EVALUATION OF RADIOPROTECTIVE EFFECT OF *GONGRONEMA LATIFOLIO* LEAF EXTRACT ON WHOLE-BODY IRRADIATED WISTAR ALBINO RATS

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF PHYSICS AND ASTRONOMY,FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.SC) IN MEDICAL PHYSICS.

SUPERVISORS: PROF. K.K. AGWU AND
PROF. R.U. OSUJI

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TITLE PAGE

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CERTIFICATION

Okeke, Izunna Stanislaus a postgraduate student in the Department of Physics and Astronomy with Registration number PG/MSc/12/63171 has satisfactorily completed the requirement for award of the degree of Master of Science (MSc) in Medical Physics.

This research work is original and has not been submitted in part or full for any other diploma or degree or professional qualification of this or any other university.

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ABSTRACT

The purpose of the study was to evaluate the radioprotective effects of *Gongronema latifolio* (GL) leaf extract on a whole-body irradiated wistar albino rats. A prospective experimental and cross-sectional design was adopted for this study and it included a control group and experimental group. Part of the control group (normal control NC) was not irradiated neither was it administered with GL extract but the other part (experimental control EC) was only exposed to graded radiation doses (GRDs). In the experimental group, the pre-treatment group (PRT) received GL extract orally before being exposed to GRDs while post-treatment group were exposed to GRDs before receiving GL extract orally.

Phytochemical analysis of GL extract was done to re-determine the bioactive constituents of the extract. Physical changes were observed and recorded in all the groups using weight loss as an index. The blood samples of the animal groups were collected before and after irradiation (IR) for following analysis namely liver function test (LFT) {which includes- Alkaline phosphase (ALP), Alanine amino-transferase (ALT), Aspatate amino-transferase (AST)}, and antioxidant enzymes tests like Malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD)}.

The result of the phytochemical analysis revealed the presence of the following bioactive agents- alkaloids (3.11mg/g), tannins (2.43mg/g), flavonoids (1.31mg/g), phenols (1.10mg/g) and saponin (0.8mg/g). Body weight of the rats exposed to 6Gy in EC (51g) significantly ($p<0.05$) decreased when compared to NC (115g) and PRT (70g) but not significantly ($p>0.05$) different from PST (60g) group. ALP mean levels recorded in rats exposed to 4Gy increased ($p<0.05$) significantly in EC (74iU/L) when compared to PRT (37iU/L), PST (43iU/L) and NC (39iU/L) group on day 8 after IR. ALT mean level for rats exposed to 4Gy elevated ($p<0.05$) significantly in EC (50iU/L) relatively to PRT (31.67iU/L), PST (38.67iU/L) and NC (37iU/L) on day 8 after IR. MDA activity levels for rats exposed to 6Gy significantly ($p<0.05$) increased in EC (70%) relatively to PRT (35%), PST (59%) and NC (36%) on day 8 after IR. For rats exposed to 2Gy, GSH % activities decreased ($p<0.05$) significantly in EC (26%) when compared to PRT (59%) and NC (69%) on day 8 after IR. For rats exposed to 4Gy, CAT % activities significantly ($p<0.05$) decreased in EC (31%), PRT (49%) and PST (44%) relatively to NC (79%) on day 8 after IR. For rats exposed to 2Gy, SOD % activities decreased significantly in EC (29.33%), PRT (50.67%) and PST (40.67) when compared to NC (75%) on day 8 after IR.

Consequently, the result obtained suggested that GL extract emeroliates oxidative stress induced by ionizing radiation, thus affirming its radioprotective potentials. The result also demonstrated that the extract was more effective in PRT group relatively to PST group

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

With the discovery of x-rays in 1895 and radioactivity in 1896, the biologic effects were also observed shortly after. Within the first six months of its use in treating patients, several cases of erythema, dermatitis and alopecia were already reported among x-ray operators and their patients. The first report of a skin cancer ascribed to x-rays was reported in 1902, followed eight years later by experimental confirmation, Bushberget al., (2002).

Radiation medicine is one of the major sources of ionizing radiation due to its numerous applications in the hospital. Other sources of radiation exposure include radon in houses, contamination from weaponstesting sites, nuclear accidents and cosmic rays.Today, ionizing radiation is not only employed in treatment of diseases and industry but also in developing new varieties of high-yielding crops and enhancing storage period of food materials. Radiotherapy is one of the common sources of ionizing radiation and more so one of the most common modality used for treating human cancer. About 80% of cancer patients need radiotherapy at some time or the other either for curative or palliative purpose, Cherupally et al., (2001). It is essentially used in the treatment of a number of malignancies, but frequently its use is limited due to its adverse effects on normal tissue.

The effects of radiation on human cells/tissue can be divided into somatic and genetic effects. Somatic effects are harms exposed individual suffer during their life time such as radiation induced cancers, opacification of the eye etc, while genetic effects are radiation induced mutation to an individual genes and DNA that can contribute to the birth defective descendants Podgorsak, (2005). Somatic effects of radiation exposure can be classified as either stochastic or non-stochastic. A stochastic effect is the effect in which the probability of the effect, rather than its severity, increases with dose. Radiation-induced cancer and genetic

effects are stochastic in nature. Stochastic effect is believed not to have a dose threshold. In non-stochastic effect, there is a threshold dose below which the effect is not seen. Cataract, erythema, fibrosis and hematopoietic damage are some of the non-stochastic effects that can result from large radiation exposure.

Radiation interactions that produce biologic changes are classified as either direct or indirect action. The change takes place by direct action if biologic macromolecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or proteins become ionized or excited by an ionizing particle or photon passing through them or near them.The DNA damages caused per Gray are about 1000 single strand breaks (SSB), 40 double strand breaks and 950 base depurination. Roughly 4.4 x 10^7 single strand breaks, 1.4 x 10^7 double strand breaks and 1.1 x 10^7base lesion per year occur per mammalian cell, Fleck, et al,.(1999). Indirect effects are the result of radiation interactions within the medium (e.g. cytoplasm or water) which creates highly reactive free radicals chemical that in turn interact with the target molecule, Bushberget al., 2002). Because 70% to 85% of the mass of living system is composed of water, the vast majority of radiation-induced damage from medical irradiation is mediated through indirect action on water molecules. Exposure of biological tissues to ionizing radiation immediately leads to ionization and excitation of their constituent atoms. The molecules where the atoms reside then dissociate, resulting in so called free radicals, Mayles et al., (2007). This free radicals are reactive oxygen species such as hyoxyl radical (OH), superoxide radicals (O_2^-), singlet oxygen and peroxyl radicals (ROO) in irradiated tissue that incite several pathophysiological changes in the body, Maurya, et al., (2011).

Free radicals can diffuse in the cell, producing damage at locations remote from their origin. They may inactive cellular mechanisms directly or via damage to genetic material (DNA and RNA), and they are believed to be the primary cause of biologic damage from low linear energy transfer (LET) radiation, Bushberg et al., (2002). It is estimated that two-thirds of DNA damage is caused indirectly by scavengeable radicals (Root and Okada, 1972), as

reported in lobachevsky, et al., (n.d).Generally ionizing radiation causes either excitation or ionization or both to atoms and molecules which lead to the following conditions.

- Generation of free radicals as mentioned earlier.
- Breaking of chemical bonds.
- Formation of new chemical bonds and cross-linkage between macromolecules.
- Damage to biomolecules (e.g. DNA, RNA, Lipids, Proteins) which controls or regulates vital cell processes.

The detrimental consequences of irradiation (IR) of cells and tissues can be encountered in cancer radiation therapy. Apart from normal tissue damage, another issue associated with cancer radiotherapy is the potential for emergency of secondary radiation-induced cancers, affecting more than 1% of patients (Hall, 2006).Severally protective mechanisms have been adopted in radiotherapy to reduce oxidative stress in patients and it includes;

- Physical protection (E.g. Conformal radiotherapy, intensity modulated radiotherapy IMRT etc).
- Biological protection (E.g. hyperfractionation and Ultrafractionation).

Attempts have also been made to protect personnel working inradiation medicine departments, radiopharmaceutical centers, nuclear power operations, aviations, uranium miners and other sources of ionizing radiation through the provision of the following; personal dosimeter, shielding devices, radiation detection equipment and other safety procedures, policies etc so as to ensure safety of patient, occupational staff and the general public. But the truth is thationizing radiation and radioactive substances are natural and permanent features of theenvironment,and thus the risks associatedwith radiation exposure can only be restricted and cannot be eliminated entirely.

Consequently, attempt to mitigate radiation toxicity in normal cells/tissues and in a whole organism are of significant clinical importance and an area of active research, considering the fact that ionizing radiation is on increase in numerous aspect of human life. There isexigency to develop and improve on another protective mechanism aside from the ones mention earlier that can mitigatenormal tissues from toxic effects of radiation.It has also been considered realizable that radiation therapy for cancer patients could be enhanced by the use of radioprotectors to protect normal tissues from unwanted radiation exposure.

Radioprotectors are compounds that are designed either to mitigate or prevent the damage caused by radiation in normal tissue. These compounds are often antioxidants and must be present before or at the time of radiation for effectiveness. It has also been found in the studies that chemical agents given after radiation exposure may assist in DNA repair activities, reduce inflammation and persistent radiation-induced oxidative stress and facilitate death pathways (apoptosis) of damaged cells, Kumud et al.,(2014). Other agents, termed mitigators, may be used to minimize toxicity even after radiation has been delivered, Deborah et al., (2010).

A number of compounds have been evaluated under the anti-irradiation drug development program, in 1948 for the first time, Patt et al., reported that cysteine is an effective radioprotector and showed that it can protect mice from harmful effects of total body x-ray irradiation when administered before radiation exposure. Badr et al., (1999) in their study suggested that melatonin administration confers protection against damage inflicted by radiation when given prior to exposure to irradiation and not after, and supports the contention that melatonin radioprotection is achieved by its ability as a scavenger for free radicals generated by ionizing radiation. The radioprotective effect of abana, following a total body irradiation was studied by Baliga et al., (2004). Their result indicates that the

radioprotective activity of abana may be due to free radical scavenging and increase GSH levelin the irradiated mice.

Several chemical compounds have been synthesized and tested for their radioprotective ability (Sweeney, 1979). The major disadvantage of some these compounds has been their high toxicity at the optimum protective dose (Sweeney,1979), which forestall their effective use in man.

Gongronema latifolium (GL) is an edible plant, less toxic, relatively cheap and available, thus, it is considered a possible radioprotective material. This study therefore aims at providing information on the radioprotective effects of GL on wistar albino rats whose whole-bodies were exposed to different doses of radiation.

1.2 Objectives of the study

- This research investigates the possible radioprotective effect of *Gongronema latifolio*(GL) extract on a whole-body irradiated wistar rats through the following specific objectives.
    1. Tore-determine the phytochemical constituent of GL extract, so as to find out the bioactive constituents of the leaves.
    2. To observe any physical changes following graded doses of radiation to wistar albino rats.
    3. To determine any radioprotective effects of GL by measuring changes in liver enzymes following exposure to graded radiation doses (GRDs).
    4. To determine lipid oxidative degradation using malondialdehyde (MDA) as an index for radiation damage in un-irradiated and radiated animal groups.
    5. To determine the scavenging of free electron activity in all the animal groups following exposure to graded radiation doses by measuring the antioxidant enzymes.

6. To compare the radioprotective effects of GL extract in both pre-treated animals and post-treated animals exposed to radiation.

1.3 Justification of the study

So far it is only few compounds that are radioprotectors registered forhuman use that has shown good radioprotective effects.However, they have significant shortcomings including relatively high toxicity and unfavorable routes of administration, which negatively affect their application and efficacy,(Lirenet al., (2010).

- This very study will be a contribution in the search for new cost effective and relatively less toxic radioprotectors.
- This study will provide information on whether *G.latifolio* can serve as prophylactic agent, mitigator or therapeutic agents, in whole-body irradiated rats.
- Results obtained in this study will also contribute significantly to the growing search for radioprotectors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual review

2.1.1 Radiation

Radiation is the energy that comes from a source and travels through material or space. Types of radiation include heat, light and sound etc. There are two kinds of radiation- ionizingand non-ionizing radiation. Ionizing radiationrefers to any type of electromagnetic radiation or sub-particles that have enough energy per quantum to liberate or remove tightly bound electron(s) from atom or molecule and non-ionizing radiation refers to any type of electromagnetic radiation that does not have enough energy per quantum to ionize atom or molecules but has sufficient amount of energy to move around atoms in a molecule or causethem to vibrate.

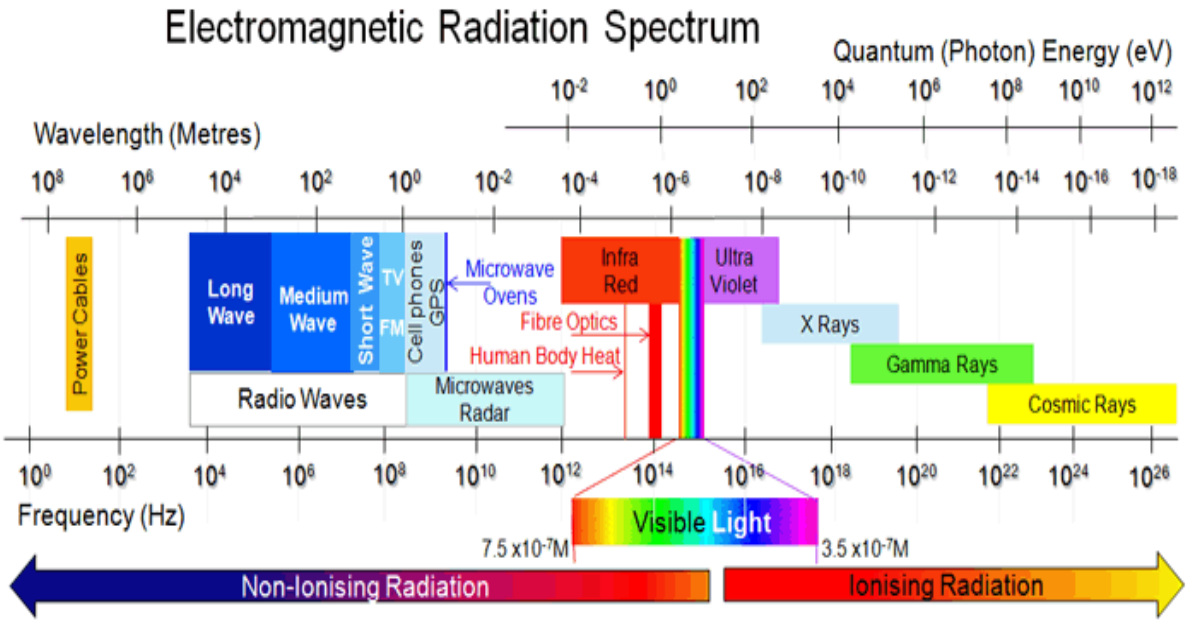


Figure 2.1: Frequencies of electromagnetic radiation and the corresponding photon energies and some of the applications for which they are used; (Source: Electropaedia-www.mpoweruk.com/radio.htm).

2.1.2 Source of exposure to ionizing radiation.

There are several sources of radiation which can be classified into two major groups- Naturally occurring radiation and anthropogenics sources.

2.1.2.1 Naturally occurring ionizing radiation sources

These includes; (a) Cosmic rays, (b) Cosmogenic radionuclides and (c) Primordial radionuclides and their radioactive decay products.

Cosmic radiation includes both the primary extraterrestrial radiation that strikes the earth's atmosphere and the secondary radiations produced by the interaction of primary cosmic rays with the atmosphere. Primary cosmic rays predominantly consist of extremely penetrating high-energy (mean energy ~10 Bev) particulate radiations, Bushberg etal., (2002). Almost all cosmic radiation (approximately 80% of which is high-energy protons) collides with our atmosphere, producing showers of secondary particulate radiations (e.g., electrons and muons) and electromagnetic radiation.

Cosmogenic radionuclides are produced when some fraction of the secondary cosmic rays collide with stable atmospheric nuclide (eg ${}^{14}_{7}N[n,p]^{14}_{6}C$).

Primordial radionuclides are the terrestrial radioactive materials that have been present on earth since its formation. Primordial radionuclides with physical half-life comparable to the age of the earth (~4.5 billion years) and their radioactive decay products are the largest sources of terrestrial radiation exposure, Bushberg et al., (2002). Primordial radio-nuclides with half-life less than 108 years have decayed to undetectable levels since their formation, whereas those with half-lives greater than 10^{10} years do not significantly contribute to background radiation levels because of their long physical half-lives (i.e. slow rates of decay), Bushberg et al., (2002).

2.1.2.2 Anthropogenic source

Technology-based source is categorized into two; (a) enhanced natural source and (b) Artificial source.

In enhanced natural source, tobacco is the largest contributor, which are estimated to produce an equivalent dose to the bronchial epithelium of ~160mSv (16 rem) for smokers Bushberg et al, (2002).Enhanced natural radiation source also include building materials, radon gas and other less important sources like mining, agricultural activities (primarily from fertilizers containing members of uranium and thorium decay series and K-40); combustible fuels including (coal and natural gas); certain ceramics.

The majority of the exposure is from medical diagnosis and therapy with small contribution from nuclear medicine. The medical use of radiation produces an annual average effective dose equivalent of ~540 μSv (54 mrem), which represent more than 95% of the total from artificial radiation source, Bushberg et al., (2002).

Other sources of artificial radiations are fallout from the atmospheric testing of nuclear weapon, nuclear power production (during mining, manufacturing of Uranium fuel, reactor operations and radioactive waste disposals.

2.1.3 Ionizing Radiation

Ionizing radiation are electromagnetic radiations or sub-particles that have enough energy per quantum to liberate electrons from an atom or molecules. Two types of radiation used in medical diagnosis and therapy are electromagnetic (EM) and particulate radiation.

EM radiation used in diagnostic imaging include: (a) Gamma rays, which emanate from within the nuclei of radioactive atoms and are used to image the distribution of radiopharmaceuticals; (b) X-rays, which are produced outside the nucleus and are used in radiography and computed tomography imaging;

Electromagnetic radiation can be described as waves and as particles. In other words, EM radiation behaves like wave and in some situation as particles. As a wave, it is characterized by the amplitude, wavelength (λ), frequency (ν), speed (c), period (T) and energy per photon (E). Where amplitude is the intensity of the wave, wavelength is the distance between any two identical points on the adjacent cycles, frequency is number of periods that occur per seconds, and period is the required to complete cycle of a wave. The speed (c), frequency (ν) and wavelength of all waves are related by

$$c = \lambda \nu \tag{1}$$

When interacting with matter, EM radiation can exhibit particle-like behavior. These particle-like bundles of energy are called photon. The energy of a photon is given by

$$E = h\nu = h\frac{c}{\lambda} \tag{2}$$

Ionizing radiation include charged particle, such as alpha particles (α^{+2}**),** protons (p^{+}**),** electron (e^{-}**),** beta particles (β) and positron (e^{+} *or* β^{+}) and uncharged particle, such as neutrons (n). Charged particles can be classified as either heavy or light charged particles. The behavior of heavy charged particles (e.g alpha particles and protons) is different from the light charged particle such as electrons and positrons. The important distinction between them is their path in matter. Electrons follow tortuous paths in matter as result of multiple scattering events caused by coulombic deflection, but heavy charge particles like alpha particle results in a dense and linear ionization track, Bushberget al., (2002). The path length (the actual distance the particle travels) of electron almost exceeds it range (range of a particle is the actual depth of penetration of in matters). Whereas the straight track of a heavy charged particle result in the path and range being nearly equal. Another important distinction between heavy and light charged particle is linear energy transfer ñLETò which is the amount of energy deposited per unit path length, expressed in units of *ev/cm*.

The LET of a charged particles is proportional to the square of the charge (Q^2) and inversely proportional to the particleôs Kinetic energy (E_k) i.e.

$$LET \quad \check{U} \quad \frac{Q^2}{E_k} \tag{3}$$

The LET of a particular type of radiation describes the energy deposition density, which largely determines the biologic consequence of radiation exposure. Generally ñhigh LETò radiation (alpha particles, protons etc) are much more damaging to tissue than ñLow LETò radiations, which include electrons (e^-, B^- and B^+) and ionizing EM radiation (gamma and X-rays).

