Radioprotective Effect of Hesperidin against Gamma-Irradiation-Induced Oxidative Stress and Biomechanical Properties of Bone in Rats

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Abstract: The radioprotective effects of hesperidin (HES), a flavonone glucoside, were investigated by using the creatine kinase (CPK), lactate dehydrogenase (LDH), asymmetric dimethylarginine (ADMA), urea, creatinine, total nitrate/nitrite (NO(x)), superoxide dismutase (SOD), glutathione peroxidase (GSHPx) activities, glutathione (GSH), malondialdehyde (MDA), calcium ion concentration and biomechanical properties of bone in rats. Eighty male albino rats were divided into four groups. The control group received 100 µL of sterile saline intra peritoneal. Rats of the second group were injected HES extract (160 mg/kg) intra peritoneal (I.P) for 3 consecutive days. Animals in the third group were administered vehicle by gastric tube for 3 consecutive days, then exposed to single dose gamma-irradiation (2Gy). The Fourth group received HES extract for 3 consecutive days; one hour later rats were exposed to gamma-irradiation. Our results revealed that, prior to irradiation HES extract produced a significant radioprotection. This was evidenced by a significant reduction in serum (CPK), (LDH), (ADMA), urea and creatinine levels as well as significant increase in serum nitrate/nitrite (NO(x)) level. Moreover, HES significantly increased renal (SOD), (GSHPx) and calcium ion concentration, and reduced (GSH) content, associated with a significant depletion in (MDA) and NO(x) levels compared to irradiated group. Additionally, treatment with HES extract led to significant break points of tibia bones compared to irradiated group. In conclusion, this study suggests that HES may serve as a potential protective agent against gamma-irradiation-induced cardio-nephrotoxicity via enhancing the antioxidant activity, biophysical mechanical properties of bone in rats and inhibition of endothelial dysfunction.

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Keywords: Hesperidin, gamma-radiation oxidative stress, calcium ions, biomechanical properties of bone.

1.Introduction:

Ionizing radiation generates reactive oxygen species in the cells. These free radicals can induce damage to critical macromolecules such as DNA. The cellular DNA damage leads to mutation and cancer (Reily, 1994) High levels of gamma irradiation can induce mortality in mammals. With respect to radiation damage to humans, it is important to protect biological systems from radiation induced geno toxicity or lethality. The main radio protective class is thiol synthetic compounds such as amifostine. Amifostine is a powerful radio protective agent compared with other agents, but this drug is limited in the use in clinical practice due to side effects and toxicity (Turrisi *et al.*, 1986; Hosseinimehr *et al.*, 2001). The search for less-toxic radiation protectors has spurred interest in the development of natural products. Recently, we reported that citrus extract protects mouse bone marrow cells against gamma irradiation. The citrus extracts contained high amounts of flavonoids (Hosseinimehr *et al.*, 2003)

Flavonoids have wide biological properties including antibacterial, antiviral, anticancer, immune stimulant and antioxidant effects (Tiwari *et al.*, 2001). Flavonoids' activities as antioxidants refer to their ability to transfer a hydrogen atom or an electron and to the possibility of their interactions with other antioxidants (Rice-Evans *et al.*, 2000). Hesperidin (HES) is a flavonone glycoside, belonging to the flavonoid family. This natural product is found in citrus species. Hesperidin was reported to have many biological effects including anti-inflammatory, antimicrobial, anti carcinogenic and antioxidant effects, and decreasing capillary fragility (Garg *et al.*, 2001)The flavonoid hesperidin may serve as a hydrogen donor for α-tocopherol radical (Hussein *et al.*, 2010)thus regenerating α-tocopherol that is a key element of redox balance in biosystems. Thus, herbal plants are considered a useful means to prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis, hepatotoxicity and other complications (Hussein *et al.*, 2008)

