (2013). Hepatic histological and histochemical alterations induced by rosuvastatin therapeutic doses. Pakistan J. Zool., 45(1):149-157.
21. Ahai L, Wycliffe M, Alistair S, Hall H, Julian H (2012). Rosuvastatin: A Review of the Pharmacology and Clinical Effectiveness in Cardiovascular Disease. Clin. Med. Insights: Cardiol.,6: 17–33.
22. Tawfik SS and Salama SF (2008). Possible protective role of carnosine against γ-radiation-induced cardiac dysfunction in mice. Egypt J. Rad. Sci. Applic., **21**(2):341- 56.
23. Abd El-Azime A and Ossman N (2013). Protective Effect of Hawthorn (Crataegus Linn) against Radiation-Induced Damage in Rats. Arab J. Nucl. Sci. and Applic., **46**(1):304-312.
24. Itemobong SE, Monday IA, Friday EU, Offiong UE (2007). Effect of folic acid and vitamin B_{12} administration on phenytoin induced toxicity in rats. Indian J. Clin.Biochem., **22**(2): 36-40.
25. Alberts AW (1988).Discovery, biochemistry and biology of lovastatin. Am. J. Cardiol., **62**:10–15.
26. Bhatia A, Gupta A (2007). Neuromonitoring in the intensive care unit. II. Cerebral oxygenation monitoring and microdialysis. Intensive Care Med., 33(8):1322-1328.
27. Weiss J and Landauer M (2003). Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicol., **189**(1-2):1-20.
28. Ping X, Junqing J, Junfeng J and Enjin J (2012). Radioprotective effects of troxerutin against gamma irradiation in mice liver. Int J Radiat Biol., 88(8): 607-612.
29. Mansour S and El-Kabany H (2009). Effects of Fructus Piperis Longi extract on fibrotic liver of gamma-irradiated rats. Chin Med., 4(2):60-67.
30. Oliinyk B, Baraboi V, Oliinyk S and Horchakova N (2001). Effect of splenosid on lipid peroxidation process and glutathione antioxidant system in rats exposed to fractionated radiation. Ukr Biokhim Zh.,73(1): 73-77.
31. Gorbunov M, Gurvich A, Kornblueh L (2000). Comparative analysis of radioholographic methods of processing radio occultation data. Radio Sci., **35**: 1025– 1034.
32. Wiseman H and Halliwell B (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J., 313(1): 17-29.
33. Kenshi K, Kouichi I, Lawrence B, Kazuo M, Hirotada F (2002). Consequences of Nitric Oxide Generation in Epileptic-Seizure Rodent Models as Studied by In Vivo EPR. Magn. Reson. Med., **48**:1051- 56.
34. Schupp N, Schmid U, Heidland A, Stopper H (2008). Rosuvastatin protects against oxidative stress and DNA damage in vitro via up regulation of glutathione synthesis**.** Atherosclerosis, **199**: 278–87.
35. Gorbunov ME, Gurvich AS, Kornblueh L (2000). Comparative analysis of radioholographic methods of processing radio occultation data. Radio. Sci., **35**: 1025– 34.
36. Lefer AM, Campbell B, Shin YK, Scalia R, Hayward R, Lefer DJ (1999). Simvastatin preserves the ischemic-reperfused myocardium in normocholesterolemic rat hearts. Circul., **100**:178-84.

Table (1): Effect of phenytoin, rosuvastatin and their combination on Serum Total Cholesterol and Triacylglycerols levels of whole body fractionated irradiated rats.