2.1.3.1 X-ray and gamma interaction with matter

When X-ray and gamma ray interact or transverse matter, four major kinds of interaction could occur, it includes; Rayleigh scattering, Compton scattering, photoelectric absorption and Pair production.

2.1.3.1.1CoherentScattering

In coherent scattering, the incident photon has an oscillating electric field with it that sets the electrons in the atoms into momentary vibration, Harold and John, (1983). The atoms electron cloud immediately radiates this energy, emitting a photon of the same energy but in a slightly different direction. In this interaction, electrons are not ejected and thus ionization does not occur. The scattering angle increases as the x-ray energy decreases.

This interaction occurs mainly with very low energy diagnostic x-rays, as used in mammography (15 to 30 keV). In soft tissue, Rayleigh scattering accounts for less than 5% of x-ray interactions above 70 keV and at most only accounts for 12% of interactions at approximately 30 keV, Bushberget al., (2002).

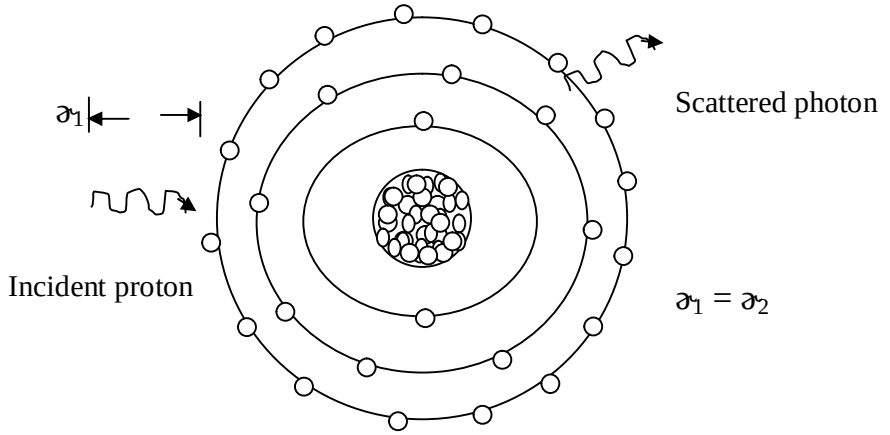


Figure 2.2: Diagram of Coherent scattering

2.1.3.1.2 Compton Scattering

This interaction is most likely to occur between incident photon and the outer (valence) electron which are not tightly bound to the atom. The electron is ejected from the atom, and the photon is scattered with some reduction in energy. The energy of the incident photon (E_o) is equal to the sum of the energy of the scattered photon (E_{sc}) and the kinetic energy the ejected electron (E_e).

$$E_o = E_{sc} + E_e \qquad (4)$$

Compton scattering results in the ionization of the atom, the ejected electron lose its kinetic energy via excitation and ionization of atoms in the surrounding material.

The scattered photonôs energy can be calculated from the incident photon energy and angle of the scattered photon (with respect to the incident trajectory)

$$\square_{\square\square} = \frac{\square_{\square}}{\square\square \frac{\square_{\square}}{\square\square\square\square\ \square\square} (\square\square\ \square\square\square\square)} \qquad (5)$$

Where ? = the angle of the scattered photon

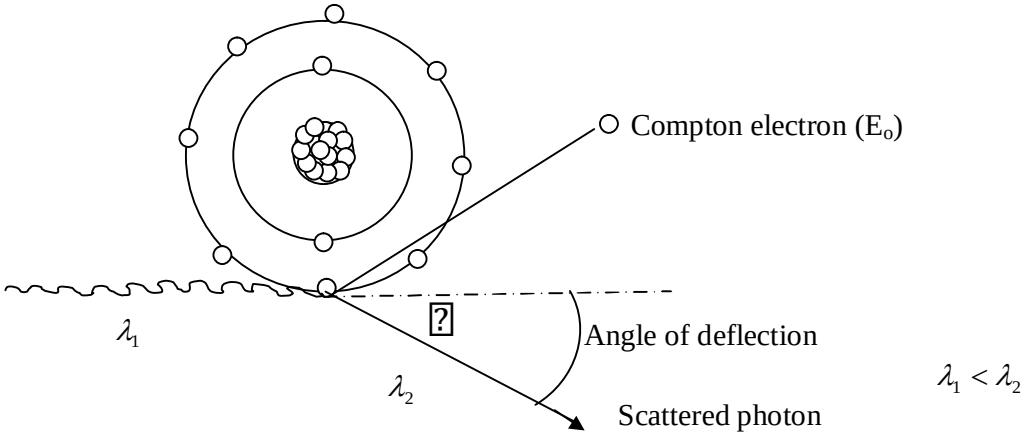


Figure2.3: Schematic Diagram of Compton scattering.

As the incident photon energy increases, both scattered photon and electrons are scattered more toward the forward direction. The incident photon energy must be substantially greater than the electrons binding energy before Compton interaction can take place.

2.1.3.1.3The Photoelectric Effect

In the photoelectric process, there is a collision between a photon and an atom resulting in the ejection of a bond electron. This process is most likely to occur if the energy of the photon is just greater than the binding energy of the electron. The K.E of the ejected photon electron (E_e) is equal to the incident photon (E_o) minus the binding energy of the orbital electron (E_b).

$$E_e = E_o - E_b \qquad (6)$$

The probability of characteristics X-ray emission decreases as the atomic number of the absorber decreases and thus does not occur frequently for diagnostic energy photon interactions in soft tissueBushberget al., (2002). The probability of photoelectric absorption per unit mass is approximately proportional to Z^3/E^3, where Z is the atomic number and E is the energy of the incident photon.

2.1.3.1.4 Pair Production

In pair production, positron under a process of energy deposition via excitation and ionization; however, when they come to rest they react violently with their antiparticles (electron). This process results in the entire rest mass of both particles be instantaneously converted to energy and emitted as two opposite (i.e.180 degrees apart) 511kev annihilation photons. According to Einsteinôs energy-mass equation.

$$E = mc^2 \quad (7)$$

511kev is the energy equivalent of the rest mass of an electron (positron or electron). Thus, there is an inherent threshold for positron decay; it is equal to the sum of the annihilation photon energies (1.02 MeV). The transition period between the parent and daughter must be greater than or equal to 1.02 MeV for positron decay to occur.

2.1.3.2 Interaction of charged particles with matter.

All charged particle (alpha particles, electrons, positrons etc) lose Kinetic energy, chiefly through interaction between the electric field of the particle and electric fields of the electrons in the material which the particle is traveling, Harold and John, (1983). These charged particles lose its kinetic energy through excitation and ionization.

2.1.3.2.1Alpha particle*(α^{2+})*

The alpha particle is an energetic helium nucleus, consisting of two neutron and two protons. Itôs therefore heavier than the electron by a factor of over 7,300 and has double the charge. Additionally, the interaction of alpha particles with matter is very strong due to alpha particles electrical charge of 2 units. Its trajectories can be deviated by both electric and magnetic field.Alpha decay can be described by the following equation

$$^{A}_{Z}X \rightarrow ^{A-4}_{Z-2}Y + ^{4}_{2}H^{2+} + (\text{Transition energy}) \quad (8)$$

Alpha particle decay results in a large energy transition and slight increase in ratio of neutron to protons ratio (N/Z).

The alpha particle emitted by radionuclei posses kinetic energies in the ranging between 4 Mev and 9 Mev. The corresponding speeds are between *1.4* and $2.1x10^{9}m/sec$. They are much lesser than the speed of beta particles in the vicinity of atoms they pass and exert much larger impulses on the orbital electron. Examples of natural occurring alpha emitters are uranium, thorium, radium, polonium etc.

2.1.3.2.2 Proton

Protons are charged particles and are relatively óheavyô compared to electron (mass of the electron Mp, is 1830 times the mass of electron (Me), Mayles et al., (2007). The interaction of proton with matter is the basis for the therapeutic potential of these beams, in particular because of their characteristics of high ionization density at the end of the range and their weak scattering.

The proton is emitted in spontaneous radioactive decay of nuclei of an element as in alpha particle, but must be given a minimum amount of energy, through a nuclear collision with another ionizing

Mechanisms of interaction for protons are: Inelastic collision with the nucleus, inelastic collision with electron and Elastic collision with the electron;

- Inelastic Interaction with the nucleus results in either a significant deflection of the incident proton associated with a nuclear reaction or bremsstrahlung. In nuclear reaction like (p,n), the production of neutrons and recoil nuclei as well as activation of the medium with gamma ray production. The probability of interaction for inelastic interactions with the nucleus creates a particular problem for radiation protection. The proportion of the nuclear interaction can be estimated by measuring the reduction in planar fluence (the number of

particles crossing a fixed plane in either direction per unit area of the plane) of the protons at different depths on matter before stopping at the end of the range is given by equation 9.

$$I = I_0 e^{-t/s} 2 \quad (9)$$

Where I_0 = the incident planar fluence, I = the planar fluence after a thickness absorber, and S = the proton mean free path length given as

$$S = A^{1/3} / 0.032 \ \ gcm^{-2} \quad (10)$$

- Inelastic interaction with atomic electrons represent the principal process by which protons lose energy along their trajectory at the energies and in the materials of clinical interest, creating atomic excitation or ionization as well as a small deflection of the incident proton.

Although it is possible for electron to lose a large fraction of its energy and be deflected through large angle in an inelastic interaction. The energy transferred by protons at each interaction is always small, the maximum possible value being approximately

$$4m / M \quad (11)$$

m = rest mass of the electron and M = the mass of the proton. The amount of energy transferred in each interaction has a probability distribution which results in energy-straggling after through a given thickness of absorber. In practice, energy and particularly range straggling for clinical proton beams can be assumed to have nearly Guassian distribution, Mayles et al., (2007). The fluctuation in the path length of clinical proton beam in water is of the order of 1-1.32 (1σ) of the range.The average loss of energy by collision per unit distance along the path of a proton $(dE/dS))$ is represented by the collision stopping power.

$$(dE/dS)) = \frac{4\pi z_{eff}^2 e^4 N_A Z}{A} \frac{1}{mv^2} \left\{ \ln \frac{2mv^2}{I(1-\beta)} - \beta^2 - \sum \left(\frac{ci}{z} \right) \right\} \quad (12)$$

z^2 = effective charge of the particle,

e = the electronic charge,

$N_A Z$ = represent the number of electrons per grams,

m and v are the mass of electron and velocity of the particle respectively,

β = the ratio of the velocity of the particle to the velocity of light,

I = mean excitation energy of the atom of the absorbing material and

$\sum\left(\frac{ci}{z}\right)$ = the density and shell correction terms.

When a proton is almost at the end of its range its capacity to ionize increases rapidly since its velocity is low giving rise to the Bragg peak**.** The amount of ionization produced by a beam of photon is a combination of the photon fluence and energy-transfer function ie the stopping power. The depth dose curve for a broad beam of heavy charged particles is known as Bragg curve.

- Elastic interaction with the nucleus causes a deviation of the incident proton with a negligible change in energy; this process is often called Rutherford scattering. The total cross section for this process decreases rapidly with the energy of the particle and differential cross-section decreases with increasing deflection angle. Most collision that involves a distant interaction of the particle with a nucleus, the nuclear charge is partially screen by the atomic electrons, and the incident particle experience only a small deflection. The multiplicity of small angle deviation along the proton path is known as multiple coulomb scattering.

The angular distribution of particles after transverse on a thin foil can be represented to first order by a Gaussian, where the mean angle of multiply scattering is given by

$$\theta_0 = 14.1\frac{z}{pv}\left\{\sqrt{\frac{L}{L_R}\left(1+\frac{1}{a}\log\left(\frac{L}{L_R}\right)\right)}\right\} \qquad (13)$$

Where z, p and v are charged number, momentum and velocity of the incident photon respectively,

L = the thickness of the scatterer,

L_R = the radiation length characteristics of scattering material, given as

$$L_R \alpha \frac{A}{NZ(Z+1)} \log(183Z^{-\frac{1}{3}}) \quad (14)$$

Where N = Avogadro number,

Z = Atomic number,

A = Atomic weight of the target material

2.1.3.2.3 Electron

As an energetic electron transverses matter, it interacts with matter through coulombs interactions with the orbital and atomic nuclei. Through these collisions the electron may lose their kinetic energy (collision and radiative losses) or change their direction travel, Podgorsak, (2005). The energy losses are described by stopping power and scattering power. The collision between the incident electron and orbital electron or nucleus of an atom may be elastic or inelastic. In an elastic collision electron is deflected from the original path and no energy loss occurs, while in an inelastic collision the electron is deflected from its original path and some of its energy is transferred to an orbital electron or emitted in the form of Bremsstralung.

- Electron-orbital electron interactions.

Coulomb interaction between the incident electron and orbital electron of an absorber results in ionization and excitation of absorber atoms. Atomic excitation and ionization result in collisional energy losses and are characterized by collision stopping power, given in equation 15

$$-\left(\frac{dE}{dX}\right)_e = \frac{2\pi e^2}{m_o v^2} NZ\left[\ln\frac{m_o vE}{2I^2(1-\beta^2)} - \ln 2(2\sqrt{1-\beta^2} - 1 + \beta) + (1-\beta^2) + \frac{1}{8}(1-\sqrt{1-\beta^2})\right] \quad (15)$$

- Electron-nucleus interactions: coulomb interactions between the incident electron and nuclei of the absorber atom results in scattering and energy loss of the electron

through production of x-ray photon. This type of energy can be characterized by radiative stopping powers.

$$-\left(\frac{dE}{dX}\right)=\frac{NEZ(Z+1)e^4}{137m_0^2c^4}\left[4\ln\frac{2E}{m_0c^2}-\frac{4}{3}\right] \quad (16)$$

E = energy of ionizing particles,

N = number of absorber atoms per cubic centimeter of medium,

Z = atomic number of the ionizing particle,

c = speed of light

m_0 = rest mass of electron,

e = electronic charge,

q = magnitude of unit of electrical charge.

Bremsstrahlung production is governed by the Larmor relationship, which states that the power p, emitted in the form of photons from an accelerated charged particles is proportional to the square of the particle acceleration a and the square of the charge q.

$$p=\frac{q^2a^2}{6\pi e_0c^3} \quad (17)$$

2.1.3.2.4 Beta Particle (β^+)

Beta particles (positron) comprise one of the most important classes of charged ionizing particles. They are actually high-speed electrons that are emitted by the nuclei of an atom as a result of energy released in a radioactive decay process involving the transformation of a proton into a neutron.

Positron decay is driven by nuclear instability caused by excess proton in radio-nuclides. Many of these radio-nuclides that decay by beta-plus (positron) emission, increases the neutron number by one. Beta-plus (positron) decay can be described by the following equation:

$$^{A}_{Z}X \rightarrow {}^{A}_{Z-1}Y + \beta^{+} + \nu \qquad (18)$$

The net result is the conversion of a proton into a neutron with the simultaneous ejection of the positron (β^{+}) and a neutrino (ν). Positron decay decreases the number of protons (atomic number) by 1 and thereby transforms the atom into a different element with an atomic number of Z-1. The number of neutrons is increased by 1; therefore, the transformation is isobaric because the total number of nucleons is unchanged. Accelerator-produced radio nuclides, which are typically neutron deficient, often decay by positron emission. Positron decay increases the *N/Z* ratio, resulting in a daughter closer to the line of stability.

The energy distribution between the positron and the neutrino is similar to that between the negatron and the antineutrino in beta-minus decay; thus positrons are poly energetic with an average energy equal to approximately *1/3* E_{max}, Bushberget al., (2002).

The neutrino and antineutrino are *antiparticles,* as are the positron and negatron. The prefix *anti-* before the name of an elementary particle denotes another particle with certain symmetry characteristics. In the case of charged particles such as the positron, the antiparticle (i.e., the negatron) has a charge equal but opposite to that of the positron and a magnetic moment that is oppositely directed with respect to spin. In the case of neutral particles such as the neutrino and antineutrino, there is no charge; therefore, differentiation between the particles is made solely on the basis of differences in magnetic moment. Other important differences between the particle and antiparticle are their lifetimes and their eventual fates. As mentioned earlier, negatrons are physically identical to ordinary electrons and as such lose their kinetic energy as they traverse matter via excitation and ionization.

When they lose all (or most) of their kinetic energy, they may be captured by an atom or absorbed into the free electron pool. Positrons undergo a similar process of energy deposition via excitation and ionization; however, when they come to rest they react violently with their

antiparticles (electrons). This process results in the entire rest mass of both particles being instantaneously converted to energy and emitted as two oppositely directed (i.e., 180 degrees apart) 51l-keV annihilation photons. According to Einstein's mass-energy equivalence formula, in equation 7

511 keV is the energy equivalent of the rest mass of an electron (positron or negatron). Therefore, there is an inherent threshold for positron decay equal to the sum of the annihilation photon energies (i.e., 2× 511 Kev; or 1.02 MeV). The transition energy between the parent and daughter nuclide must be greater than or equal to 1.02 MeV for positron decay to occur.

2.1.3.3 Neutron interactions

Neutrons are uncharged particles; they do not interact with electrons and therefore do not directly cause excitation and ionization. But however, interact with atomic nuclei, sometimes librating charged particles or nuclear fragment that can directly cause excitation and ionization, Bushberget al., (2002). Neutrons often interact with light atomic nuclei (e.g. H, C, O) via excitation and ionization.

In tissue, energetic neutrons interact primarily with the hydrogen in water, producing recoil protons (hydrogen nuclei). Neutrons may also be captured by atomic nuclei. In some cases the neutron is remitted, in other case the neutron is retained, converting the atom to a different nucleus.

2.1.4 Biological Effects of Ionizing Radiation on tissue/cells

When human cells are exposed to ionizing radiation, fundamental physical effects between radiation and the atom or molecules develops first and the possible biological impair to cell functions follow afterwards. The biological effect of ionizing radiation results mainly from damage to the DNA, which is the most critical target within the cell. Never the less, there are

also other site in the cell that when impaired may lead to cell death, Podgorsak, (2005). Ionizing radiation can directly or indirectly have effect on human cells.

2.1.4.1 Direct action in cell impairment by Radiation

In direct action the radiation interacts directly with the critical target inthe cell. The atoms of the target itself may be ionized or excited throughCoulomb interactions, leading to the chain of physical and chemical events thateventually produce the biological damage.Direct action is a dominant process in the interaction of high LET particles with biological material.

2.1.4.2 Indirect action in cell damage by ionizing Radiation.

In indirect action,the absorption of radiation by water molecules results in an ion pair (H_2O^+, H_2O^-). The H_2O^+ ion is produced by the ionization of H_20, whereas the H_20^- ion is produced via capture of a free electron by water molecules. These ions are very unstable; each dissociates to form another ion and a free radical.

$$H_20^+ \longrightarrow H^+ + OH^*,$$

$$H_20^- \longrightarrow H^* + OH^-$$

Free radicals are extremely reactive chemical species that can undergo a variety of chemical reaction. It can combine with other free radicals to form non-reactive chemical species such as water (eg $H^+ + OH^+ = H_2O$) in which case no biologic damage occurs, or with each other to form other molecules such as hydrogen peroxide (e.g. $OH^* + OH^* = H_2O_2$), which are highly toxic to the cells.

It is these free radicals that break the chemical bonds and produce chemical charges that lead to biological damage. Free radicals are highly reactive molecules because they have an unpaired valance electron. About two thirds of the biological damage by low LET radiations such as X-rays or electrons is due to indirect action.

2.1.4.3 Organ response to ionizing radiation

The response of organ system to ionizing radiation do not depends only on the dose, dose rate and linear energy transfer (LET) of the radiation but also on the relative radiosensitivities of the cells that comprise both functional parenchyma and supportive stroma, Bushberget al., (2002). In other words, the response is measured in terms of morphologic and functional changes of the organ system as whole rather than simple changes in cell survival kinetics.The response of organ system after irradiation occurs over a period of time whose onset and period of expression are inversely proportional to the dose. The higher the dose, the shorter the interval before the physiologic manifestations of the damage becomes apparent (latent period) and the shorter the period of expression during which the full extent of radiation-induced damage is evidenced. There are practical threshold doses below which no significant changes are apparent.