Bones are living tissues and continue to change throughout life. During child hood and adolescence, bone increase in size and mass, it continue to add more mass until around age 30 where it reached peak bone mass then the more bone loss will be delayed

with aging. Therefore, it is particularly important to consume adequate calcium and vitamin D throughout infancy, childhood, and adolescence (Liebschner, 2004) .Bone density is generally expressed in terms of grams of mineral per volume or area. In any given individual, it is determined by peak bone mass. Osteoporosis is defined as a systemic bone decrease characterized by insufficient bone strength that predisposes to a higher risk of bone fracture (Wettergreen *et al.*, 2005).Mechanical properties of bone are basic parameters, which reflect the structure, function of bone, and can be measured by testing completely anatomical units or specimens prepared to isolate particular structural components. Within this context, the fracture of bone can represent failure of whole bone at the structural level and bone tissue at the material level. The mechanical behavior of bone in normal physiological situations is similar to that of an elastic material with no visible change in external appearance. However, Bone can be degraded and retain its morphological features for an indefinite period. Unlike inorganic materials, bone has adaptive mechanisms, which give the tissue the ability to repair itself, altering its mechanical properties and morphology in response to increased or decreased function. (Patel, 1969).Mechanical properties vary significantly around the periphery and along the length of the bone, vary between can cellos and cortical bones and also between locations of the bone. Bone also exhibit selector mechanical effects both in vivo and in vitro, where an electric potential is generated in bone as a result of mechanical stress. (Brown & Ferguson., 1980)

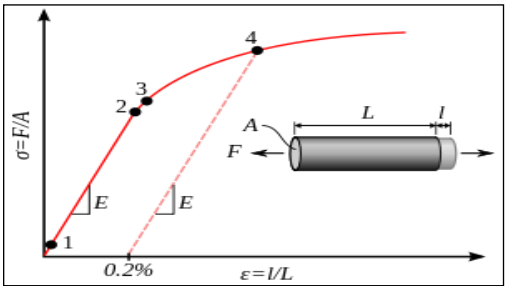

Figure. (1): Typical yield behavior for non-ferrous alloys, 1: True elastic limit, 2: Proportionality limit, 3: Elastic limit, 4: Offset yield strength (Behiri & Bonfield., 1980)

Tension and compression are forces applied in a perpendicular to the surface or a plane within an object. Tensile forces result in elongation and narrowing deformation. Tensile testing can be one of the most accurate methods for measuring bone properties, provided that force is applied without inducing a coupled bending moment. However, compressive tests tend to be less accurate than tensile tests due to end effects imposed on the specimen during the test (Behiri & Bonfield., 1980).Young's modulus is the ratio between the longitudinal stress to the longitudinal strain

Young's modulus (E) = Tensile stress σ = F/A
Longitudinal strain € $\Delta L/L_0$

Aim of the work:

The aim of this study was to investigate the radio protective effect of HES against 2Gy γ-rays on the structural as biophysical properties of bone, oxidative stress and endothelial dysfunction in rats.

2.Materials and methods:

1-Experimental Animals:

In the present work 80 male albino rats, each of average weight 110 ±10 gm were purchased from the Faculty of Veterinary Medicine, Cairo University. Rats were housed in accordance to the principles outlined in ‘‘The Guide for The Care and Use of Laboratory Animals’’ prepared by VACSERA -Giza-Egypt, and given standard rats pellets and water *ad libitum*

2-Chemicals and experimental design:

HES were purchased from Sigma Aldrich, Egypt. It was in phosphate buffered saline (pH 7.6). Rats were injected intra peritoneal (I.P) for all experiments. The Fourth group received HES (160 mg/kg) according to (Gadkariem *et al.*,2010) for 3 consecutive days, one hour later rats of groups C and D were exposed to single dose γ-irradiation (2Gy γ-rays) as shown in table (1).

Table (1): The experimental design.

Groups	Experimental conditions
G(A)	Normal (received 100 µL of sterile saline by i.p*).
G(B)	Animals were injected with dose of HES (160 mg/ kg(i.p))
G(C)	Animals exposed into cobalt-60 γ-radiation (total dose of 2Gy γ-rays)
G(D)	Animals were injected with dose of HES (160 mg/ kg (i.p)) and exposed Whole body into cobalt-60 γ-radiation (total dose of 2Gy γ-rays)

3-Gamma Radiation Facility:

Whole-body gamma irradiation was performed at the National Centre for Radiation

Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using a cobalt-60 γ-radiation source. The source-to-skin distance was 80 cm with a dose rate of 1.03 Gy /min at room temperature (23+2°C). The rats were irradiated with a total dose of 2Gy γ-rays (Hosseinimehr *et al.*, 2006).