Parameters / Drugs & Doses	Total Cholesterol		Triacylglycerols	
	Absolute Value (mg/dl)	%Of irradiated Control	Absolute Value (mg/dl)	% Of irradiated Control
Normal Control (Saline)	61.97 ± 3.45	65.22	58.09 ± 3.38	88.96
Irradiated Control (2Gy /day for 3days)	95.01 [a] ± 5.81	100.00	65.30± 2.16	100.00
Irradiation(2Gy /day for 3days) +Phenytoin (60mg/kg)	84.43 [a] ± 2.62	88.86	65.20 ± 2.09	99.85
Irradiation (2Gy /day for 3days) + Rosuvastatin (1.25mg/kg)	76.20 [b] ± 4.09	80.20	59.73 ± 1.60	91.47
Irradiation (2Gy /day for 3days) +Phenytoin (60mg /kg) +Rosuvastatin (1.25mg/kg)	115.80 [a, b, c, d] ± 4.51	121.88	91.00 [a, b, c, d] ± 5.28	139.36

N=8 rats per group.
Each value represents the mean± S.E. of the mean.
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
a: Significantly different from normal control at p<0.05.
b: Significantly different from Irradiated control at p<0.05.
c: Significantly different from Irradiation+Phenytoin at p<0.05.
d:Significantly different from Irradiation+Rosuvastatin at p<0.05.
e: Significantly different from Irradiation+Phenytoin+ Rosuvastatin at p<0.05.

Table (2): Effect of phenytoin, rosuvastatin and their combination on serum Lipoproteins (HDL-C, LDL-C and VLDL-C) of whole body fractionated irradiated rats.

Parameters / Drugs &Doses	HDL-C		LDL-C		VLDL-C	
	Absolute Value (mg/dl)	%Of irradiated Control	Absolute Valu (mg/dl)	% Of irradiated Control	Absolute Value (mg/dl)	% Of irradiated Control
Normal Control (Saline)	50.59 ± 2.48	95.83	12.48 ± 1.09	52.50	11.62 ±0.68	88.97
Irradiated Control (2Gy /day for 3days)	52.79 ± 3.42	100.00	23.77± 0.83	100.00	13.06 ±0.43	100.00
Irradiation(2Gy /day for 3days) + Phenytoin(60mg/kg)	54.27± 3.19	102.80	24.21 ± 1.05	101.85	13.04 ±0.42	99.84
Irradiation(2Gy /day for 3days) + Rosuvastatin (1.25mg/kg)	48.23± 2.86	91.36	12.87± 0.68	54.14	11.95±0.32	91.50
Irradiation(2Gy /day for 3days) + Phenytoin (60mg/kg) +Rosuvastatin (1.25mg/kg)	58.16 ± 4.93	110.17	23.65 ± 2.19	99.50	18.20 [a, b, c,d] ±1.06	139.46

N=8 rats per group.
Each value represents the mean± S.E. of the mean.
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
a: Significantly different from normal control at p<0.05.
b: Significantly different from Irradiated control at p<0.05.
c: Significantly different from Irradiation+Phenytoin at p<0.05.
d:Significantly different from Irradiation+Rosuvastatin at p<0.05.
e: Significantly different from Irradiation+Phenytoin+ Rosuvastatin at p<0.05.

Table (3): Effect of phenytoin, rosuvastatin and their combination on Risk Factor and Atherogenic Index of whole body fractionated irradiated rats.

Parameters / Drugs & Doses	Risk factor		Atherogenic Index	
	Absolute Value	% Of irradiated Control	Absolute Value	% Of irradiated Control
Normal Control (Saline)	1.23± 0.05	67.58	0.23± 0.04	30.26
Irradiation (2Gy /day for 3days)	1.82± 0.14	100.00	0.76± 0.15	100.00
Irradiation (2Gy /day for 3days) +Phenytoin (60mg/kg)	1.52± 0.11	83.52	0.52± 0.11	68.42
Irradiation (2Gy /day for 3days) +Rosuvastatin (1.25mg/kg)	1.61± 0.19	88.46	0.61± 0.19	80.26
Irradiation (2Gy /day for 3days) +Phenytoin(60mg/kg) +Rosuvastatin (1.25mg/kg)	2.11 [a]± 0.25	115.93	1.14 [a]± 0.29	150.00

N=8 rats per group.
Each value represents the mean± S.E. of the mean.
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
a: Significantly different from normal control at $p<0.05$.
b: Significantly different from Irradiated control at $p<0.05$.
c: Significantly different from Irradiation+Phenytoin at $p<0.05$.
d:Significantly different from Irradiation+Rosuvastatin at $p<0.05$.
e: Significantly different from Irradiation+Phenytoin+ Rosuvastatin at $p<0.05$.