Human body consists of cells of differing radio sensitivities and a large radiation dose delivered acutely yields greater cellular damage than when the same dose is delivered over a protracted period. When the whole body is subjected to a large acute radiation exposure, a characteristic clinical response known as acute radiation response (ARS) occurs. ARS is an acute illness caused by irradiation of the entire or whole body by a high dose penetrating radiation in very short period of time, CDC, (2014). It is usually a combination of sub-syndromes occurring in stages over a period of hours to weeks after the exposure, as the injury to various tissues and organ systems is expressed. Bushberget al., (2002). There are three classic ARS syndromes;

- Bone marrow or hematopoietic syndrome: the full syndrome will usually occur with a dose between 0.7 and 10Gy (70-1000rads) though mild symptoms may occur as low as 0.3Gy or 30rads.

- Gastrointestinal (GI) syndrome: the full syndrome will usually occur with a dose greater than approximately 10Gy (1000rads) although some symptoms may occur as low as 6Gy or 600rads.
- Cardiovascular (CV) / Central Nervous system (CNS) syndrome: the full syndrome will usually occur with a dose greater than approximately 50Gy (5000 rads) although some symtoms may occur as low as 20Gy or 2000rads

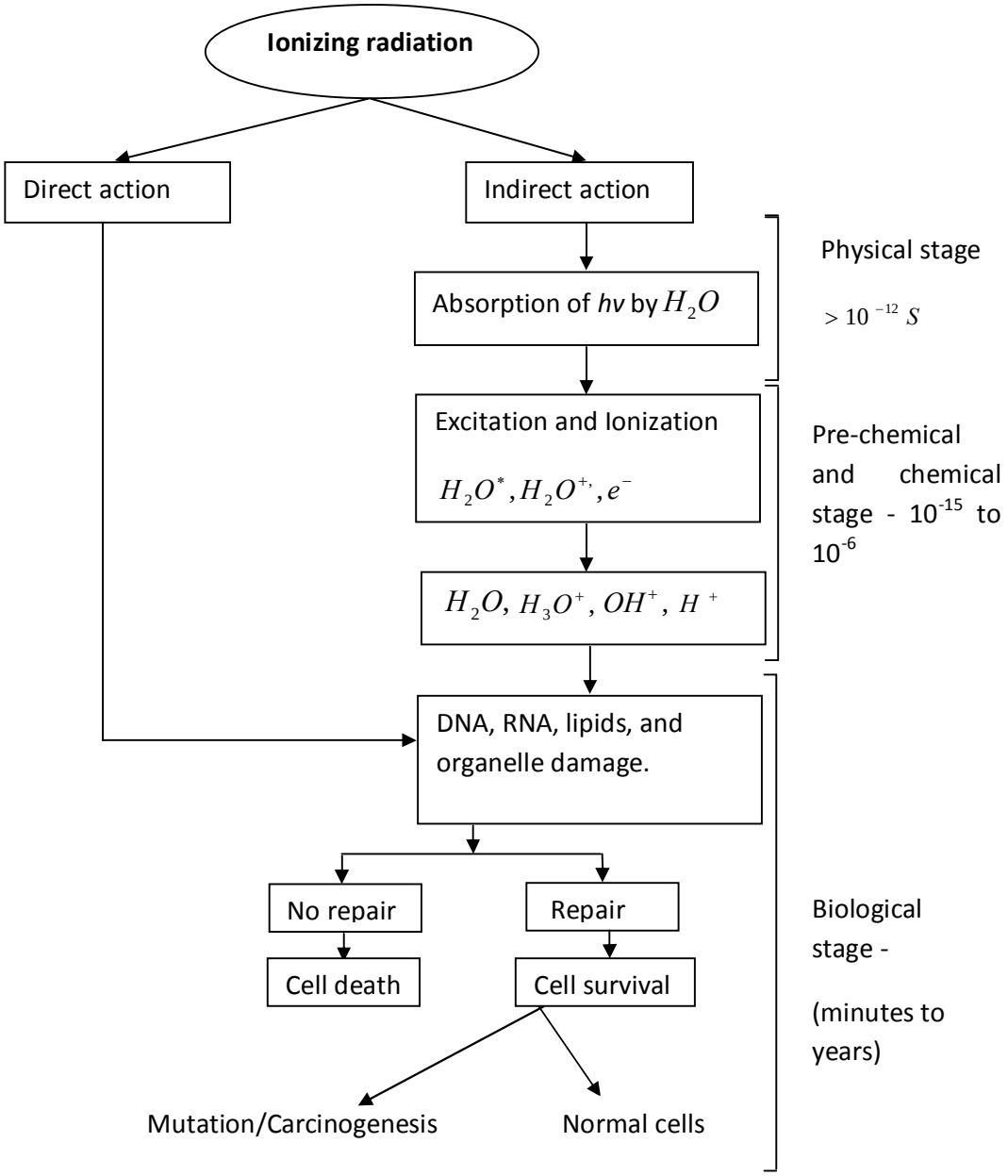


Figure 2.4 Chains of cellular events occurring in the cell/tissue after ionizing radiation exposure.

2.1.4.4Nine possible outcomes when a cell is irradiated

- No effect
- The cell delayed from going into division (Division delay).
- Apoptosis: Dying of cell before it can divide

- Reproductive failure: The cell dies when attempting the first subsequent mitosis
- Genomic instability: The cell dies when attempting the cut or subsequent mitosis.
- Mutation: the cell survives but contains a mutation
- Transformation: The cell survives but the mutation leads to a transformed phenotype and possible carcinogensis.
- Bystander effects: An irradiated cell can send signals to neighboring unirradiated cells and induce genetic damage in them.
- Adaptive responses: The irradiated cell is stimulated to react and become more resistant to subsequent irradiation.

2.2 Radioprotector

Radioprotectors are agents, that when given before or during radiation exposure, reduces the likelihood of early and/or late effects of radiation from developing. These compounds are often antioxidants. The agents can be classified into three categories (1) Prophylactic agents (ii) Mitigators and (iii) therapeutic agents(Stone etal., (2004) as cited in (Maurya, 2011).

i. Prophylactic agents are administered before radiation exposure to mitigate damage to cell / tissue.

ii. Mitigatorsarespecified agents that are administered during or after radiation exposure with the aim of preventing or reducing the action of radiation tissues before the appearance of symptoms.

iii. Therapeutic agents are administered after radiation exposure to treat or facilitate recovery from various aspect of the acute radiation syndrome (ARS) and the delayed effects of radiation exposure(Weiss and Landauer, 2009 as cited in (Maurya, 2011).

2.2.1Mechanisms of action for radioprotector

- Scavenging of free Radicals.
- The sulphydryl group may act by chemically reacting with free radicals generated by indirectly ionizing radiation and preventing their interaction with DNA.
- Improvement of DNA repair.
- Detoxifying the radiation induced reactives.
- Promoting the recovering of hematopietic and immune functions
- Reduction in lipid perioxidation
- Up regulates antioxidiant enzymes such as catalase (CAT), superoxide dismutase (SOD), Glutathione (GSH) etc.

2.3 Gongronema Latifolium

Gongronema latifolium belong to family of Asclepiadaceae, locally known as ñutaziò and ñarokekeò in the southeastern and southwestern parts of Nigeria.The plant is perennial, edible and has stems which are soft and pliable.It is a tropical rainforest plant primarily used as spice and vegetable in the traditional folk practice (Ugochukwu and Babady, 2002; Ugochukwu and Elekwa, 2003). It has been used since olden times in Nigerian ethnomedicine for the management of diabetes mellitus and high blood pressure (Ugochukwu and Elekwa,2003).

Studies have been carried out and the anti-hyperglycemic, and anti-hypercholesterole activities of the leaves of *G.latifolio* in both normal and diabetic rats have been reported; Ugochukwu and Elekwa, 2003). Effiong et al., (2012), recorded that acute toxicity of *Gongronema latifolium*intraperitoneally and calculated the lethal dose (LD50) to be 1500mg/kg.

Figure 2.5: Fresh leaves of Gongronema latifolio

2.2 Empirical Review

Recently researchers have adopted different models to evaluate the radioprotective effects some plants material, fruits, and herbal preparation effects of ionizing radiation and radioprotectors on animals.

Gharib, (2013) studied the protective role of onion oil on hepatotesticular oxidative damage induce by gamma irradiation in rats. The results showed a significant increase in serum acid phosphatase (ACP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to control. The level of superoxide (SOD), catalase (CAT) and glutathione (GSH) significantly decreased in irradiated group. But supplementation of onion oil result in significant recovery in GSH content, GPx and SOD while CAT activity still significantly decrease than the normal.

Pre-treatment and post-treatment of ascorbic acid in mice from radiation induced lethal gastrointestinal damage was investigated by Yasutoshi et al., (2013). Ascorbic acid (250mg/kg/day) was orally administered for three days before irradiation, one shot of

engulfment 250mg/kg at 8hours before irradiation and post-treatment of (250mg/kg/day) 7days after irradiation. Survival was 20% for pretreatment, 20% for engulfment and 0% for post treatment was recorded in the study. However, combination therapy using ascorbic acid, including pretreatment, engulfment and post-treatment, rescued all of the mice from lethal abdominal radiation, and was accompanied by remarkable improvement in the gastrointestinal damage (100% survival).

Thulasi (2013) evaluated the radioprotective effect of polysaccharide (PS) isolated from the mushroom *Ganoderma lucidium* against radiation induced intestinal damage. He compared radioprotective effects of PS with that of clinical used radioprotective drug amifostine (WR-2721) at 300mg/kg body-weight intraperitoneally, 30minutes before irradiation. The result showed that depletion of GSH level in jejuna mucosa was restored significantly by PS and amifostine administration. Similarly MDA level was maintained normal by PS and amifostine administration when compared to radiation alone treated group.

Menon and Nair (2013), explored Ayurvedic formulations(Brahma Rasayana (BRM) and Chyavanaprash (CHM) as therapeutic radioprotectors by analyzing their ability to restore the cellular antioxidant status and enhancing repair of radiation induced DNA damages. The antioxidant status in various tissues of mice was restored when these formulations were orally administered, following whole-body exposure to gamma radiation.

The radioprotective activity of Basil in albino rats was examined by Farag (2012), following gamma irradiation. The result showed that gamma rayscaused a significant increase in serum level of alanine and aspartate aminotransferase, alkaline phosphatase, gamma glutamul transpeptidase and significant decrease in reduced glutathione, superoxide dismutase (SOD) and catalase (CAT), serum sex hormones levels testosterone (T). The Basil extract (BAE) administered orally to rats significantly modulated all the radiation induced biochemical alterations.

Rehab and Ibrahim, (2012), investigated the radio-protective effect of *Spirulina*algae*a*gainst oxidative stress and tissue injury caused by gamma radiation. The results showed significant increase in MDA, AST, ALT, and GGT level in the irradiated rats. Treatment of rats with spirulina for days before acute irradiation significantly abolished radiation induced elevation in liver MDA level and significantly maintained hepatic GSH content and CAT close to the control values.

The role of antioxidant properties of celery against lead acetate induced hepatotoxicity and oxidative stress in irradiated rats was studied by Nadia, (2012). Results showed that combined treatment of lead acetate and radiation caused an increase in liver enzymes-aspartate aminotransferase (ALP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), with reduction in activity of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). However, Celery administration ameliorated increased liver enzymes as well as improved the decreased level of GSH, CAT and SOD activities.

Sang et al., (2010) studied the radioprotective effect of pre and post-treatment of *Hesperdin* and *curdlan* on gamma induced cellular damage and oxidative stress in liver of sprague-Dawley rats. The resultshows that whole-body radiation resulted in an increase in serum AST, ALT, ALP, liver LPO as well as decrease in the liver of SOD, CAT, GPx and GSH, Vit-C and Vit-E post 2 days irradiation. The result further shows that pre and post-treatment with Hesperdin and Curdlan for 2 days did not offer any significant protection, administration of Hesperidin and curdlan orally for 7days post irradiation was found to restore the altered levels of the above parameters in serum and liver tissue to near normalcy.

Abdou and Abbas (2009), evaluatedpre- and post-treatments of diphenyl dimethyl bicarbonate (DBB) effectsas a probable hepato-protector in rats against whole-body gamma irradiation. The pre- and post-treated animals with DBB against radiation showed earlier and potent improvement since it increased total protein and albumin level to near that of the

control. On the other hand, DBB reduced the altered activity of AST, ALT and ALP earlier than the irradiated group. Hence they concluded that all the treatments with DBB have a hepato-radioprotective effect and the pre- and post-treatment has the most potent effect against the alterations induced by irradiation.

Thepossible radioprotective action of *rosmarinus officinalis* leave. in Swiss albino Mice were investigated by Garima and Goyal, (2007). The result showed a significant increase in lipid peroxidation level and a decline in reduced glutathione level in the blood of irradiated animals. Conversely, prior treatment of animals with rosemary extract exhibited a significant decrease in lipid peroxidation level and an increase in the glutathione content. There results suggested the radioprotective effect of rosemary extract on hematological and biochemical alterations in mice.

Nwanjo et al. (2006) studied the anti-lipid peroxidation of*Gongronema latifolio (*GL)in streptozotocin-induced diabetic rats. They also carried acute toxicity (LD_{50}) test of the extractorally to define the range ofthe lethal dose and the safe range for the extract. The result recorded lethal dose(LD_{50}) to be 1050 ± 45 mg/kg of body weight and established that doses up to 500 mg/kg of bodyweight were observed to be safe dose (with no death recorded).

Baliga et al. (2004) evaluated the effect of *Abana* (a polyherbal drug) on radiation-induced sickness and mortality in mice exposed to 7 Gy to 12 Gy of gamma irradiation. The evaluation includes treatment of mice with *Abana* I hr before irradiation, 30 days post-irradiation survival,and DDW+ Irradiation, radiation tolerance and dose modification facto. Treatment ofmice with abana before irradiation caused a significant depletion in lipid peroxidation followed by a significantelevation in GSH concentration in the liver of mice at day 31 post-irradiation. The result also shows that *Abana* scavenged OH, DPPH, ABTS and NO in a concentration dependent manner in vitro.

Various synthetic compounds containing thiols have been developed and proven to be highly effective but due to their high toxicities and side effects like nausea, vomiting, skin lashes, itching, dizziness etc, at optimum level, research and development (R&D) is geared towards the development and discovering radioprotector which is readily available, cost effective and less toxic.

Gongronema latifolio is local plant which is readily available and cost effective. Studies carried out in the past have shown that it possesses antidiabetic, antihyperglycemic, and antilipidemic properties, Atangwho et al., (2009), Nwanjo et al., (2009). However, there is no information on its radioprotective potential; therefore, this study was undertaken to explore the possible use of gongronema latifolio extract as a radioprotector against wistar albino rats exposed to graded dose of radiation under pre and post-radiation scenario.

CHAPTER THREE

DESIGN, MATERIALS AND METHODS

3.1 Design

An Experimental design was adopted for this study. The design includes two control groups namely the normal control (NC) and experimental control (EC), and two experimental groupsthe pre-treatment (PRT)and post-treatment (PRT) irradiation group.

3.1.2 Location of study

The research took place in the following locations- University of Nigeria, Nsukka, University of Nigeria, Enugu campus and University of NigeriaTeaching Hospital, Ituku Ozalla Enugu state.

3.1.3 Target population

A specific breed of rats (wistar albino rats) was used for the research. This strain of rats was chosen for this research because they accurately reflect human physiology and also mimics human disease precisely.Most laboratory animals (rats) have the same set of organs- heart, lungs, liver and soon which work in the same way as they do in human, Giridharan et al., (2000).

3.1.4 Sample size

The sample size was determined based on the method of Rahab and Ibrahim (2012).A total number of 30 Wistar Albino rats, comprising 17 males and 13 females, weighing 130g-150g, between the ages of 16 to 20weeks were used in the study.This number of rats was chosen to maximize the chance of uncovering a specific mean difference in each sub-group and to also used to determine the statistical significant difference among the sub-groups.

3.1.5 Animal selection and handling

All the rats used in this study were purchased from the animal house of the department of zoology, University of Nigeria Nsukka, Enugu state. Prior to the studies the animals were allowed to acclimatize for 14days under standard environmental conditions with ambient temperature 22± 2^{o}C, air humidity of 50 ±10%, and light-darkness cycle 12/12hrs at the University of Nigeria Enugu campus animal house. The rats were housed in standard cages and fed with a standard Growerôs mash rat pellets and water *ad libitum*, throughout the experiment. All animal experiments were in conformity with National Institute of Health, Guide for care and use of laboratory Animals (NIH publication 85-23, 1985).

3.1.6 Sources of data

1. Observation of physical changes in all the groups following exposure to graded radiation doses (GRDs) and using weight loss as a major index.
2. Measurement of liver function enzymes in all the groups as an indication of hepatoprotective potential of Gongronema latifolio extract following exposure to graded radiation doses.
3. Measurement of lipid peroxidative stress in all the groups as an index for radiation damage following exposure to graded radiation doses.
4. Measurement of antioxidant enzymes in all the groups so as to estimate the scavenging of the free radicals activity of gongronema latifolio extract following graded doses of radiation.

3.2 Materials

- Gongronema latifolio leaves
- Liver function test kits
- Antioxidant enzyme test kits

- Linear accelerator.
- Weighing balance

3.2.1 Gongronema latifolio collection and identification

The fresh leaves of Gongronema latifolium used for this study were bought from Ogige market, Nsukka local government area of Enugu state. The leaves were botanically identified by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka Enugu state Nigeria.

3.2.2 Instruments/Equipment

The instruments/equipments used were obtained from University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu, Department of Biochemistry, UNN and other scientific shops in Nsukka. The equipments used for this study includes Linear accelerator with serial number 151315 (manufacture by Elekta precise treatment System, UK), centrifuge800D (Vickas Ltd, England), Colorimeter LCD-52, (El, scientific co. India), Spectrophotometer E312 (Jenway, UK), Oven (Gallenkamp, England), Refrigerator (thermocool, England), Pasteur pipette (Pyrex England), Water bath (Gallankamp England) and weighing balance (Vickas Ltd, England).

3.2.3 Chemicals

The chemicals and reagents used were of analytical grade and its includes absolute ethanol, Glacial acetic acid, sodium dodecyl sulphate (SDS), Mayerôs reagent, ethyl acetate, aluminum chloride solution, 1% thiobarturic acid (produced by BDH, England), Ascorbic acid and dichromate acetic acid were produced by May & Baker, England, Chloroform, trichloroacetic acid, potassium dichromate were produced by Sigma Aldrich Germany while adrenalin, picric acid, lead acetate solution were produced by Merck Darmstadt, Germany.

3.3 METHODS

3.3.1 Preparation of *Gongronema latifolio*

The leaves Gongronema latifolio were washed and chopped into smaller bits with a knife. The leaves were freeze-dried and pulverized into fine powder using electric mill. The pulverized samples were packed in air-tight plastic container.

3.3.2 Ethanol extraction of *Gongronema latifolio*

One thousand grams (1000g) of the pulverized sample was weighed and macerated in 2.5 litres of ethanol with thorough shaking at regular intervals for 72hours at room temperature (26-28^{o}C). The resulting extract was filtered using Whatman No. I filter paper. The filtrate was concentrated using rotary evaporator to obtain 16.2 g of the ethanol leaves extract. The extract was stored in an air-tight plastic container in the refrigerator and used for the study.

3.3.3 Qualitative phytochemical analysis of *Gongronema latifolio* extract.

The qualitative analysis of the leaves of the GL ethanol extract was done using standard procedures to identify the phytochemical constituents according to the methods of Harborne (1998), Trease and Evans (1983).

3.3.3.1 Test for tannins

Dried powdered sample (0.1 g) was boiled in 4 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added and observed for a color change which indicates the presence of tannins.