4-Determination of Serum Lactate De hydrogenase (LDH) activity:

Lactate dehydrogenate activity was estimated in serum by commercially available LDH Hesperid in Alleviates Doxorubicin-Induced Cardio toxicity in Rat skit (Linear Chemicals, S.L., Spain) according to the method of (Whitaker ., 1969). Using this method, lactate dehydrogenate (LDH) catalyzes the reduction of pyruvate to lactate in the presence of reduced nicotine amide adenine dinucleotid (NADH) at pH 7.5. The reaction is monitored kinetically at 340 nm using an UV-visible spectro photometer (Shimadzu, Japan) by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+ which is proportional to the activity of LDH present in the sample.

5-Estimation of oxidative stress biomarkers in cardiac tissues:

Twenty-four hours after the last dose of the specific treatment, animals were anesthetized with ether, and blood samples were obtained by kidney puncture and serum was separated by centrifugation (Sorvall TC centrifuge, Hamburg, Germany) at 750 g at room temperature for 10 min. Serum urea and creatinine were determined according to the methods of (Hallet and Cook, 1971) and (Bonsenes and Taussky, 1945) respectively. Serum creatinin phosphokinase (CPK) was determined according to the methods of (Swanson and Wilkinson, 1972) and (IFCC, 1980). Serum total nitrate/nitrite (NO(x)) was measured as stable end product, nitrite, according to the method of (Miranda *et al.*,2001). ADMA was estimated using a standard enzyme linked immune sorbent assay (ELISA) method according to the manufacturer's instructions(Immunodiagnostic AG, Bensheim/Germany (Kidneys were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized in ice-cold 0.15 MTris-KCl buffer (pH 7.4) to yield a 20 % (w/v) homogenate using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were used for the determination of malondialdehyde (MDA) level, glutathione peroxides (GSHPx) and superoxide dismutase (SOD) activities, total glutathione (GSH) content, and total nitrate/nitrite (NO(x)). The homogenates were centrifuged at 800 g for 5 min at 4ºC to separate the nuclear debris. The supernatant obtained was centrifuged (Eppendorf AG, centrifuge 5804R, Hamburg, Germany) at 15000g for 30 min at 4ºC to get the post mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity. Reduced glutathione (GSH) and malondialdehyde (MDA) levels in kidney homogenates were determined spectrophotometrically using the methods of (Ellman, 1959) and (Buege and Aust, 1978), respectively. Total nitrate/nitrite (NO(x)) was measured as the stable end product, nitrite, according to the method of (Miranda *et al.*, 2001). The activities of GSHPx and SOD were determined according to the methods of (Lawrence and Burk 1976) and (Minami and Yoshikawa (1979), respectively.

6-Bone Calcium Concentration:

Bone specimens are extracted for calcium assays and put in furnace to obtain bone ash. Each specimen is weighted before analysis then prepared by dissolving in 10% nitric acid over a period of 24 hrs. Calcium assays are preformed via Atomic Absorption Spectrophotometer. Calcium ions form a violet complex with o-cresolophthale in complex one in alkaline solution. The intensity of violet color of this complex measured at 560 nm is proportional to the calcium concentration in the sample. (Wettergreen *et al.*,2005).

7-Biomechanical Measurement:

Biomechanical properties measurements of biomaterials such as bone are ascertained by performing carefully designed laboratory instrument that replicates as nearly as possible the service conditions. Many factors should be considered during the test as nature of the biomaterial, the type of the applied stress and its duration, and also the environmental conditions. Special local hand-made instrument is manufactured according to bone biomechanical characteristics and its visco elastic properties. The main role of this instrumental structure was to determine the stress-strain behavior curves of the bone specimens and its load-unload hysteresis loops. The system is consisted of electronic digital input circuit connected to rotating capacitor coaxial with a frictionless wheel. Special rope of negligible expansion was wrapped around the wheel with two free ends; one end connected to the pan of the loads and the other end was fastened to the bone specimen which was clamped to a fixed point. (An., 2000).