Table (4): Effect of phenytoin, rosuvastatin and their combination on serum AST and ALT Levels of whole body fractionated irradiated rats.

Parameters / Drugs & Doses	AST		ALT	
	Absolute Value (U/L)	% Of irradiated Control	Absolute Value (U/L)	% Of irradiated Control
Normal Control (Saline)	34.30 ± 2.70	84.07	15.80 ± 1.80	68.10
Irradiated Control (2Gy /day for 3 days)	40.80 ± 2.20	100.00	23.20 ± 1.80	100.00
Irradiation(2Gy/day for3 days) +Phenytoin (60mg/kg)	50.00 ± 4.40	122.55	44.70 [a, b] ± 2.70	192.67
Irradiation(2Gy/day for3 days) +Rosuvastatin (1.25mg/kg)	46.50 ± 3.50	133.97	33.50 [a] ± 3.80	144.40
Irradiation(2Gy/day for3 days) +Phenytoin (60mg/kg) +Rosuvastatin (1.25mg/kg)	57.00 [a] ± 4.40	139.71	50.00 [a, b, c, d] ± 3.60	215.52

N=8 rats per group.
Each value represents the mean± S.E. of the mean.
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
a: Significantly different from normal control at $p<0.05$.
b: Significantly different from Irradiated control at $p<0.05$.
c: Significantly different from Irradiation+Phenytoin at $p<0.05$.
d:Significantly different from Irradiation+Rosuvastatin at $p<0.05$.
e: Significantly different from Irradiation+Phenytoin+ Rosuvastatin at $p<0.05$.

Table (5): Effect of phenytoin, rosuvastatin and their combination on serum MDA, NO Levels and Blood SOD activity of whole body fractionated irradiated rats.

Parameters / Drugs & Doses	MDA		NO		SOD	
	Absolute Value (nmol/ml)	% Of irradiatd Control	Absolute Value (U/ml)	% Of irradiated Control	Absolute Value (U/ml)	% Of irradiated Control
Normal Control(Saline)	33.60±3.34	43.42	37.87± 1.95	33.40	23.36 ±2.93	168.42
Irradiated Control (2Gy /day for 3days)	77.38 [a] ±5.66	100.00	113.40 [a] ± 4.1	100.00	13.87 [a] ±1.13	100.00
Irradiation(2Gy /day for3days) + Phenytoin(60mg/kg)	54.59 [a, b] ±3.14	70.55	57.97 [a, b] ±0.5	51.12	24.06 [b] ±0.90	173.47
Irradiation(2Gy /day for3days) + Rosuvastatin (1.25mg/kg	48.29 [b] ±1.45	62.41	67.49 [a, b] ± 4.9	59.51	24.67 [b] ±1.25	177.87
Irradiation(2Gy /day for3days) +Phenytion (60mg/kg) +Rosuvastatin(1.25mg/k	61.98 [a] ± 4.43	80.11	117.50 [a, c, d] ±3.28	103.62	14.94 [a, c, d] ±1.09	107.71

N=8 rats per group.
Each value represents the mean± S.E. of the mean.
Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.
a: Significantly different from normal control at $p<0.05$.
b: Significantly different from Irradiated control at $p<0.05$.
c: Significantly different from Irradiation+Phenytoin at $p<0.05$.
d:Significantly different from Irradiation+Rosuvastatin at $p<0.05$.
e: Significantly different from Irradiation+Phenytoin+ Rosuvastatin at $p<0.05$.