3.3.3.2 Test for alkaloids

A quantity, 0.2g of the sample was boiled with 5ml of 2 % HCl on a steam bath. The mixture was filtered and 1ml portion of the filtrate was treated with 2 drops of the following reagents

(i) Dragendorffôs reagent: An orange precipitate indicates the presence of alkaloids.

(ii) Mayerôs reagent: A creamy-white precipitate indicates the presence of alkaloids.

(iii) Wagnerôs reagent: A reddish-brown precipitate indicates the presence of alkaloids.

(iv) Picric acid (1 %): A yellow precipitate indicates the presence of alkaloids.

3.3.3.3 Test for saponins

A known quantity, 0.1 g of the sample was boiled with 5 ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests.

(i) Emulsion test: A quantity, 1 ml of the filtrate was added to two drops of oil. The mixture was shaken and observed for the formation of emulsion which indicates the presence of saponins.

(ii) Frothing test: A quantity, 1 ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth which indicates the presence of saponins

3.3.3.4 Test for flavonoids

A given quantity, 0.2 g of the sample was heated with 10 ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered, and the filtrate was used for the following tests.

(i) Ammonium test: 4 ml of the filtrate was shaken with 1ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicates the presence of flavonoids

(ii) Aluminium chloride test: A quantity, 4 ml of the filtrate was shaken with 1 ml of 1 % aluminium chloride solution and observed for light yellow coloration that indicates the presence of flavonoids.

3.3.3.5 Test for phenols

A known volume, 5 ml of folin ciocalteu reagent and 4ml of aqueous sodium carbonate were added to 0.5 ml of extract. Appearance of blue color indicates the presence of phenols.

3.3.4. Quantitative determination of phytochemicals present in of the ethanolic extract of *Gongronema latifolio*

3.3.4.1 Determination of Tannins

The method of Swain (1979) was used for the determination of the tannin content of *Gongronema latifolio* extract. A quantity, 0.2 g of finely ground sample was measured into a 50 ml beaker. About 20 ml of 50 % methanol was added and covered with paraffin and placed in a water bath at 77-80 ^{0}C for 1 hour and stirred with a glass rod to prevent bumping. The extract was filtered using a double layer of Whatman No. 1 filter paper into a 50 ml volumetric flask, then, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17 % Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water and allowed to stand for 20 mins, when a bluish-green coloration developed. Standard tannic acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above. The absorbance of the tannic acid standard solutions as well as samples were read after color development at 760 nm. The tannin content was calculated using the formular:

$$\% \text{ of Tannin} = \frac{\text{[illegible]} \times \text{[illegible]} \times (\text{[illegible]})}{\text{[illegible]} \times \text{[illegible]}} \quad (19)$$

3.3.4.2 Determination of Alkaloids

The alkaloid quantity was determined using method of Harborne (1998). A 200ml of 10% acetic acid in ethanol was added to 5g of the sample in 250ml beaker and was allowed to stand for 2-4hours before filtering. The filtrate was concentrated to (1/4) of the original volume on a hot plate. A conc. ammonium hydroxide was added drop wise to the filtrate until a precipitate is formed. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue on the filter paper was dried in an oven at 60^0Cfor 30minutes and weighed. The percentage of alkaloid was calculated as follows, using equation 20;

$$\% \text{ of alkaloid} = \frac{W_2 - W_1}{W_0} \times 100 \quad (20)$$

W_1= weight of filter paper alone, W_2=weight of the paper +alkaloid precipitate and W_0= weight of the sample used.

3.3.4.3 Determination of Flavonoid

This was determined using method AOAC, (1970). A 10g of sample was extracted at room temperature and filtered using wheatman No1 filter paper. The filtrate was transferred in a crucible and evaporated to dryness in a water bath. The percentage of flavonoid was calculated as follows, using equation 19;

$$\% \text{ of flavonoid} = \frac{W_2 - W_1}{W_0} \times 100 \quad (21)$$

W_1, W_2& W_0 as in above

3.3.4.4 Determination of phenol

The total phenolic content of the sample was determined using the method of Oyedemi etal., (2010). An aliquot of the extract (0.5 ml) was mixed with 2.5 ml of 10 % Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (75 % w/v). The resulting mixture was vortexes for 15 seconds and incubated at 40^{o}C for 30 minutes for color development. The absorbance of the sample was measured spectrophotometrically at 765 nm. Total phenolic content was expressed as mg/g tannic acid equivalent from the calibration curve.

3.3.4.5 Determination of Saponin

The spectrophotometric method of Brunner (1984) was used for the estimation of saponins in the plant sample. A portion (0.1g) of ground sample was weighed into a 25 ml beaker and 10 ml of ethanol was added. The mixture was vortexed on a mechanical shaker for 5 hrs to ensure uniform mixing. After, it was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40 % solution of magnesium carbonate was added. The mixture obtained with magnesium carbonate was again filtered to obtain a clear, colourless solution. Then, 1 ml of the colorless solution was pipetted into a 50 ml volumetric flask and 2 ml of 5 % $FeCl_3$ solution was added and made up to mark with distilled water and was allowed to stand. Standard saponin (0-10 ppm) was prepared from saponins stock solution. The standard solutions were treated similarly with 2 ml of 5 % $FeCl_3$. The absorbance of the sample as well as standard saponin solution was read after color development on a spectrophotometer at a wavelength of 380 nm.

$$\% \text{ of Saponin} = \frac{([illegible] \times [illegible] \times [illegible] \times [illegible])}{[illegible]} \quad (22)$$

3.3.5Experimental protocols

The 30 wistar albino rats were randomly divided into two major (2) groups;

- Control group - Group A ï Normal control
  Group B- Experimental control

- Experimental group - Group C ï Pre-treatment irradiation
  Group D ï Post-treatment irradiation

There are three rats in group A while group B, C and D contains nine rats each and were further divided into three sub-groups each. For group B- B2, B4, & B6, group C- C2, C4, & C6, and group D- D2, D4, & D6. Each sub-group was divided and tagged base on the dose of radiation administered. That is, B2, C2 & D2 all received 2Gy dose each, and B4, C4 & D4 all received 4Gy dose each while B6, C6 & D6 all received 6Gy dose each.

1. Group A - the wistar albino rats in this group were not administered with *Gongronema latifolio* extract and were not irradiated. They were used to monitor and compare both the physical and biochemical changes in rats of groups ï C & D.
2. GroupB - the wistar albino rats in these subgroups were exposed to graded radiation doses in the order of 2Gy, 4Gy, and 6Gy without administration of *Gongronema latifolio*extract.They were used to monitor and compare both the physical and biochemical changes with animals in groups ï A,C & D.
3. Group C- the wistar albino rats in these sub-groups received250mg/Kg of body weight extract orally, once a day for seven consecutive daysbefore they were exposed to graded radiation doses in the order of 2Gy, 4Gy, and 6Gy. After irradiation the

animals in this group were monitor for another 7days for radiation sickness and were compared with animals in A, B and D.

4. Group D - the wistar rats in these sub-groups were first exposed to graded radiation doses in the order of 2Gy, 4Gy, and 6Gy and afterwards 250mg/kg of body weight extract was administered orally, once a day for seven consecutive days. The rats were monitored for seven days for radiation sickness and were also compared to A, C, and D.

Table 3.1 Experimental protocol table

Animal Groups	**Conditions**
Group A(Normal control)	Untreated with GL extract and unirradiated
Group B (experimental control)	**Irradiated animals without treatment**
B2	2Gy
B4	4Gy
B6	6Gy
Group C (Pre-treatment)	**Treated before IR**
C2	250mg/kg body of weight for 7days +2Gy
C4	250mg/kg body of weight for 7days +4Gy
C6	250mg/kg body of weight for 7days +4Gy
Group D (Post-treatment)	**Treated after IR**
D2	2Gy+250mg/kg body of weight for 7days
D4	4Gy+250mg/kg body of weight for 7days
D6	6Gy+250mg/kg body of weight for 7days

3.3.6Sample collection

The blood sample of all the rats in Normal control, Experimental control, Pre-treatment and Pretreatment groups were collected 2 weeks after acclimatization period (Before IR), day 1 and day 8 after IR) for measurement of the following biochemical parameters- alkaline phosphatase ALP, alanine amino-transferase ALT, aspartate amino-transferase AST, malondialdehyde MDA, glutathione GSH, catalase CAT and superoxide dismutase SOD.

Method of Harkness and Wargner, (1983), was adopted for blood sample collection. The blood samples were collected through the ocular puncture and were allowed to clot. After which the samples were centrifuged to obtain serum which was used for the analysis.

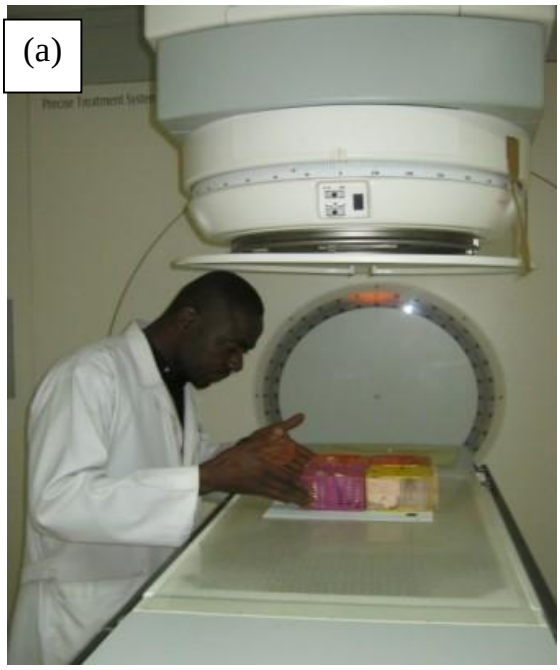
3.3.7Extract dose selection

The Gongronema latifolio extract dose given to the rats waschosen based on work done by Nwanjo et al.,(2006). They carried-out LD_{50}toxicity test of GL extract and recorded it to be 1050±45 and doses up to 500mg/kg bodyweight were observed to be safe.

3.3.8 Irradiation of rats

The animals were whole-body irradiated using 6 mV photon beam, Elekta linear accelerator at radiotherapy unit, Radiation Medicine Department, University of Nigeria Teaching Hospital, Enugu. The animals were immobilized in special well ventilated plastic cage (1mm thick). Graded doses (2Gy, 4Gy, and 6Gy) of radiation were given to the animals at dose rate of 245mu/min. Rats that are to receive 2Gy, were given 202MU, weighted 1:1, parallel opposed for posterior-anterior, those rats that are to receive 4Gy, were given 404MU, weighted 1:1 parallel opposed for posterior-anterior, while rats that are to receive 6Gy, were given 606MU, weighted 1:1 parallel opposed for anterior-posterior fields.Six rats were irradiated at the same time at a field size of 22.5cm x 18cm at a source surface distance (SSD) of 95.5cm. All irradiation was done under the same temperature (23.2^0C), pressure (984.5hPa), attenuation factor (0.9982), and treatment set-up (source axis distance). After irradiation the animals were sorted to their various group cages.

Fig 3.1 a&b: Wistar albino rats after acclimatization period.



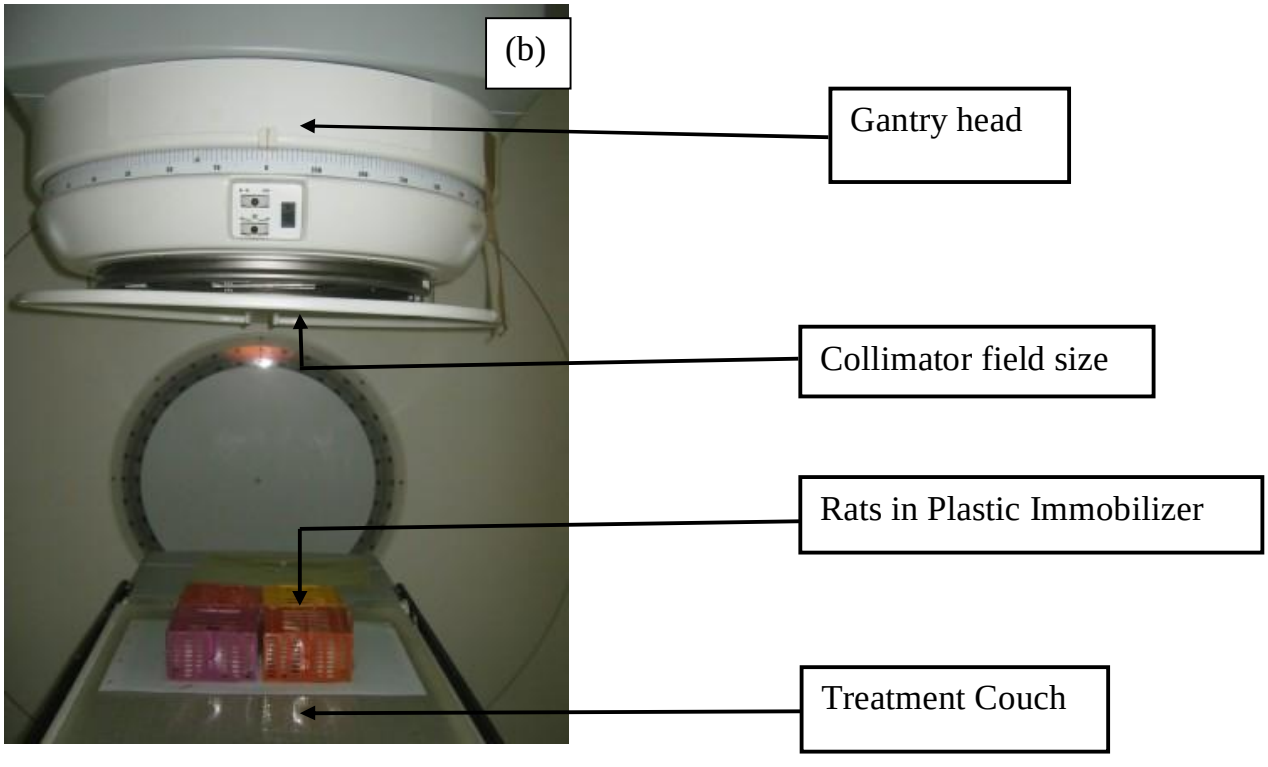


Fig 3.2a&b: Aligning rat Immobilizer with the collimator field size of the LINAC with the help of laser light

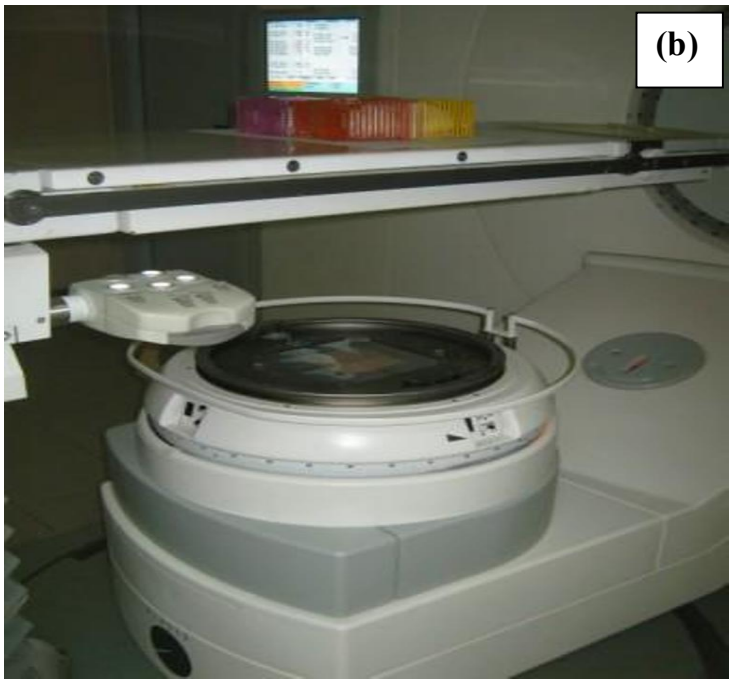


Fig 3.3 a&b: Repositioning of LINAC gantry head for posterior Irradiation.

Fig: 3.6 Oral administration of Gongronema latifolio extract

3.3.9 Determination of liver function test (ALP, ALT &AST), lipid peroxidation (MDA) and scavenge of free radical activities parameters (GSH, CAT & SOD).

3.3.9.1 Determination of Alkaline phosphatase (ALP)

Determination of ALP was based on method of Rec. GSCC (1972). The principle of this method is based on the reaction involving serum alkaline phosphatase and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turn pink that can be determined spectrophotometrically.

P-nitrophenylphosphate + H_20 --------→ ALP ------→ PO_4^{2-} + P-nitrophenol (pink at pH=9.8)

Method: The blank and sample test tubes were set up in duplicates and 0.05ml of sample was pipette into the sample test tubes. 0.05ml of distilled water was pipetted into the blank tube.

Three milliliters (3.0ml) of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one minute intervals.

Calculation: alkaline phosphatase activity was calculated as follows using equation 23:

$$\text{Activity of ALP (in iU/L)} = \frac{\text{[illegible]}}{\text{[illegible]}} \qquad (23)$$

3.3.9.2Determination of Alanine Aminotransferase (ALT)

Method of Schmidt and Schmidt, (1963) was adopted in determining ALT level. ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The color intensity is measured against the blank at 540nm.

Method: The blank and sample test tubes were set up in duplicates. 0.1ml of serum was pipetted into the sample tubes. To these were added 0.5ml buffer solution containing phosphate buffer, L-alanine and Ǚ-oxoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37^0C ml and pH 7.4. A volume, 0.5ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to both tubes while 0.1ml of sample was added to sample blank tube. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25 ^{0} C. Five milliliters of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 540nm.

3.3.9.3 Determination of Aspartate Aminotransferase (AST)

Method of Schmidt and Schmidt, (1963), was also used in determining AST level. This is measured by monitoring the concentration of oxaloacetate hydrazones formed with 2, 4-dinitrophenylhydrazine. The color intensity is measured against the blank at 546nm.

Method: The blank and sample test tubes were set up in duplicates. A volume, 0.1ml of serum was pipetted into the sample tubes and 0.5ml of reagent 1 was pipette into both sample

and blank tubes. The solutions were thoroughly mixed and incubated for exactly 30 minutes at 37^0C ml and pH 7.4. 0.5ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added into all the test tubes followed by 0.1ml of sample into the blank tubes. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25^0C and 5.0ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 546nm.

3.3.9.4 Determination of lipid peroxidation

Lipid peroxidation in the liver was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelsson (1968). In brief, 0.1 ml of liver homogenate (10 %w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 15 % trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min, and cooled. The amount of malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm against reference blank. Percentage inhibition was calculated using the equation 24:

$$\text{lipids \% Inhibition} = \frac{\square\square\square\square\square}{\square\square\square\ \square\square\square} \qquad (24)$$

Where $\square_{\square}$ is the absorbance of the control and $\square_{\square}$ is the absorbance of the sample extract.

3.3.9.5Determination of reduced glutathione activity

Reduced glutathione was determined using the modified method of Ellman (1951). A volume, 0.1ml of the sample was mixed with 0.9ml of distilled water in a beaker. Sodium sulphate of volume 0.02ml was also added, shaken and allowed to stand for 2mins at RT. A volume, 0.02ml of Lithium Sulphate (20%), 0.2ml of 20% $NaCO_3$ and 0.2ml of phosphor-18-tungstic acid were also added to the beaker, it was shaken and allowed to stand for 4mins

while observing for maximum color development. A volume, 2.5ml of 2% sodium sulphite was also added and the absorbance was taken at 680nm, within 10mins a blank (0.1m H_2O) was also set up.The absorbance was measured at 412 nm. The percentage inhibition of GSH was calculated using equation 25:

$$\% \text{ glutathione inhibition} = \frac{\{\text{absorbance of uninhibited sample} - \text{absorbance of inhibited sample} \times 100\}}{\text{absorbance of uninhibited sample}} \quad (25)$$

3.3.9.6 Determination of catalase activity

Catalase activity was assayed according to the method of Pari and Latha (2004). The percentage inhibition was done spectrophotometrically following decrease in absorbance at 620 nm. The liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuged at 5000 rpm. The reaction mixture consisted of 0.4 ml of hydrogen peroxide (0.2 M), 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of liver homogenate (10 % w/v). The reaction of the mixture was stopped by adding 2 ml of dichromate-acetic acid reagent (5 % K2Cr2O7 prepared in glacial acetic acid). The changes in the absorbance was measured at 620 nm over 3 min at 1 min interval and recorded. Percentage inhibition was calculated using the equation 26.

$$\% \text{ Catalaseinhibition} = \frac{\{\text{absorbance of uninhibited sample} - \text{absorbance of inhibited sample} \times 100\}}{\text{absorbance of uninhibited sample}} \quad (26)$$

3.3.9.7Determination of superoxide dismutase activity

This was determined using the method of Xin et *al.* (1991). Superoxide dismutases (SOD) are enzymes that catalyses the conversion of two superoxides into hydrogen peroxide and oxygen.