1-Stress-Strain Behavior:

Each bone specimen diameter is measured at three levels by Vernier caliperin (mm± 0.01mm) then average diameter is considered (Mensun, 1991).The mass of the loads (in Kg) is multiplied by the acceleration due to gravity 9.80m/s^2 to get the axial applied force. The axial stress calculated by dividing the axial force by the cross sectional area (πr^2) of the bone specimen and given by

Tensile Stress (σ) =Force /area (N/m^2)

The tensile force is applied by uploading of loads on the pan and calculating its stress value. This applied stress leaded to extend the bone specimen length, and due to this extension the wheel rotated and changed the effective area of the capacitor and in turn the frequency is also changed. Calculate the axial changes on the bone specimen length in terms of frequency and by dividing these value by the

original length in term of frequency also, one can get the longitudinal strain. It is produced due to the tensile stress and given by

Longitudinal Strain (€) = Change in length/original length

$$€ = \Delta L/L_0$$

The stress-strain behavior for each bone sample is performed by applying tensile stress on bone specimen till the breaking point and measure the strain then plotting the stress values on y-axis and its corresponding strain values on the x-axis.

8-Statistical analysis:

Results were expressed as mean ± SEM. The intergroup variation was measured byone way analysis of variance (ANOVA) followed by Turkey's Multiple comparison test. Statistical significance was considered at $p < 0.05$.

3.Results:

Table 2 shows the effects of HES, 2Gy γ-irradiation and their combination on serum creatinine phosphor kinase (CPK), lactate dehydrogenate (LDH), creatinine and urea. Gamma-irradiation (2Gy γ-rays) induced a significant increase in CPK and LDH activities and significant increase in the levels of serum urea and serum creatinine compared to control ($P < 0.001$). Administration of HES for 3 consecutive days before irradiation significantly reduced the activities of CPK and LDH, and the levels of urea and creatinine in serum ($P < 0.001$) compared to the irradiated group (Table 2).

Table 2: Effect of HES irradiation (IR, 2Gy γ-rays) and their combination on serum creatine phosphokinase (CPK) and lactate dehydro genase (LDH) activities, creatinine and urea levels

Groups	LDH (IU/L)	CPK (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)
G(A)Control	1080+50.12	477+40.2	59+2.6	0.91+0.03
G(B) HES	1099+26.9#	401.5 +20.2#	50.9+0.654#	0.92+0.02#
G(C) IR	2256+151.3*	995.3 +22.2*	69.9+3.0*	3.9+0.09*
G(D)HES+IR	1335+96.9#	454.5 +30.2#	50.8+0.64#	1.391+0.08#

Data are presented as mean ± SEM, n = 20. * and # indicate significant changes from control and IR respectively at $P \leq 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

Table 3 shows the effects of HES, 2Gy γ-irradiation and their combination on levels of GSH, MDA, and NO(x), the activity of SOD and GSHPx in kidney tissues. Gamma-irradiation exposure resulted in significant (59.9 %, 20 % and 44.3 %)decrease in SOD and GSHPx activities and GSH content and significant (69.5 % and 119 %) increase in MDA and NO(x), respectively, as compared to the control group. Treatment with HES for 3 consecutive days prior to irradiation resulted in a significant (98.9 %, 20.3 % and 65.9 %)increase in the activities of SOD and GSHPx and in GSH content, respectively, as compared with the irradiated group, and significant (33.6 % and 50.1 %) decrease in MDA and NO(x) levels, respectively, compared to the irradiated group.

Table 3: Effect of HES irradiation (IR, 2 Gy γ-rays) and their combination on the levels of malondialdehyde (MDA), total nitrate/nitrite (NO(x)) and reduced glutathione (GSH), Superoxide dismutase (SOD) and Glutathione peroxidase (GSHPx) activities in rat kidney tissue.

Groups	MDA (nmol/g tissue)	SOD (µg/g tissue)	GSH (µmol/g tissue)	GSHPx (mol/min/g tissue)	NO(x) (µmol/g tissue)
G(A)Control	178.3+2.1	99.36+1.7	0.159+0.001	0.47+0.003	27.9+0.7
G(B) HES	159.5+3.9#	95.6+1.9#	0.163+0.001#	0.46+0.002#	28.8+1.8#
G(C) IR	299.3+8.3*	49.3+0.8*	0.096+0.001*	0.44+0.001*	59.9+1.6*
G(D) HES+IR	200.5+11.9#	89.9+1.9*#	0.152+0.001*#	0.46+0.003	36.9+1.5#

Data are presented as mean ± SEM, n = 20. * and # indicate significant changes from control and IR respectively at $P \leq 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

Figures 2 and 3 show the effects of HES, irradiation and their combination on serum NO(x) and ADMA. Gamma-irradiation exposure resulted in a significant increase in the level of serum ADMA and significant decrease in serum NO(x) level compared to control ($P < 0.001$). Administration of HES for 3 consecutive days before irradiation significantly reduced the level of serum ADMA and the increase in serum NO(x) level ($P < 0.001$) compared to the irradiated group.

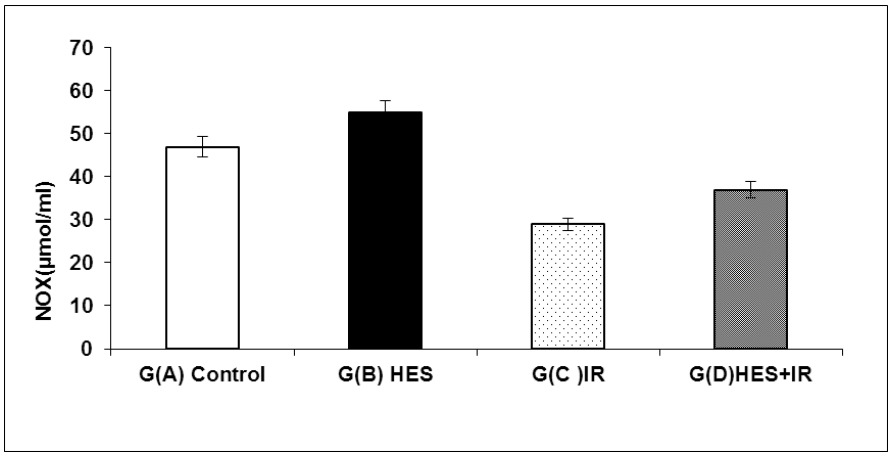

Figure 2: Effect of HES, irradiation (IR, 2 Gy γ-rays) and their combination on serum total nitrate nitrite NO(x)).)

Data are presented as mean ± SEM, n = 20*.and # indicate significant changes from control and IR respectively at $P \leq 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

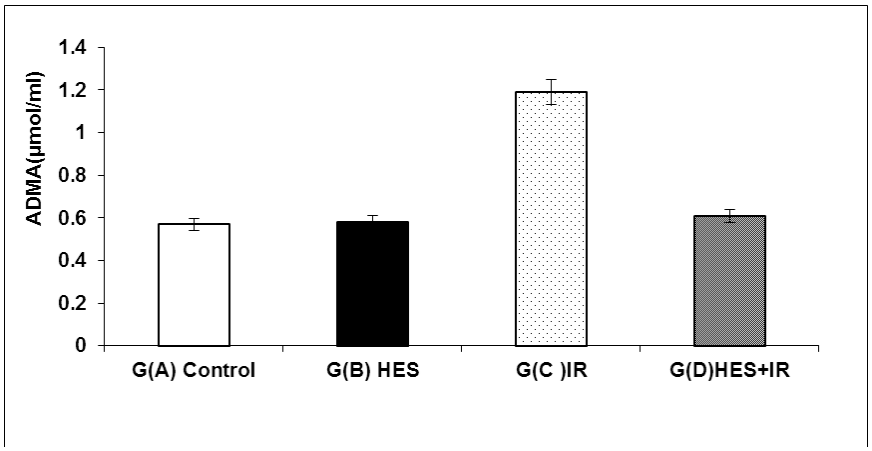

Figure 3: Effect of HES, irradiation (IR, 2 Gy γ-rays) and their combination on serum asymmetric dimethyl arginine (ADMA).