The benefit here is that hydrogen peroxide is substantially less toxic than superoxide. Erythrocyte superoxide dismutase (SOD) activities serve as antioxidant enzymes. The principle of SOD activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction.

A quantity, 0.01g of adrenalin was dissolve in 17ml of distilled water and 0.1ml of serum and 0.9ml of phosphate buffer (pH 7.8) were taken in triplicates in 2.5ml buffer. A volume, (0.3ml) adrenaline solution was added and mixed inside the cuvette. The changing rate of absorbance was used to determine superoxide dismutase activity. The change in absorbance was recorded at 560 nm. Percentage inhibition was calculated using equation 27:

$$\text{SOD \% inhibition} = \frac{\{\text{[illegible]} \times \text{[illegible]}\}}{\text{[illegible]}} \quad (27)$$

3.3.10Statistical analysis

Statistical analysis was carried out using IBMSPSS version 20 software. Data were presented as mean ± standard deviation (SD). The standard deviations were carried out for the mean data obtained to analyze the degree or measure of dispersion in the set of data recorded. ANOVA in conjunction with tukeyȏs honest significance different (HSD) test were used to determine significance difference ($p<0.05$) among the animal groups- A, B, C and D. A value of $p<0.05$ was taken as the level of significance but when $p>0.05$ it is considered to be non-significant. For body weight loss, data that is significantly different ($p<0.05$)from normal group (NC) was denoted by(*), those that are significantly different ($p<0.05$) from Pre-treatment group (PRT) were denoted by (À) while those that are significantly different ($p<0.05$) from Post-treatment (PST) were denoted by (ὶ). For alkaline phosphate,alanine amino-transferase, aspartate amino-transferase, malondialdehyde, catalase, glutathione and superoxide dismutase parameters, data that are significantly different ($p<0.05$) from normal control weredenotedwith (*), data that are significantly different ($p<0.05$) from PRT and

PSTgroups were denoted with (À) while data that are not significantly ($p>0.05$) different from NC group was denote with (ï). Clustered bar chart was also used to compare means of PRTand PST groups against normal control group. While error bar were used to indicate the error or uncertainty in the measured data.

CHAPTER FOUR

Results and Discussion

4.1 Phytochemical analysis

Table 4.1 Bioactive phytochemicals present in*Gongronema latifolio* extract.

Bioactive agents	Mean ±SD (in mg/g)
Alkaloids	3.11±0.03
Tanins	2.43±0.02
Flavonoids	1.31±0.02
Phenols	1.10±0.01
Saponins	0.80±0.10

Alkaloids were the highest bioactive phytochemical present (3.11±0.026mg/g) and saponins lowest (0.8±0.100mg/g) as shown in the table 4.1.

4.2 Physical Observation

Table 4.2 Variation of body weight of all the animal groups, before and after graded doses of radiation

Body weight (g)									
	for 2Gy, Mean ± SD			for 4Gy, Mean ± SD			for 6Gy, Mean ± SD		
Groups	Before IR. (N=3)	Day 1 after IR (N=3)	Day 8 after IR (N=3)	Before IR (N=3)	Day 1 after IR (N=3)	Day8 after IR (N=3)	Before IR (N=3)	Day 1 after IR (N=3)	Day 8 after IR (N=3)
NC	111± 6.5	119± 7.6	124± 4.8	109± 4.8	110± 4.7	113± 5.2	110± 8.8	111± 10.2	115± 7.8
EC	110± 8.9	106± 3.5	68± 2.8*À×	111 ±6.1	109± 3.5	68± 3.5*À	108± 6.4	107± 8.41	51± 3.0*À
PRT	109± 9.2	106± 8.4	90± 5.0*	112± 5.7	105± 4.1	87± 4.3*	112± 6.0	111± 6.2	70± 1.0*
PST	110± 9.6	116± 6.3	85± 4.5*	110± 8.8	106± 3.7	72± 2.0*	110± 2.6	109± 1.8	60± 4.1*

**Significantly different (p<0.05) from NC, †significantly different (p<0.05) from PRT, ×significantly different from PST*

In table 4.2 body weight of all the rats exposed to 2Gy dose in experimental control EC (68g), pretreatment PRT (90g) and post-treatment PST (85g) decreased significantly ($p<0.05$) when compared to normal control NC (124g)group on day 8 after irradiation (IR). The table further shows that decrease in body weight for rats exposed to 4Gy in EC (68g) were significantly ($p<0.05$) different from NCgroup (113g) and PRT (87g), but not significantly different ($p>0.05$) from PST (72g) group on day 8 after IR. For rats exposed to 6Gy dose, the table also shows that the body weight of animals in EC (51g) decreased significantly ($p<0.05$) when compared to NC (115g) and PRT groups but not significantly different ($p>0.05$) from PST (60g) animal group.

4.3 Biochemical parameters

4.3.1 Alkaline phosphatase (ALP) parameters.

Table 4.3.1 Variation of alkaline phosphatase mean level for all the animal groups, before and after graded doses of radiation

ALP (iU/L)									
	ALP 2Gy, MEAN ±SD			ALP 4Gy, MEAN ±SD			ALP 6Gy, MEAN ±SD		
Group	Before IR (n=3)	Day 1 after IR (n=3)	Day 8 after IR (n=3)	Before IR (n=3)	Day 1 after IR (n=3)	Day 8 after IR (n=3)	Before IR (n=3)	Day 1 after IR (n=3)	Day 8 after IR (n=3)
NC	38.00± 2.00	37.33± 4.10	39.00± 1.00	38.00± 2.00	37.33± 4.10	39.00± 1.00	38.00± 2.00	37.33± 4.10	39.00± 1.00
EC	33.67± 3.51	59.33± 11.80ï	67.00± 4.00*À	34.67± 2.51	75.00± 2.00*	74.00± 3.46*À	40.67± 1.52	75.00± 7.00*À	80.00± 2.00*
PRT	32.00± 4.00	46.67± 5.03ï	42.00± 2.00ï	41.00± 1.00	46.67± 4.93ï	37.00± 4.00ï	31.33± 3.05	48.33± .057ï	43.33± 3.00ï
PST	32.00± 1.00	65.67± 10.06*	48.00± 8.00ï	35.67± 2.51	72.00± 2.64*	43.00± 7.00ï	31.67± 1.52	57.00± 4.00*	52.00± 3.00*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST, ˣ Not significantly (p>0.05) different from NC*

In table 4.3.1, it can be observed that rats exposed to 2Gy dose,that, there was a significant increase (p<0.05)in ALP mean level in EC group (67.00iU/L),when compared to NCgroup (39.00iU/L) and significantly different (p<0.05) from PRT (42.00iU/L) and PST (48.00iU/L)groupson day 8 after IR. However, ALP level for rats exposed to 2Gy in PRT and PST groups were not significantly different (p>0.05) from NCgroup on day 8 after IR. ALP level for rats exposed to 4Gy and 6Gy show that rats in EC increased significantly (P<0.05) when compared to NC, PRT and PST groups on day 1 and 8 after IR. However, PRT and PST were not significantly different (p>0.05) from NC group, except for PSTgroup in rats exposed to 6Gy (52.00iU/L) that was significantly different (P<0.05) from NC (38.33iU/L) onday 8 after irradiation.

4.3.2: Alanine aminotransferase (ALT) parameter

Table 4.3.2 Variation of alanine amino-transferase mean level for all the animal groups, beforeand after graded doses of radiation

ALT (iU/L)									
ALT 2 Gy, MEAN ±SD				ALT 4Gy, MEAN ±SD			ALT 6Gy, MEAN ±SD		
Group	Before IR (n=3)	Day 1 after IR (n=3)	Day 8 after IR (n=3)	Before IR (n=3)	Day 1 after IR (n=3)	Day 8 after IR (n=3)	Before IR (n=3))	Day 1 after IR (n=3)	Day 8 after IR (n=3)
NC	36.67± 2.51	36.33± 3.51	37.00± 1.00	36.67± 2.51	36.33± 3.51	37.00± 1.00	36.67± 2.51	36.33± 3.51	37.00± 1.00
EC	37.00± 5.19	43.67± 4.50ï	62.00± 5.00*À	36.67± 1.52	45.67± 2.51*	50.00± 2.00*À	36.67± 3.51	69.33± 2.5*	69.67± 7.50*À
PRT	35.00± 4.58	37.00± 2.00ï	27.00± 2.00ï	38.33± 2.08	41.00± 1.73ï	31.67± 2.51ï	29.00± 1.00	43.33± 3.51ï	41.33± 9.45ï
PST	33.33± 4.50	41.00± 7.00ï	28.00± 1.00ï	38.33± 1.15	44.00± 4.35ï	38.67± 4.50ï	31.00± 1.00	67.00± 2.00*	45.67± 2.51ï

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST,ˣNot significantly (p>0.05) different from NC*

In table 4.3.2,rats exposed to 2Gy demonstrated thatALT mean levels in EC (43.67iU/L) group increased significantly (P<0.05) when compared to NCgroup (36.33iU/L), PRT (27.00iU/L) and PST(28.00iU/L)groups on day 8 after IR. Nevertheless, PRT (37.00iU/L and 27.00iU/L)& PST (41.00 and 28.00iU/L) groupswere not significantly different (p>0.05) from NC group on day1 and 8 after IR.

The tables also shows that rats exposed to 4Gy and 6Gy, the ALT mean level increased significantly (p<0.05) on day 1 after irradiation in EC, relatively to NC. On day 8 after radiation exposure, ALP mean level in EC group increased further and was significantly (p<0.05) different from NC, PRT and PST. However, PRT and PST were not significantly different (p>0.05) from NC on day 8 after IR.

4.3.3 Aspatate aminotransferase (AST) parameter.

Table 4.3.3 Variation ofaspartate amino-transferase mean level for allthe animal groups, before and after graded doses of radiation

AST (iU/L)									
AST 2 Gy, MEAN ±SD				**AST 4Gy, MEAN ±SD**			**AST 6Gy, MEAN ±SD**		
Group	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3
NC	38.33± 2.51	36.00± 2.00	33.00± 1.00	38.33± 2.00	36.00± 2.00	33.00± 1.00	38.33± 2.51	36.00± 2.00	33.00± 1.00
EC	37.33± 6.11	63.00± 4.00*	63.00± 4.00*	38.67± 6.11	59.67± 7.50*	63.00± 4.00*À	37.67± 8.14	55.67± 3.51*	63.00± 4.00*À
PRT	34.00± 2.00	31.67± 4.50ï	30.00± 1.00ï	38.67± 2.51	41.67± 6.50ï	33.67± 2.51ï	31.67± 3.78	45.33± 3.51*	35.33± 1.15ï
PST	32.00± 2.0	60.33± 1.52*	45.33± 2.08*	38.67± 2.08	58.33± 2.08*	44.00± 3.00*	30.00± 3.60	57.33± 2.08*	44.00± 3.00*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST, ˣ Not significantly (p>0.05) different from NC*

Table 4.3.3 indicated that the AST mean level in EC (63.00iU/Land63.00iU/L) group increasedsignificantly (p<0.05) when compared to NC (36.00 and 33.00iU/L) group on day 1 and 8 after 2Gy dose radiation exposure. However, there was no significant (p>0.05) difference between animals in NC (38.33iU/L& 36.00iU/L) and PRT (31.67iU/L and 30.00iU/L) groups on day 1 and 8 after2Gy dose exposure. The result also indicated significant increase (p<0.05) in EC group (63.00iU/L) when compared to NC(33.00iU/L) and PRT (31.67iU/L) groupon day 8, for rats exposed to 4Gy. Table 4.3.3 alsofurther revealed that rats exposed to 6Gy dose, the AST mean level in EC (55.67iU/L), PRT (45.33iU/L) and PST (57.33iU/L)groups increasedsignificantly (p<0.05) when compared to NCgroup (36.00iU/L) on day 1 after radiation exposure.

4.3.4 Malondialdehyde (MDA) parameters.

Table 4.3.4 Variation of malondialdehyde mean activity for all the animal groups, before and aftergraded doses of radiation

MDA (% Inhibition)									
MDA 2Gy, MEAN ±SD				MDA 4Gy, MEAN ±SD			MDA 6Gy, MEAN±SD		
Group	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3
NC	38.00± 2.00	37.00± 1.00	36.33± 1.73	38.00± 2.00	37.00± 1.00	36.33± 1.52	38.00± 2.00	37.00± 1.00	36.33± 1.52
EC	40.00± 2.00	61.00± 3.00*	67.00± 6.00*	39.67± 1.52	79.67± 1.57*	83.00± 2.00*À	42.33± 2.08	63.67± 4.50*	70.67± 5.50*
PRT	37.67± 2.51	46.67± 3.51*	39.67± 3.51ï	42.67± 0.57	63.67± 5.50*	45.00± 13.00ï	40.00± 1.00	38.67± 4.50ï	35.00± 5.00ï
PST	37.00± 1.00	67.00± 1.00*	59.67± 9.50*	39.00± 1.00	77.00± 3.60*	48.00± 8.18ï	34.00± 2.00	64.33± 2.51*	59.00± 1.00*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST,ˣ Not significantly (p>0.05) different from NC*

In table 4.3.4, MDA mean activities for rats exposed to 2Gyincreasedsignificantly (p<0.05) in EC (61.00% and 67.00%), PRT (46.67%) and PST (67.00% and 59.67%)groups when compared to NCgroup (37.00% and 36.00%) for day 1 and 8 after IR, with the exception of PRTgroup (39.67%) that was not significantly different (p>0.05) from NC on day 8 after IR. For rats exposed to 4Gy, significant increase (p<0.05) was observed in the MDA mean activities in ECgroup (79.67% and 83.00%), on day 1 and 8 after IR when compared to NC (37.00% and 36.33%) and PRT (63.67% and 45.00%) groups. However, on day 8 after irradiation, PRT (45.00%) and PST (34.00%)groups were not significantly different (p>0.05) from NC (36.33%). The table further demonstrated that 6Gy dose exposed rats, the MDA mean activities in EC (63.67% and 70.67%) and PST (64.33% and 59.00%) groups significantly increased (p<0.05) when compared to NC (37.00% and 36.67%), for day 1 and 8

after IR. Radiation induced increase in MDA level were significantly reduced ($p<0.05$) in PRT (38.67% and 35.00%), on day 1 and 8 after IR.

4.3.5Glutathione (GSH) parameter

Table 4.3.5Variation of glutathione mean activities for all the animalgroups, before and after graded doses of radiation

GSH (% inhibition)									
GSH 2Gy, MEAN±SD				**GSH 4Gy, MEAN±SD**			**GSH 6Gy,MEAN±SD**		
Group	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3
NC	69.33± 2.51	68.67± 2.08	69.00± 1.00	69.33± 2.51	68.67± 2.08	69.00± .00	69.33± 2.51	68.67± 2.08	69.00± 1.00
EC	66.67± 3.51	33.67± 2.51*	26.67± 2.51*À	68.00± 2.00	32.33± 2.08*	29.67± 2.88*À	65.00± 1.00	39.33± 0.57*À	35.67± 2.51*
PRT	66.33± 1.52	48.33± 0.57*	59.67± 3.51ὶ	62.00± 100	55.67± 2.51*	59.67± 1.52*	68.00± 1.00	56.67± 7.50*	60.00± 7.00ὶ
PST	70.33± 1.52	31.67± 4.50*	46.00± 13.00*	65.00± 2.00	31.00± 5.00*	44.67± 4.50*	73.67± 2.51	40.33± 1.52*	42.33± 4.04*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST,˟ Not significantly (p>0.05) different from NC*

Table 4.3.5shows that the GSH mean activities forrats exposed to 2Gy, 4Gy and 6Gy doses, significantly decreased (p<0.05) in EC, PRT and PSTgroups relatively to NC group on day 1 after IR, but PRT animal group exposed to 2Gy (59.67%) and 6Gy (60%) on day 8, were not significantly different from NC (69%).

Catalase (CAT) parameter

Table 4.3.6Variation of catalasemean activities for all the animal groups, before and graded doses of radiation

CAT (% Inhibition)									
CAT 2Gy, MEAN ±SD				CAT 4Gy, MEAN ±SD			CAT 6Gy, MEAN±SD		
Group	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3
NC	81.33± 1.52	77.00± 3.00	79.00± 2.64	81.33± 1.52	77.00± 3.00	79.00± 2.64	80.33± 1.52	77.00± 3.00	79.00± 2.64
EC	79.00± 3.00	36.67± 2.51*	39.67± 2.51*À	76.00± 5.00	26.00± 2.00*	31.00± 5.00*	76.67± 4.50	34.67± 1.52*	38.33± 1.52*
PRT	81.33± 1.52	55.67± 2.08*	51.00± 1.00*	77.00± 2.54	42.67± 5.68*	49.67± 8.50*	77.33± 4.61	45.33± 2.51*	60.33± 8.50*
PST	79.00± 2.64	34.33± 3.51*	52.67± 5.50*	78.33± 3.78	30.67± 5.03*	44.00± 6.00*	76.33± 10.97	33.00± 5.00*	41.00± 8.00*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST, ˣ Not significantly (p>0.05) different from NC*

In table 4.3.5, for rats exposed to 2Gy dose, significant decrease (p<0.05) was recorded in EC (36.67% and 39.67%), PRT (55.67% and 51%), and PST (34.33) and (52.67)groups relatively to NCgroup (77% and 79%) on day 1 and 8 after radiation exposure. However, increase in CAT mean activities for rats in PRT and PST groups were not significant (p>0.05)when compared to NC groups. The table also shows that rats exposed to 4Gy and 6Gy, the CAT mean activitieslevel in EC groupdecreased (p<0.05) when compared to NC group on day 1 and 8 after IR. But radiation induced decrease in CAT mean activities was significantly attenuated (p<0.05)in PRT group when compared to rats in EC group.

4.3.7 Superoxide dismutase (SOD) parameters

Table 4.3.7Variation of superoxide dismutase mean activities for all the animalgroups, before and after graded doses of radiation

SOD (% Inhibition)									
SOD 2Gy, MEAN±SD				**SOD4Gy, MEAN±SD**			**SOD 6Gy, MEAN±SD**		
Group	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3	Before IR n=3	Day 1 after IR n=3	Day 8 after IR n=3
NC	78.00±2.00	80.67± 2.51	79.00± 1.00	78.00± 2.00	80.67± 2.51	79.00± 1.00	78.00± 2.00	80.67± 2.51	79.00± 1.00
EC	74.67± 8.40	36.00± 0.00*	30.00± 2.00*	74.33± 4.72	32.33± 5.68*	29.33± 4.04*À	72.00± 2.64	29.00± 5.29*	34.00± 2.00*À
PRT	76.00± 4.00	57.67± 8.50*	39.67± 3.51*	65.67± 1.52	47.00± 5.00*	50.67± 3.51*	70.67± 6.65	53.00± 5.00*	63.67± 2.51*
PST	82.00± 1.00	37.33± 5.50*	61.00± 8.00*	76.33± 3.51	35.67± 2.51*	40.67± 6.50*	77.0± 2.00	30.00± 4.00*	50.33± 7.37*

**Significantly different (p<0.05) from NC,† Significantly different (p<0.05) from PRT and PST,ˣ Not significantly (p>0.05) different from NC*

In table 4.3.7, there were significant decrease (p<0.05) in SOD mean activities in EC, PRT and PST groups relatively to NC on day 1 and 8after radiation exposure to 2Gy, 4Gy and 6Gy doses. On day 8, rats exposed to 4Gy and 6Gy in EC group were not only significantly different (p<0.05) from NC but also from rats in PRT and PST groups.