Data are presented as mean ± SEM, n = 20*.and # indicate significant changes from control and IR respectively at $P \leq 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

Table 4 shows the effects of HES, 2Gy γ irradiation and their combination on levels of calcium concentration (mg/dl) in bone .Gamma-irradiation exposure resulted in significant (47.2 %) decrease in average Ca^{+2} concentration (mg/dl) as compared to the control group. Treatment with HES for 3 consecutive days prior to irradiation resulted in a significant (90.9 %) increase in average Ca^{+2} concentration (mg/dl),as compared with the irradiated group. The effect of gamma irradiation with or without HES on the induction of the average value of break points of tibia bones for each group is shown in Table 4. The frequency of break points of tibia bones for each group was increased in group (D) of rats irradiated with 2 Gy γ-irradiation compared with the control treated with normal saline. There was a drug dose–response effect of HES in the reduction of break points of tibia bones for group (D).The maximum reduced break points of tibia bones for group was observed in rats treated with HES at a dose of 160 mg/kg (Figure 4). All these changes in the calcium ion concentration affected the mechanical properties of the bone and caused stiffness of bone as can be noticed from table (4), figures(4&5).

Table (4): Average calcium concentration (mg/dl) in bone & average break point values for each group.

Treatment	Calcium (mg/dl) (mean+S.E.M)	Average break points (Stress/10^5 N/m^2) (mean+S.E.M)	Longitudinal Strain /10^{-3} (mean+S.E.M)
G(A)	11.12+0.12	1.66+ 0.09	2.45+0.06
G(B)	6.98+0.66	1.75+ 0.18	2.11+0.21
G(C)	5.32+0.65	0.8+ 0.09	0.9 +0.05
G(D)	10.11+0.01	1.55+ 0.19	1.999+0.11

Data are presented as mean ± SEM, n = 20*.and # indicate significant changes from control and IR respectively at $P \leq 0.0001$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

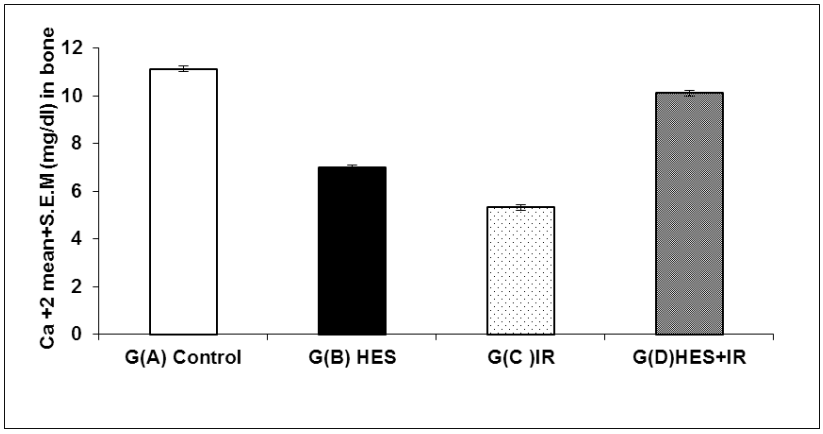

Figure (4): Effect of HES, irradiation (IR, 2Gyγ-rays) and average calcium concentration (mg/dl) in bone for each group.

Data are presented as mean ± SEM, n = 20*.and # indicate significant changes from control and IR respectively at $P \leq 0.0001$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

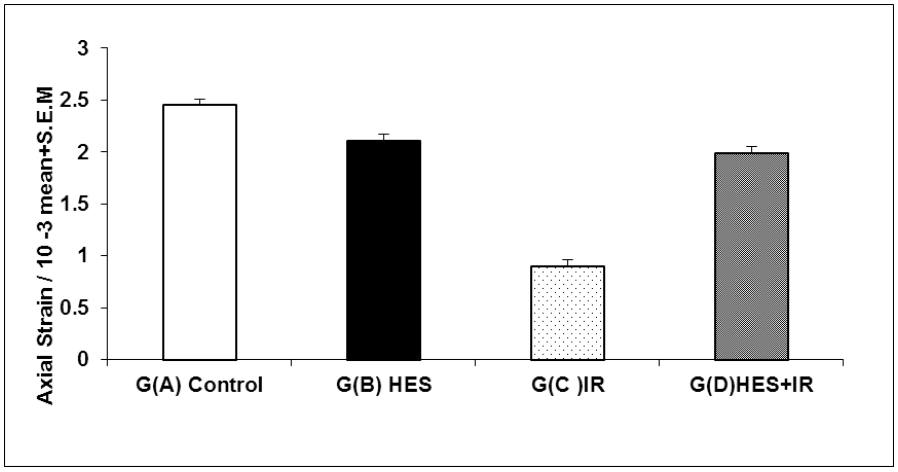

Figure (5): Effect of HES, irradiation (IR, 2Gyγ-rays) and average break point for each group.

Data are presented as mean ± SEM, n = 20*.and # indicate significant changes from control and IR respectively at $P \leq 0.0001$ using ANOVA followed by Tukey-Kramer as a post ANOVA test**.**

4.Discussion:

The results of this study demonstrated the protective effects of HES, a flavonone, against genotoxicity and toxicity induced by γ-irradiation in rat bone marrow cells. However, synthetic compounds, mainly thiol compounds, have good radio protective effects, but they are limited in their use by side effects. Natural compounds, including flavonoids, may play a role in scavenging free radicals, such as hydroxyl radicals generated by γ-rays in cells. Ionizing radiation generates free radical damage in DNA and induces genotoxic effects and

death in the cells (Reily ., 1994 and Pietta 2000) There is a possibility that pre-treatment with flavonoids could induce protection against oxidative stress. Orienting and Vicenin, two flavonoids, protect rats against chromosomal aberration induced by γ-irradiation when administrated before 2 Gy γ-rays (Devi *et al.*, 1998)

Ionizing radiation is known to induce oxidative stress through generation of ROS in an imbalance in pro-oxidant, anti oxidant status in the cells (Bhosle *et al.*, 2005). In the present study, Gamma-irradiation caused a marked increase in serum activities of LDH and CPK, levels of creatinine,urea and ADMA in parallel with a significant decrease in NO(x) level. These data agree with that reported in previous studies, which reported that IRR caused a significant increase in CPK and LDH activities (Sridharan and Shyamaladevi, 2002) .The excessive production of free radicals and lipid per oxides might have caused the leakage of cytosolic enzymes such as lactate dehydrogenase, creatine kinase and phosphates. Also, it could induce lipid per oxidation of cell membranes structure by oxygen derived free radicals leading to ionic leakage through cellular membranes and excessive calcium influx with ensuring cellular dysfunction and death from calcium overload (Ramadan *et al.,* 1997). Increase in serum urea was due to increase in glutamate de hydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetics activity leading to increase in urea concentration (Ramadan *et al.*, 2001).Treatment with HES for 3 consecutive days prior to irradiation ameliorated the activates of serum CPK and LDH and the levels of serum creatinine and urea.

In the present study, the γ-irradiated rats showed a significant increase in serum ADMA concomitantly with a significant decrease in NO(x) level. The effect is probably mediated by oxidative stress (Maas *et al.*, 2007,Heba., 2013).In agreement with our results, previous studies of (Schnabel *et al.,* 2005;Busch *et al.*, 2006; Ueda *et al.,* 2007) and Wilcox 2012) have reported elevated ADMA levels and decreased NO(x) levels in states of cardiovascular diseases and chronic kidney disease in human and rat and also in response of endothelial cells to ionizing radiation (Lanza *et al.*, 2007). Elevated levels of ADMA inhibit NO synthesis and therefore impair endothelial function (Sibal *et al.,* 2010). Reduction of NO(x) levels might be due to both decreased production and increased consumption, with possible endothelial dysfunction and vascular impairment (Soloviev *et al.,* 2003).In addition to improvement of serum cardiac enzymes like LDH and CK, hesperidin also ameliorated the altered oxidative stress biomarkers. Hesperidin markedly increased the reduced glutathione (GSH) levels and augmented the superoxide dismutase (SOD) activity in heart tissues that was attenuated by doxorubic in treatment. Our results are matched with that of (Tirkey *et al.,* 2005, Heba., 2013).