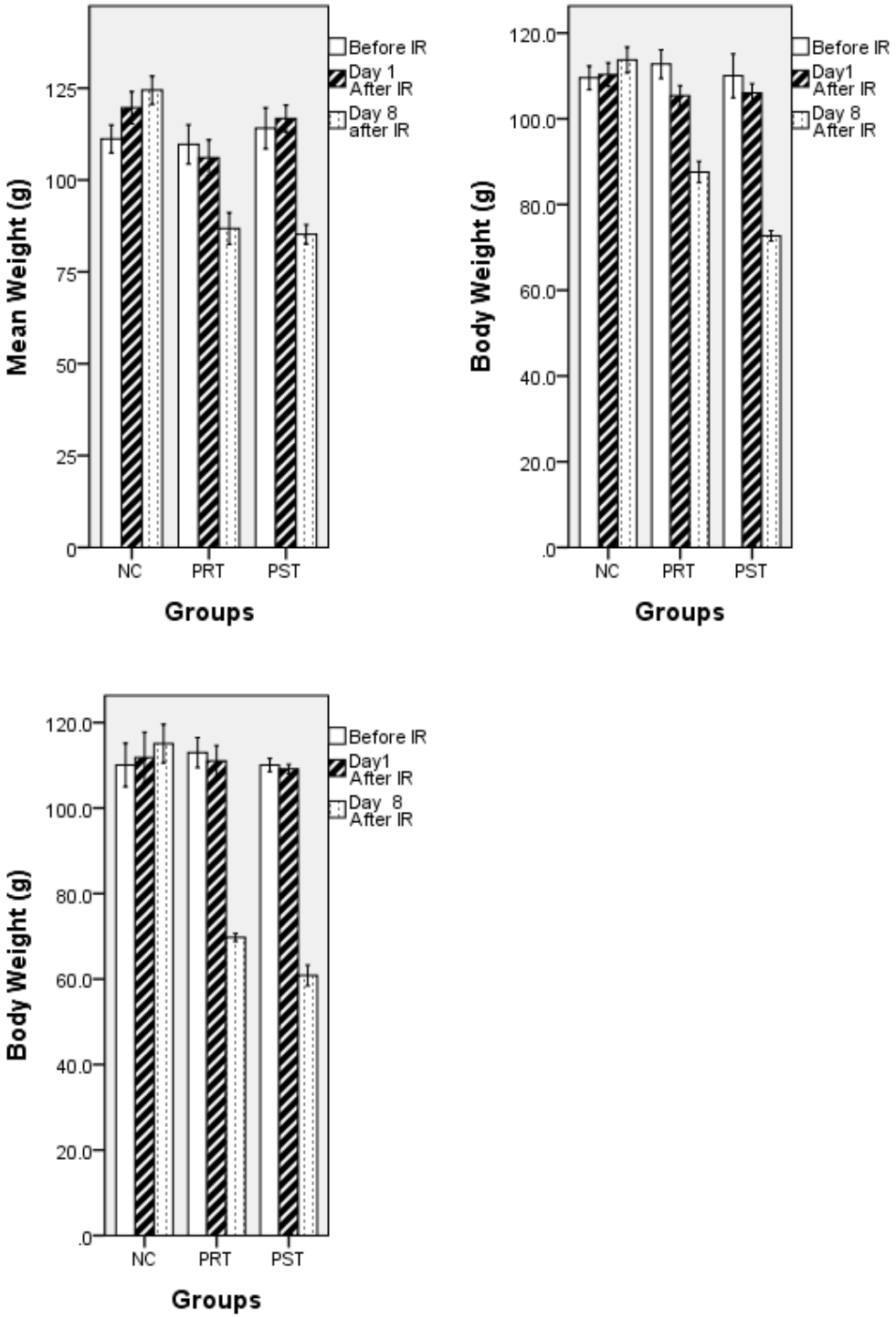


Fig 4.1.1 Comparing mean Body weight of rats in PRT and PST groups against NC group for 2Gy, 4Gy and 6Gy radiation dose respectively

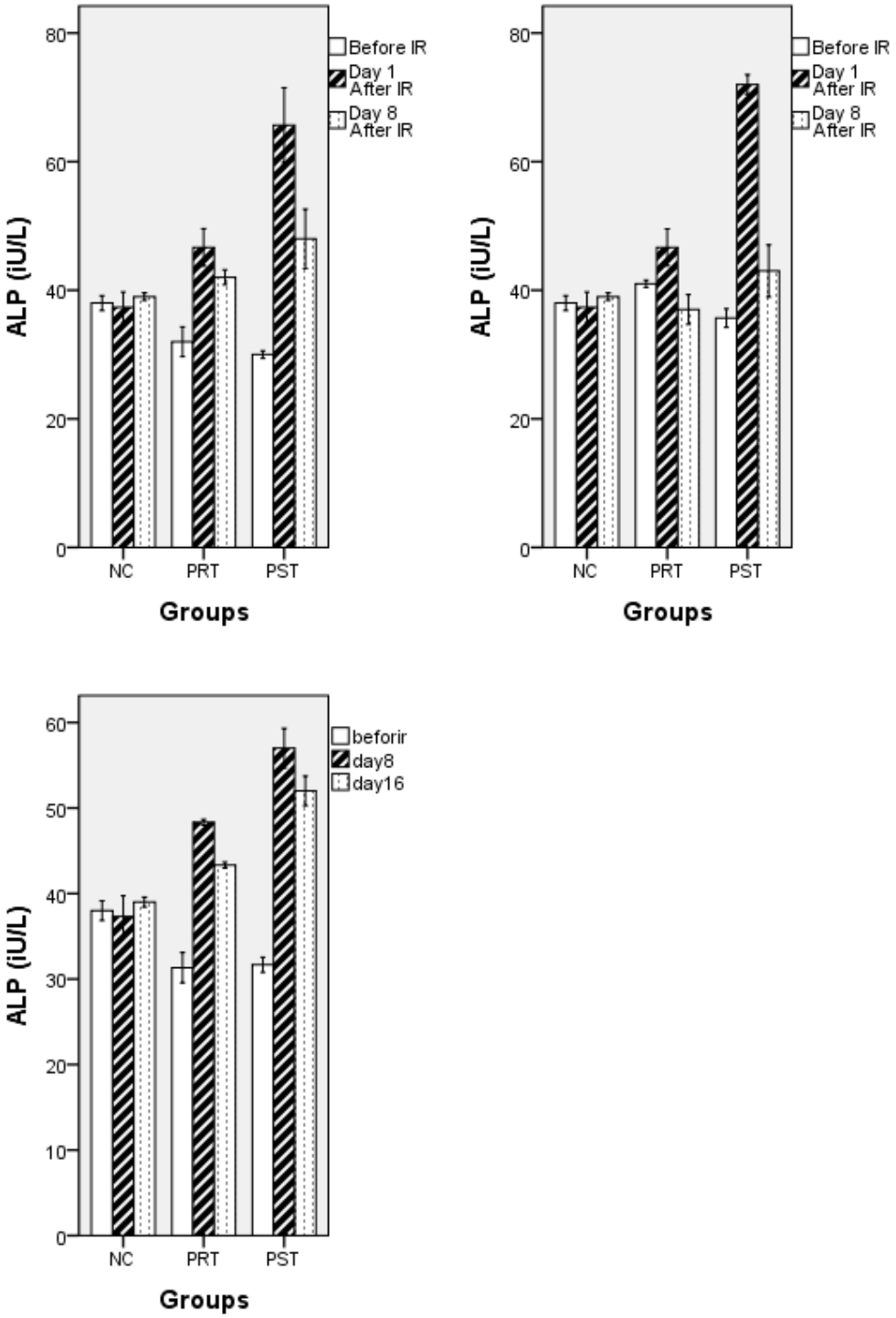


Fig 4.1.2 Comparing Alkaline phosphate mean levels in PRT and PST groups against NC group for 2Gy, 4Gy and 6Gy radiation dose respectively

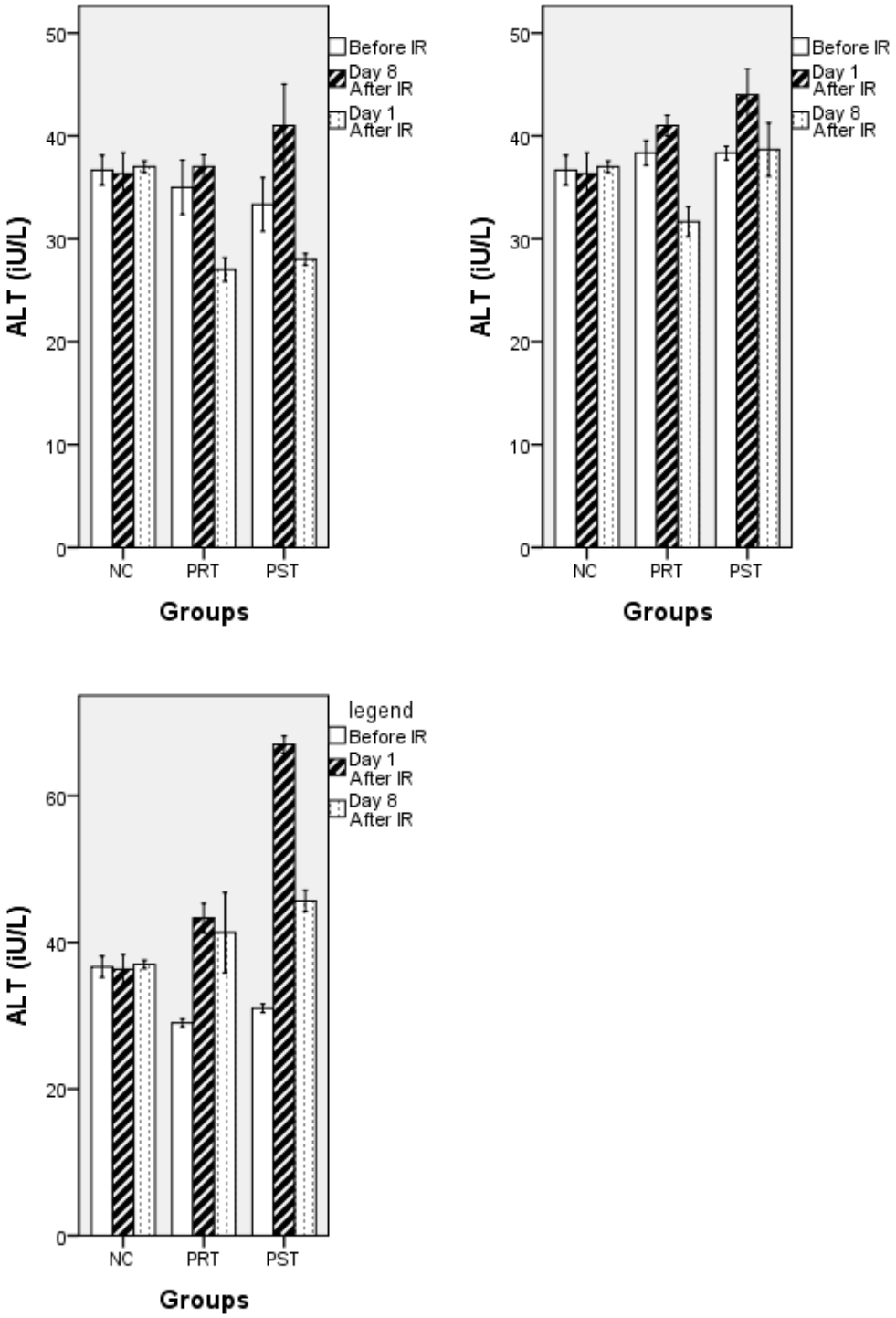


Fig 4.1.3 Comparing Alanine amino-transferase mean levels in PRT and PST groups against NC group for 2Gy, 4Gy and 6Gy radiation dose respectively

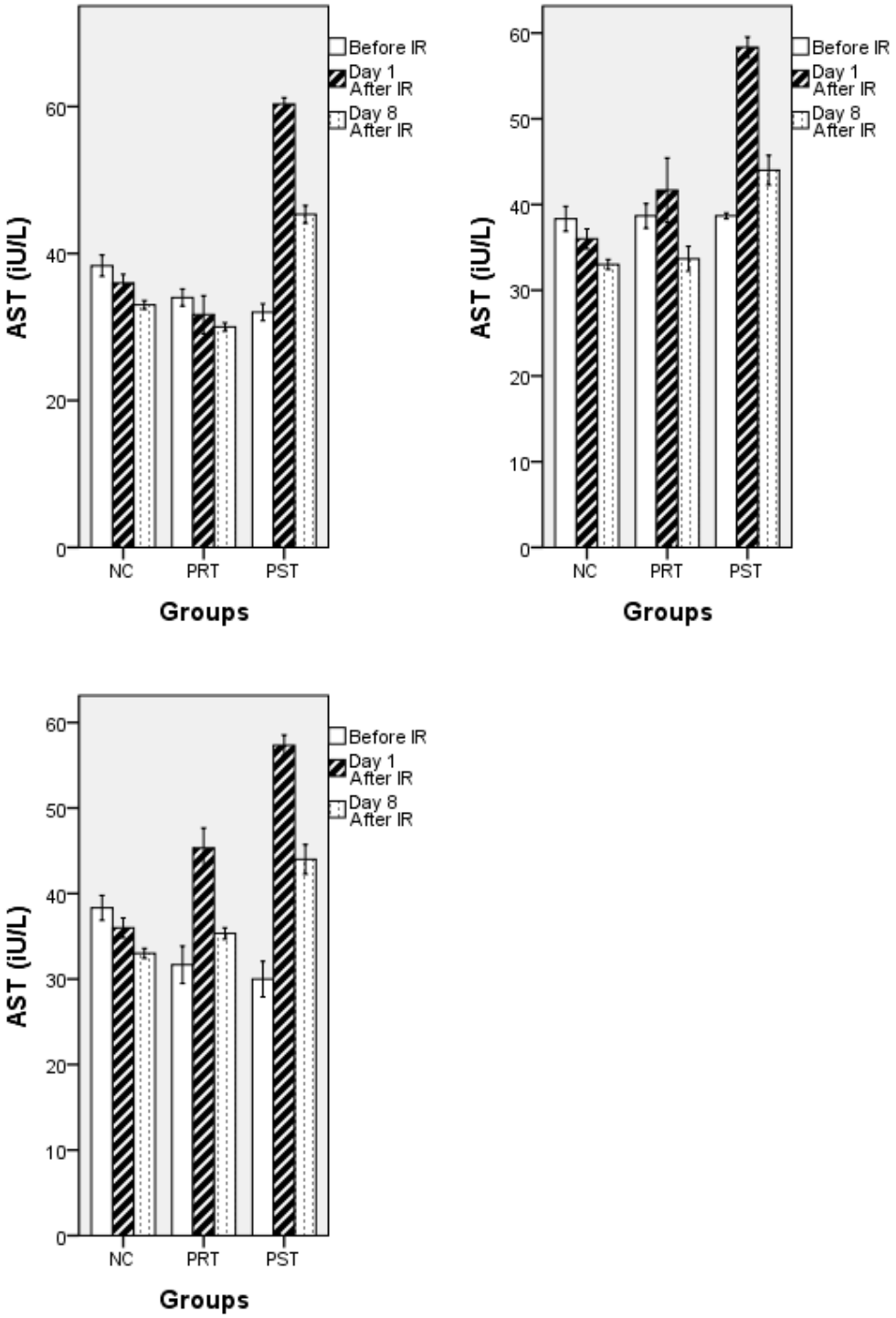


Fig 4.1.4 Comparing Aspartate amino-tranferase mean levels in PRT and PST groups against NC group for 2Gy, 4Gy and 6Gy radiation dose respectively

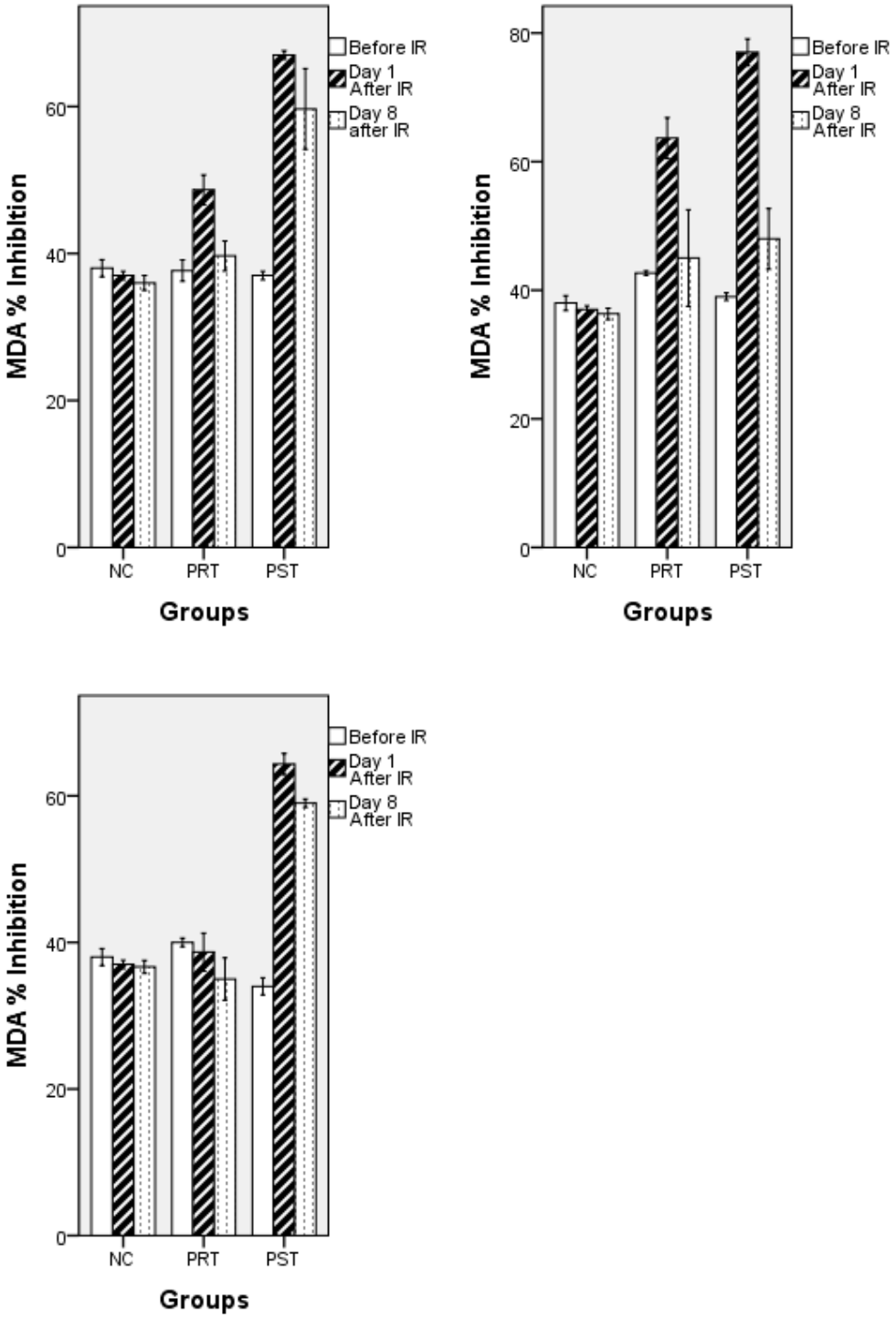


Fig 4.1.5 Comparing Malondialdehyde mean activity levels in PRT and PST groups against NC for 2Gy, 4Gy and 6Gy radiation dose respectively