HES is known to enhance the release of nitric oxide (NO) from endothelial cells of the rat aorta and kidney, and to protect the heart from injury via up-regulation of endothelial NO synthesis (eNOS) expression (Razavi *et al.,* 2005; Hare and Stamler, 2005), resulting in Ca^{+2} channel inhibition, activation of cardiac potassium channels and protection against ischemia-reperfusion injury (Wilcox., 2012; Szelid *et al.,* 2010). These findings suggest that some of the observed effects of HES are possibly mediated through its antioxidant property. Consistent with previous studies (Mansour and Hafez, 2012; Pradeep *et al.,* 2012), the present study showed a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides and NO(x) levels in renal tissues after whole body gamma-irradiation. In agreement with previous studies and in line with the findings derived from human studies, doxorubicin in our model led to severe cardio myopathy as indicated from the increase in serum activities of cardiac enzymes such as lactate dehydrogenase and creatine kinase (Gómez *et al.,* 1998;Mohamad *et al.,* 2009).Ionizing radiation is known to induce oxidative stress through generation of ROS in an imbalance in pro-oxidant, anti oxidant status in the cells (Bhosle *et al.,* 2005 ;Heba., 2013). The increase in lipid peroxidation levels in γ-irradiated rats might be due to the interaction of free radicals with poly un saturated fatty acids in the phospholipids portion of cellular membranes (Spitz *et al.,* 2004;Prasad *et al.,* 2005). The decrease in the activities of SOD and GSHPx and the decreased level of GSH might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation (Kregel and Zhang, 2007). Among naturally occurring flavonoids, HES has been pharmacologically evaluated as a potential anti carcinogenic agent because of its antioxidant activity (Yang *et al.,* 1997: Tanaka *et al.,* 2000). Other biological effects include immune-modulation, treatment of venous in sufficiency and scavenging of peroxy nitrite as a reactive oxidant (Garg *et al.,* 2001: Kim *et al.,* 2004). HES has also protected against photo induced breakage of DNA (Yoshikawa *et al.,* 2004). However, the antioxidant capacity of HES is not as high as that of other flavonoids such as quercetin and myricetin (Rice-Evans *et al.,* 1996; Bonina *et al.,* 1996) Other mechanisms probably contribute to its radio protective effects. Thus, further experiments are needed to explain the molecular mechanism of HES protective effects. All these

changes in the calcium ion concentration affected the mechanical properties of the bone and caused stiffness of bone as can be noticed from table (4) and figures (4&5).The molecular mechanism of the radio protective effects of HES is not clear. It has been reported that flavonoids have antioxidant and chelating properties. These poly phenols are excellent scavengers of free radicals due to the high reactivity of their hydroxyl substituent (Pietta *et al.,* 2000). Free-radical scavenging is apparently responsible for the inhibitory effect of flavonoids such as rutin, morin, quercetin and genestin on the clastogenic activity induced by γ-irradiation in mice (Shimoi *et al.,* 1994). Among naturally occurring flavonoids, HES has been pharmacologically evaluated as a potential anti carcinogenic agent because of its antioxidant activity (Yang *et al.,* 1997; Tanaka *et al.,* 2000).Other biological effects include immune-modulation, treatment of venous insufficiency and scavenging of peroxy nitrite as a reactive oxidant (Garg *et al.,* 2001; Kim *et al.,* 2004). HES has also protected against photo induced breakage of DNA (Yang *et al.,* 1997; Heba., 2013). It is possible that HES protects bone marrow cells with its antioxidant activity. However, the antioxidant capacity of HES is not as high as that of other flavonoids such as quercetin and myricetin (Rice- *et al.,* 1996; Bonina *et al.,* 1996).Other mechanisms probably contribute to its radio protective effects. Thus, further experiments are needed to explain the molecular mechanism of HESs protective effects.

In Conclusion:

Our results demonstrate that HES gives significant protection to rats bone against the clastogenic effects of gamma irradiation.

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