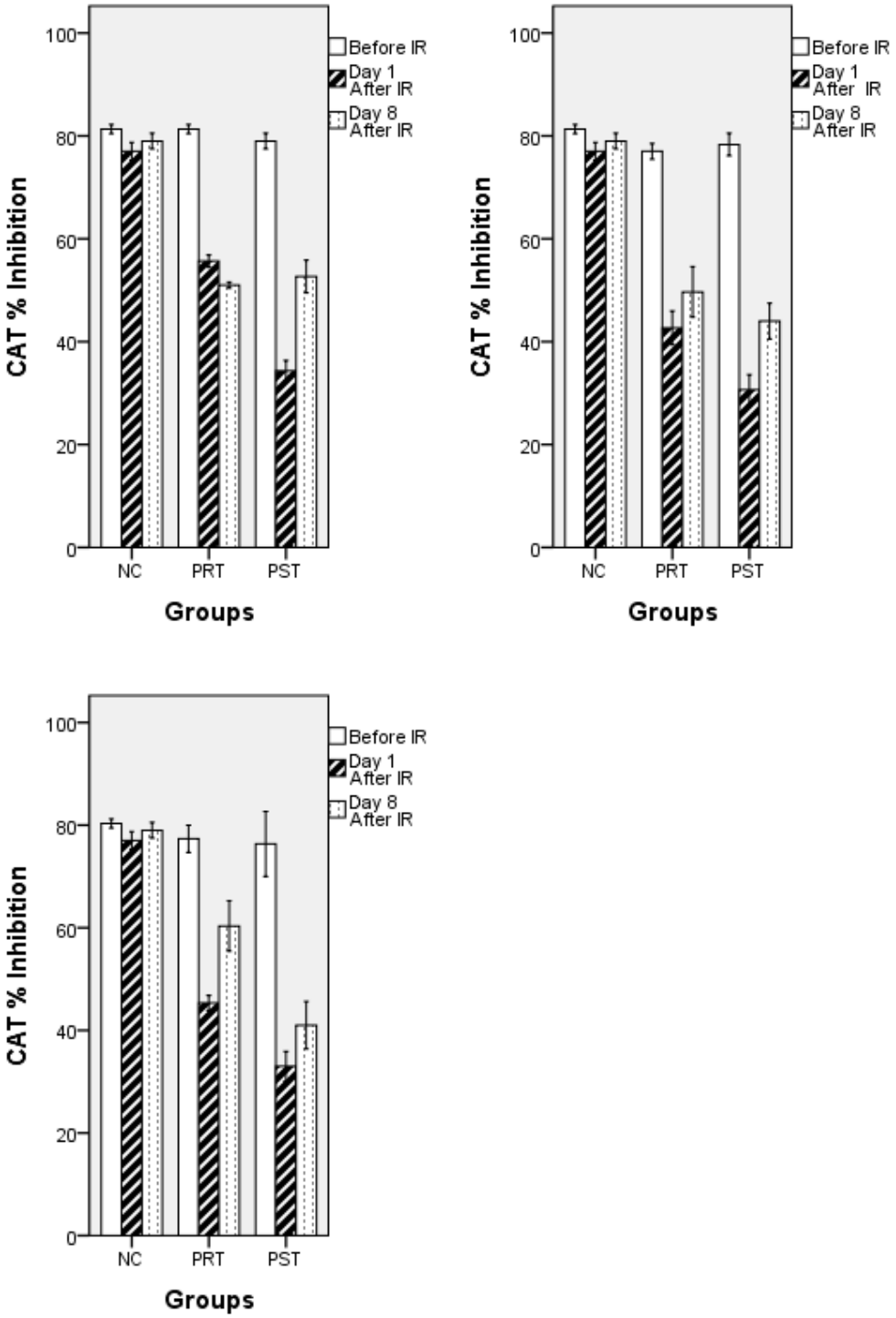


Fig 4.1.6 Comparing Catalase mean activity levels in PRT and PST groups against NC group for 2Gy, 4Gy and 6Gy radiation dose respectively

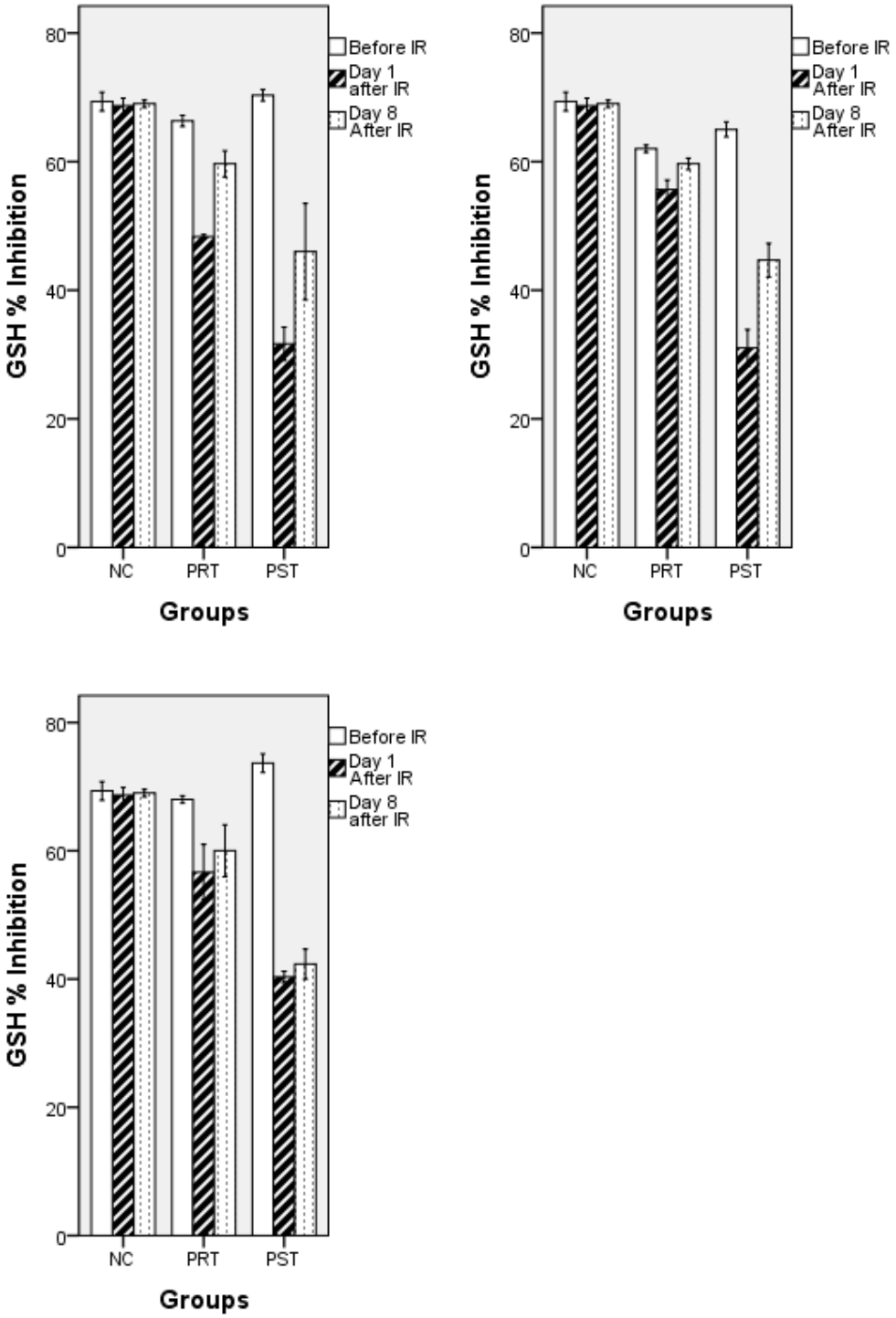


Fig 4.1.7 Comparing Glutathione mean activity levels in PRT and PST groups against NC for 2Gy, 4Gy and 6Gy radiation dose respectively

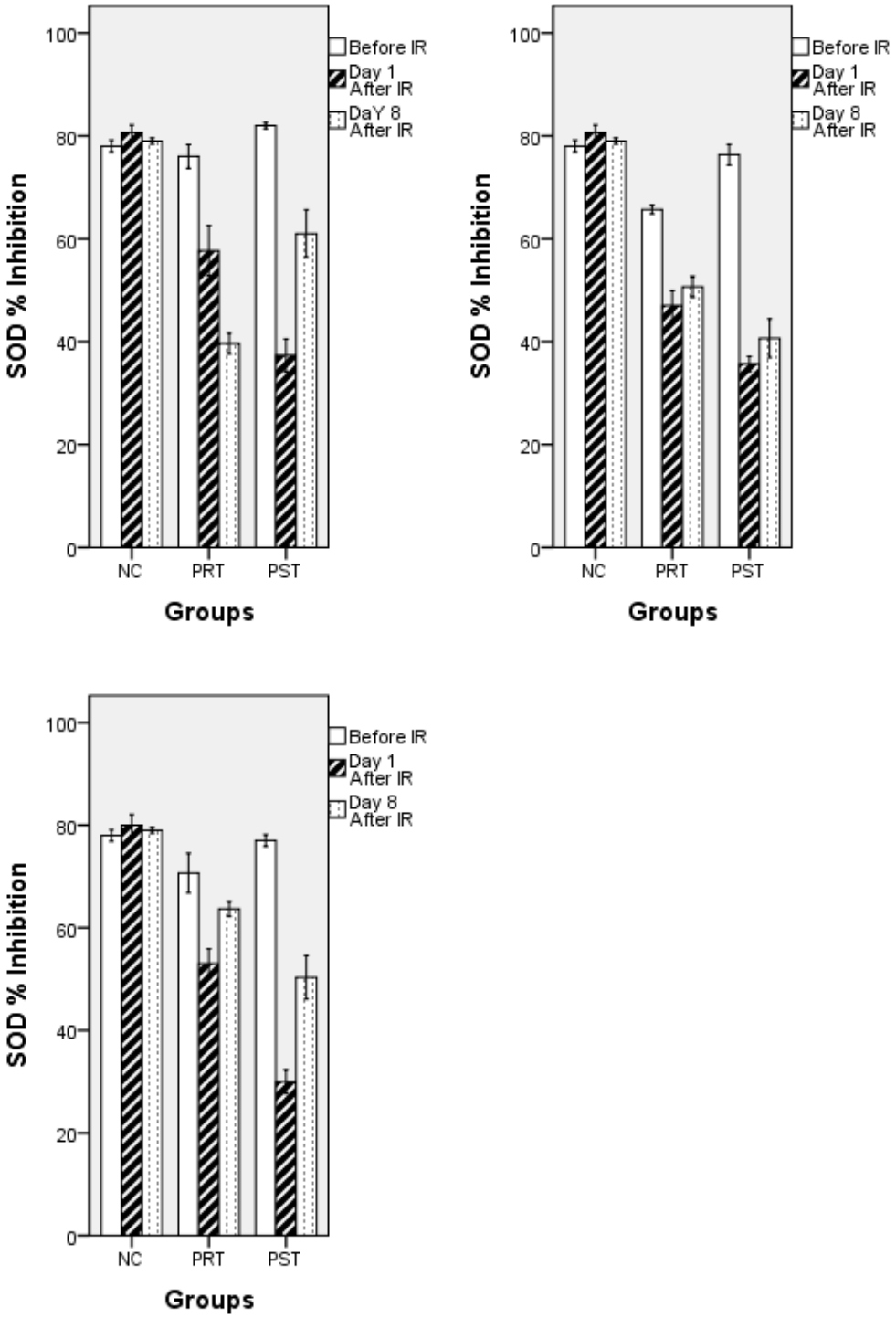


Fig 4.1.8 Comparing Superoxide dismutase mean activity levels in PRT and PST groups against NC for 2Gy, 4Gy and 6Gy radiation dose respectively

4.5 Discussion

In radiation medicine, radiotherapy is one of the most common therapies for treating cancer and tumor cells. During this process human body can be subjected to undesirable tissue/cell injury which could lead to several health complications like cancer, cataract, etc. This is in contrast with main goal set by radiation oncologist, which is the use of radiation to shrink tumors and kill cancerous cells while maintaining minimum acceptable injury to the surrounding tissue. Injury to tissue/cells may occur as result of the following lapses- human error, computer error (programming error), poor quality control and assurance, poor dosimetric measurements, mechanical faults etc. Advancement in scientific and technological know-how have also further increase the radiation oxidative stress in humans, due to the fact that exposure to low level radiation has become common during medical diagnostic procedures, space or air travel, cosmic radiation and the use of certain electronic gadgets.

Radiation damage to mammalian cells could be lethal damage (which is irreversible, irreparable and leads to cell death) and sublethal damage (this can be repaired within months, days or hours). When biological system is exposed to ionizing radiation, it induces oxidative stress via production of reactive oxygen species ROS which includes super oxide (O_2^-, OOH) Hydrogen peroxide ($H_2O_{2)}$), Hydroxyl radical (^-OH), and reactive nitrogen species RNS which includes nitrogen oxide (NO_2), dinitrogen trioxide (N_2O_3) and Nitric oxide *(NO).* These free radicals are atoms or group of atoms that have unpaired electron, and due to its state, they are highly reactive and are capable of altering all biological molecules including lipids, DNA etc.

For these reasons, this present study is designed to explore the possible radioprotective effects of *gongronema latifolio* (GL) extract on a whole-body radiation induced oxidative stress on a wistar albino rats. The quantitative phytochemical analysis shows thatGLextract contains the following alkaloids, tanins, flavonoids, phenols, saponin andalkaloids. Alkaloids

were the highest bioactive phytochemicals present and saponins the lowest. Atangwho et al., (2009), also reported the presence of flavonoids, alkaloids, tannins, saponins and polyphenols. They recorded tannins to be the highest and polyphenols the lowest. The variation in quantity of bioactive agents could be as result of environmental factors. The presence of the following bioactive agents indicates that the GL extract possesses some bioactive agents which could serve as antioxidants.This probably suggests that the *Gongronema latifolio* extract may have the ability to scavenge for free radicals due to the presence of alkaloids, flavonoids and polyphenols which are the main source of antioxidants in plant. Tannins have also shown to possess some medicinal properties, (Ekeanyanwu et al., 2010).

Biological systems are naturally protected from reactive oxygen species (ROS) and reactive nitrogen species (RNS) by antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), gluthathione peroxidase (GPx) etc and other endogenous sources such as ascorbic acid (vitamin C), uric acid, glutathione (GSH) etc. Majority of toxic effects of ionizing radiation to liver are due to ROS and RNS.

After irradiation some of the following physical changes were observed in experimental groups within 2 ï 4 days: - ruffling of hair, diarrhea, loss of appetite, paralysis and weight loss. The frequency of occurrence was more in experimental control and post-treatment groups. The result of weight loss revealed significant decline in the body weight of rats exposed to whole body radiation at different graded doses The result obtained by Oluwatosin, (2009) also indicates that the body weight of animals exposed to radiation significantly decreased ($p<0.05$) few weeks after been exposed to radiation when compared to normal animals. This decrease in body weight may be as a result of acute radiation syndrome (ARS) or what can be referred as a radiation sickness (e.g. diarrhea, loss of appetite, nausea etc). The major cause of this syndrome is depletion of immature parenchymal stem cells in specific

tissues. It could also be as a result of destructive and irreparable changes in gastro-intestinal tract (GIT) and bone marrow which could lead to dehydration and diarrhea, CDC, (2014). However, rats pretreated with *Gongronema latifolio* showed more significant recovery in the radiation induced weight loss relatively to post-treated rats. This suggests that *Gongronema latifolio* extract ameliorated radiation induced weight loss in rats exposed to radiation.

This study carried out liver function tests (LFTs) by observing the activities of the following parameters- alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase in normal control (NC), experimental control (EC), pre-treatment (PRT) and post-treatment (PST) groups. These parameters measure the excretory and synthetic functions of liver and indicate an injury, impaired functions or damage to liver in irradiated animals, Oluwatosin, (2009).

The result obtained in this work revealed significant elevation in ALP level of rats exposed to whole-body radiation at different graded doses. Rats treated with GL before (Pre-treatment group) and after Irradiation (Post-treatment group) showed significant recovery relatively to rats in Experimental control group on day 8 after IR. However, ALP mean level in Post-treated rats exposed to 6Gy dose remained significantly different from NC control on day 8 after IR. This could be as a result of dose dependent nature of ionizing radiation.

The observed effect of ionizing radiation on the level of Alanine Aminotransferase (ALT) significantly elevated in Experimental control when compared to Normal control on day 8 after Radiation exposure. Katanyatanon et al. (2008) found that ALT level was within the normal range suggesting no measurable radiation-induced hepatic injury of general tissue at dose 5Gy whole-body irradiation when followed up to 8 days. Interestingly, ALT level in rats Pre-treated and Post-treated with GL recorded full recovery on day 8 after IR. In other words, there was no significant difference in ALT level of rats treated with GL before and after radiation exposure when compared to Normal control.

Whole-body exposure of rats at different graded doses of radiation induced significant increase in the Aspartate Aminotransferase level on day 8 post IR. However,AST level in Pre-treated rats displayed significant decreased in radiation induced increase in AST level on day 8 post IR, but decrease in AST level in Post-treated rats remained significant different from the Normal control on day 8 after IR.

Increase in ALP level in the rats exposed to different graded doses of radiation maybe due to pathological alteration in biliary flow and damage to the liver cell membranewhile increase in AST and ALT levels could be attributed to the drastic dysfunction of hepatic cells as a consequence of radiation interaction with the membrane of cells and also related to extensive breakdown of liver parenchyma. The significant elevation in ALT, AST and ALP levels in the rats exposed to graded radiation doses relatively to those in normal control (NC) is in agreement with the findings made by Farag (2013), and Abdou and Abbas (2009) who recorded significant increase in AST, ALT and ALP serum activities of gamma treated rats. The results obtained in LFT tests shows that administration of *Gongronema latifolio* extract resulted in a reduction of high ALP, ALT and AST levels induced by radiation in most of the rats in PRT and PST groups thus suggesting the radioprotective and hepatoprotective abilities *Gongronema latifolio* extract.

This study also measured Malondialdehyde MDA (which is an index for lipid peroxidation) activity level in across all the groups. MDA acts as sensitive bio-marker for oxidative stress that occurs as part of the pathogenesis of various diseases, Oluwatosin, (2009). Whole-body exposure of rats at different graded doses of radiation demonstrated significant increase in MDA activity level on day 1 & 8 post IR.This result is consistent with the result obtained by Rahab and Ibrahim, (2012), who recorded significant increase in the MDA level in irradiated animal groups. Thevarious degree of significant elevation in the MDA level in the rats exposed to radiation indicates the level of oxidative degradation. This could be as result of

long lived free-radical attack on the cell membrane and lipids. The Pre-treated rats showed significant decreased in radiation induced increase in MDA level on day 8 post IR. On the other hand, decrease in MDA level in Post-treated rats remained significant different from the Normal control on day after IR. MDA result obtained suggests that *Gongronema latifolio* extract reduces oxidative stress especially when itôs in the biological system prior to radiation exposure.

Also assessed in this work are antioxidant enzymes- Glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD). This study recorded significant decline in GSH level for rats exposed to different graded doses of radiation. This result is in harmony with those of Sang et al., (2010), Abd-Elraheim et al., (2015), they recorded significant decrease ($p<0.05$) in GSH and GPx levels. Rats Pre-treated with GL significantly increased GSH level but could not restore GSH level close to normal on day 8 post IR. However, there was no statistical difference in GSH level of rats post-treated with GL compared to Experimental control. The decrease in mean level of GSH activity in the whole-body irradiated rats may have resulted in GSH being directly utilized as antioxidant i.e. by neutralizing free radicals induced in rats exposed to graded doses of radiation.

The CAT level indicated high significant decrease in the rats exposed to whole-body radiation at different graded doses. Rats treated with GL before and after Irradiation showed significant recovery on day 8 after IR. The SOD level also showed high significant decline in rats exposed to whole body radiation at different graded doses. Rats treated with GL before and after Irradiation also demonstrated significant recovery on day 8 after IR. SOD and CAT results are consistent with the result obtained by Baliga et al., (2004), Sang et al., (2010) and Farag (2013). They recorded significant decrease ($p<0.05$) in CAT and SOD activities level in irradiated animals. This suggests that SOD and CAT activity level in pre-treatment and post-treatment groups were able to mob up free radicals generated in rats exposed to graded

radiation doses. SOD catalyzes dismutation of super oxide ion (O_2^-) and converts it to H_2O_2 while CAT decomposes H_2O_2 to H_2O and O_2.

The statistical analysis result provides uswith significant evidence that pre- and post-treatment of rats with *Gongronema latifolio* has potent effects against radiation induced injury. It is noteworthy to suggest that oxidative stress caused by radiation were ameliorated due to the antioxidant activities of *Gongronema latifolio* extract. In other words, GL extract potential to enhance the antioxidant state in the rats exposed to graded doses of radiation affirms its radioprotective effects.

4.6 LIMITATIONS / CHALLENGES

The major challenges encountered in the course of this research work revolve round the problems of lack of standard laboratory. This includes use of obsolete equipment, poor animal house facilities and managements, Poor access and high cost of accessing radiation medical equipment, little or no access to isolation and characterization equipment.

Other challenges include bottleneck in getting laboratory approvals, lack of access to funds or research grants from Government Agencies, Corporate Organizations, Foundations and Trust, Educational institutes etc for a research of this nature.

CHAPTER 5

Conclusion and Recommendation

5.1 Conclusion

We have successful evaluated radioprotective effects of *Gongrenema latifolio*(GL)on whole-body irradiated wistar albino rats. Hence we conclude that this current study find*Gongrenema latifolio*extractradioprotective against radiation induce oxidative stress. However itôs mechanism of action is not full known yet but its radioprotective effects may be credited to its antioxidants and free radicals scavenging properties.

GL may have advantage over well-known and available radioprotectors considering the significant result achieved with very low dose of extract (250mg/kg) and also within a short duration of treatment.

TheGL radioprotective effects have shown to be more effective in pre-treatment group when compared to post-treatment group. As a result, supplementation of antioxidant will be of great importance in radiotherapy and to astronauts. It should be encourage for patients before and after undergoing radiotherapy and individuals that may have been exposed to ionizing radiation by accident or as result of occupational predisposition. Complementing of GL extract in radiotherapy and radiation medicine should be encouraged for safe application of radiation technology.

5.2 Recommendation

- It is recommended that further work should be done with GL leaf adopting different approach like calculating dose reduction factor, survival rate and also extending pre-treatment and post-treatment period from weeks to months.
- It is also recommended that other biochemical parameter like Glutathione Peroxidase (GPx), vitamin-C and vitamin-E, should also be evaluated.

- Further studies are required to evaluate the protective effect of GL on macromolecules like DNA, RNA and also on other tissues.
- Finally there is need to isolate and characterize the active agent(s) responsible for itsradioprotective effects.

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APPENDICES

APPENDIX A: Calculation Of Graded Radiation Doses Administered To Rats In Experimental Control, Pre-Treatment And Post- Groups

Source axis distance (SAD) technique was adopted for treatment modality.

PDD - percentage depth dose = 88.70%

SSD - source surface distance = 95.5cm

Depth - 4.5cm

Dmax - 1.3cm

Tissue maximum ratio TMR = $\frac{\square\square\square \times (\square\square\square\square\square\square\square\square)^{\square}}{\square\square\square \times (\square\square\square\square\square\square_{\square\square})^{\square}}$

$$TMR = \frac{\square\square.\square\square \times \square\square\square\square}{\square\square\square \times \square\square\square\square.\square\square} = 0.9466$$

Peak Dose = $\frac{\square\square\square\square\square\square\square\square\square\square\square \times \square\square\square\square \times \square\square\square\square \square\square\square\square\square\square}{\square\square \square}$

Where field factor = 0.957

a) For 2Gy = 200cGy

Peak dose = $\frac{\square\square\square \times \square.\square\square\square}{\square.\square\square\square\square}$ = 202cGy =202MU

b) For 4Gy = 400cGy

Peak dose = $\frac{\square\square\square \times \square.\square\square\square}{\square.\square\square\square\square}$ = 404cGy = 404MU

c) For 6Gy = 600cGy

Peak dose = $\frac{\square\square\square \times \square.\square\square\square}{\square.\square\square\square\square}$ = 606.6cGy = 606.6MU

APPENDIX B: Descriptive table.

Descriptive table for ALP 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	33.67	3.512	2.028	24.94	42.39	30	37
	PRT	3	32.00	4.000	2.309	22.06	41.94	28	36
	PST	3	30.00	1.000	.577	27.52	32.48	29	31
	Total	12	33.42	3.942	1.138	30.91	35.92	28	40
Day 1 after IR	NC	3	37.33	4.163	2.404	26.99	47.68	34	42
	EC	3	59.33	11.846	6.839	29.91	88.76	52	73
	PRT	3	46.67	5.033	2.906	34.16	59.17	42	52
	PST	3	65.67	10.066	5.812	40.66	90.67	55	75
	Total	12	52.25	13.552	3.912	43.64	60.86	34	75
Day 8 after IR	NC	3	39.00	1.000	.577	36.52	41.48	38	40
	EC	3	67.00	4.000	2.309	57.06	76.94	63	71
	PRT	3	42.00	2.000	1.155	37.03	46.97	40	44
	PST	3	48.00	8.000	4.619	28.13	67.87	40	56
	Total	12	49.00	12.030	3.473	41.36	56.64	38	71

Descriptive table for ALP 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	34.67	2.517	1.453	28.42	40.92	32	37
	PRT	3	41.00	1.000	.577	38.52	43.48	40	42
	PST	3	35.67	2.517	1.453	29.42	41.92	33	38
	Total	12	37.33	3.114	.899	35.35	39.31	32	42
Day 1 after IR	NC	3	37.33	4.163	2.404	26.99	47.68	34	42
	EC	3	75.00	2.000	1.155	70.03	79.97	73	77
	PRT	3	46.67	4.933	2.848	34.41	58.92	41	50
	PST	3	72.00	2.646	1.528	65.43	78.57	70	75
	Total	12	57.75	17.126	4.944	46.87	68.63	34	77
Day 8 after IR	NC	3	39.00	1.000	.577	36.52	41.48	38	40
	EC	3	74.00	3.464	2.000	65.39	82.61	72	78
	PRT	3	37.00	4.000	2.309	27.06	46.94	33	41
	PST	3	43.00	7.000	4.041	25.61	60.39	36	50
	Total	12	48.25	16.136	4.658	38.00	58.50	33	78

Descriptive table for ALP 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	40.67	1.528	.882	36.87	44.46	39	42
	PRT	3	31.33	3.055	1.764	23.74	38.92	28	34
	PST	3	31.67	1.528	.882	27.87	35.46	30	33
	Total	12	35.42	4.582	1.323	32.51	38.33	28	42
Day 1 after IR	NC	3	37.33	4.163	2.404	26.99	47.68	34	42
	EC	3	75.00	7.000	4.041	57.61	92.39	68	82
	PRT	3	48.33	.577	.333	46.90	49.77	48	49
	PST	3	57.00	4.000	2.309	47.06	66.94	53	61
	Total	12	54.42	14.902	4.302	44.95	63.89	34	82
Day 8 after IR	NC	3	39.00	1.000	.577	36.52	41.48	38	40
	EC	3	80.00	2.000	1.155	75.03	84.97	78	82
	PRT	3	43.33	.577	.333	41.90	44.77	43	44
	PST	3	52.00	3.000	1.732	44.55	59.45	49	55
	Total	12	53.58	16.741	4.833	42.95	64.22	38	82

Descriptive table for ALT 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	36.67	2.517	1.453	30.42	42.92	34	39
	EC	3	37.00	5.196	3.000	24.09	49.91	31	40
	PRT	3	35.00	4.583	2.646	23.62	46.38	30	39
	PST	3	33.33	4.509	2.603	22.13	44.53	29	38
	Total	12	35.50	3.989	1.151	32.97	38.03	29	40
Day 1 after IR	NC	3	36.33	3.512	2.028	27.61	45.06	33	40
	EC	3	43.67	4.509	2.603	32.47	54.87	39	48
	PRT	3	37.00	2.000	1.155	32.03	41.97	35	39
	PST	3	41.00	7.000	4.041	23.61	58.39	34	48
	Total	12	39.50	5.036	1.454	36.30	42.70	33	48
Day 8 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	62.00	5.000	2.887	49.58	74.42	57	67
	PRT	3	27.00	2.000	1.155	22.03	31.97	25	29
	PST	3	28.00	1.000	.577	25.52	30.48	27	29
	Total	12	38.50	14.933	4.311	29.01	47.99	25	67

Descriptive table for ALT 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	36.67	2.517	1.453	30.42	42.92	34	39
	EC	3	36.67	1.528	.882	32.87	40.46	35	38
	PRT	3	38.33	2.082	1.202	33.16	43.50	36	40
	PST	3	38.33	1.155	.667	35.46	41.20	37	39
	Total	12	37.50	1.834	.529	36.33	38.67	34	40
Day 1 after IR	NC	3	36.33	3.512	2.028	27.61	45.06	33	40
	EC	3	45.67	2.517	1.453	39.42	51.92	43	48
	PRT	3	41.00	1.732	1.000	36.70	45.30	40	43
	PST	3	44.00	4.359	2.517	33.17	54.83	39	47
	Total	12	41.75	4.595	1.326	38.83	44.67	33	48
Day 8 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	50.00	2.000	1.155	45.03	54.97	48	52
	PRT	3	31.67	2.517	1.453	25.42	37.92	29	34
	PST	3	38.67	4.509	2.603	27.47	49.87	34	43
	Total	12	39.33	7.377	2.130	34.65	44.02	29	52

Descriptive table for ALT 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	36.67	2.517	1.453	30.42	42.92	34	39
	EC	3	36.67	3.512	2.028	27.94	45.39	33	40
	PRT	3	29.00	1.000	.577	26.52	31.48	28	30
	PST	3	31.00	1.000	.577	28.52	33.48	30	32
	Total	12	33.33	4.053	1.170	30.76	35.91	28	40
Day 1 after IR	NC	3	36.33	3.512	2.028	27.61	45.06	33	40
	EC	3	69.33	2.517	1.453	63.08	75.58	67	72
	PRT	3	43.33	3.512	2.028	34.61	52.06	40	47
	PST	3	67.00	2.000	1.155	62.03	71.97	65	69
	Total	12	54.00	15.255	4.404	44.31	63.69	33	72
Day 8 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	69.67	7.506	4.333	51.02	88.31	62	77
	PRT	3	41.33	9.452	5.457	17.85	64.81	34	52
	PST	3	45.67	2.517	1.453	39.42	51.92	43	48
	Total	12	48.42	14.222	4.106	39.38	57.45	34	77

Descriptive table for AST 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.33	2.517	1.453	32.08	44.58	36	41
	EC	3	37.33	3.512	2.028	28.61	46.06	34	41
	PRT	3	34.00	2.000	1.155	29.03	38.97	32	36
	PST	3	32.00	2.000	1.155	27.03	36.97	30	34
	Total	12	35.42	3.450	.996	33.22	37.61	30	41
Day 1 after IR	NC	3	36.00	2.000	1.155	31.03	40.97	34	38
	EC	3	63.00	4.000	2.309	53.06	72.94	59	67
	PRT	3	31.67	4.509	2.603	20.47	42.87	27	36
	PST	3	60.33	1.528	.882	56.54	64.13	59	62
	Total	12	47.75	14.919	4.307	38.27	57.23	27	67
Day 8 after IR	NC	3	33.00	1.000	.577	30.52	35.48	32	34
	EC	3	63.00	4.000	2.309	53.06	72.94	59	67
	PRT	3	30.00	1.000	.577	27.52	32.48	29	31
	PST	3	45.33	2.082	1.202	40.16	50.50	43	47
	Total	12	42.83	13.710	3.958	34.12	51.54	29	67

Descriptive table for AST 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.33	2.517	1.453	32.08	44.58	36	41
	EC	3	38.67	6.110	3.528	23.49	53.84	32	44
	PRT	3	38.67	2.517	1.453	32.42	44.92	36	41
	PST	3	38.67	.577	.333	37.23	40.10	38	39
	Total	12	38.58	3.029	.874	36.66	40.51	32	44
Day 1 after IR	NC	3	36.00	2.000	1.155	31.03	40.97	34	38
	EC	3	59.67	7.506	4.333	41.02	78.31	52	67
	PRT	3	41.67	6.506	3.756	25.50	57.83	35	48
	PST	3	58.33	2.082	1.202	53.16	63.50	56	60
	Total	12	48.92	11.619	3.354	41.53	56.30	34	67
Day 8 after IR	NC	3	33.00	1.000	.577	30.52	35.48	32	34
	EC	3	63.00	4.000	2.309	53.06	72.94	59	67
	PRT	3	33.67	2.517	1.453	27.42	39.92	31	36
	PST	3	44.00	3.000	1.732	36.55	51.45	41	47
	Total	12	43.42	12.887	3.720	35.23	51.60	31	67

Descriptive table for AST 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
baseline	NC	3	38.33	2.517	1.453	32.08	44.58	36	41
	EC	3	37.67	8.145	4.702	17.43	57.90	32	47
	PRT	3	31.67	3.786	2.186	22.26	41.07	29	36
	PST	3	30.00	3.606	2.082	21.04	38.96	27	34
	Total	12	34.42	5.712	1.649	30.79	38.05	27	47
Day 1 after IR	NC	3	36.00	2.000	1.155	31.03	40.97	34	38
	EC	3	55.67	3.512	2.028	46.94	64.39	52	59
	PRT	3	45.33	4.041	2.333	35.29	55.37	41	49
	PST	3	57.33	2.082	1.202	52.16	62.50	55	59
	Total	12	48.58	9.346	2.698	42.64	54.52	34	59
Day 8 after IR	NC	3	33.00	1.000	.577	30.52	35.48	32	34
	EC	3	63.00	4.000	2.309	53.06	72.94	59	67
	PRT	3	35.33	1.155	.667	32.46	38.20	34	36
	PST	3	44.00	3.000	1.732	36.55	51.45	41	47
	Total	12	43.83	12.525	3.616	35.88	51.79	32	67

Descriptive table for MDA 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	40.00	2.000	1.155	35.03	44.97	38	42
	PRT	3	37.67	2.517	1.453	31.42	43.92	35	40
	PST	3	37.00	1.000	.577	34.52	39.48	36	38
	Total	12	38.17	2.038	.588	36.87	39.46	35	42
Day 1 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	61.00	3.000	1.732	53.55	68.45	58	64
	PRT	3	48.67	3.512	2.028	39.94	57.39	45	52
	PST	3	67.00	1.000	.577	64.52	69.48	66	68
	Total	12	53.42	12.243	3.534	45.64	61.20	36	68
Day 8 after IR	NC	3	36.00	1.732	1.000	31.70	40.30	35	38
	EC	3	67.00	6.000	3.464	52.10	81.90	61	73
	PRT	3	39.67	3.512	2.028	30.94	48.39	36	43
	PST	3	59.67	9.504	5.487	36.06	83.28	50	69
	Total	12	50.58	14.569	4.206	41.33	59.84	35	73

Descriptive table for MDA 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	39.67	1.528	.882	35.87	43.46	38	41
	PRT	3	42.67	.577	.333	41.23	44.10	42	43
	PST	3	39.00	1.000	.577	36.52	41.48	38	40
	Total	12	39.83	2.167	.626	38.46	41.21	36	43
Day 1 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	79.67	1.528	.882	75.87	83.46	78	81
	PRT	3	63.67	5.508	3.180	49.99	77.35	58	69
	PST	3	77.00	3.606	2.082	68.04	85.96	73	80
	Total	12	64.33	17.895	5.166	52.96	75.70	36	81
Day 8 after IR	NC	3	36.33	1.528	.882	32.54	40.13	35	38
	EC	3	83.00	2.000	1.155	78.03	87.97	81	85
	PRT	3	45.00	13.000	7.506	12.71	77.29	32	58
	PST	3	48.00	8.185	4.726	27.67	68.33	41	57
	Total	12	53.08	19.737	5.697	40.54	65.62	32	85

Descriptive table for MDA 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	38.00	2.000	1.155	33.03	42.97	36	40
	EC	3	42.33	2.082	1.202	37.16	47.50	40	44
	PRT	3	40.00	1.000	.577	37.52	42.48	39	41
	PST	3	34.00	2.000	1.155	29.03	38.97	32	36
	Total	12	38.58	3.554	1.026	36.33	40.84	32	44
Day 1 after IR	NC	3	37.00	1.000	.577	34.52	39.48	36	38
	EC	3	63.67	4.509	2.603	52.47	74.87	59	68
	PRT	3	38.67	4.509	2.603	27.47	49.87	34	43
	PST	3	64.33	2.517	1.453	58.08	70.58	62	67
	Total	12	50.92	13.996	4.040	42.02	59.81	34	68
Day 8 after IR	NC	3	36.67	1.528	.882	32.87	40.46	35	38
	EC	3	70.67	5.508	3.180	56.99	84.35	65	76
	PRT	3	35.00	5.000	2.887	22.58	47.42	30	40
	PST	3	59.00	1.000	.577	56.52	61.48	58	60
	Total	12	50.33	16.093	4.646	40.11	60.56	30	76

Descriptive table for CAT 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	81.33	1.528	.882	77.54	85.13	80	83
	EC	3	79.00	3.000	1.732	71.55	86.45	76	82
	PRT	3	81.33	1.528	.882	77.54	85.13	80	83
	PST	3	79.00	2.646	1.528	72.43	85.57	76	81
	Total	12	80.17	2.290	.661	78.71	81.62	76	83
Day 1 after IR	NC	3	77.00	3.000	1.732	69.55	84.45	74	80
	EC	3	36.67	2.517	1.453	30.42	42.92	34	39
	PRT	3	55.67	2.082	1.202	50.50	60.84	54	58
	PST	3	34.33	3.512	2.028	25.61	43.06	31	38
	Total	12	50.92	18.108	5.227	39.41	62.42	31	80
Day 8 after IR	NC	3	79.00	2.646	1.528	72.43	85.57	76	81
	EC	3	39.67	2.517	1.453	33.42	45.92	37	42
	PRT	3	51.00	1.000	.577	48.52	53.48	50	52
	PST	3	52.67	5.508	3.180	38.99	66.35	47	58
	Total	12	55.58	15.324	4.424	45.85	65.32	37	81

Descriptive table for CAT 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	81.33	1.528	.882	77.54	85.13	80	83
	EC	3	76.00	5.000	2.887	63.58	88.42	71	81
	PRT	3	77.00	2.646	1.528	70.43	83.57	74	79
	PST	3	78.33	3.786	2.186	68.93	87.74	74	81
	Total	12	78.17	3.639	1.050	75.85	80.48	71	83
Day 1 after IR	NC	3	77.00	3.000	1.732	69.55	84.45	74	80
	EC	3	26.00	2.000	1.155	21.03	30.97	24	28
	PRT	3	42.67	5.686	3.283	28.54	56.79	38	49
	PST	3	30.67	5.033	2.906	18.16	43.17	26	36
	Total	12	44.08	21.146	6.104	30.65	57.52	24	80
Day 8 after IR	NC	3	79.00	2.646	1.528	72.43	85.57	76	81
	EC	3	31.00	5.000	2.887	18.58	43.42	26	36
	PRT	3	49.67	8.505	4.910	28.54	70.79	41	58
	PST	3	44.00	6.000	3.464	29.10	58.90	38	50
	Total	12	50.92	19.033	5.494	38.82	63.01	26	81

Descriptive table for CAT 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	80.33	1.528	.882	76.54	84.13	79	82
	EC	3	75.67	4.509	2.603	64.47	86.87	71	80
	PRT	3	77.33	4.619	2.667	65.86	88.81	72	80
	PST	3	76.33	10.970	6.333	49.08	103.58	64	85
	Total	12	77.42	5.775	1.667	73.75	81.09	64	85
Day 1 after IR	NC	3	77.00	3.000	1.732	69.55	84.45	74	80
	EC	3	34.67	1.528	.882	30.87	38.46	33	36
	PRT	3	45.33	2.517	1.453	39.08	51.58	43	48
	PST	3	33.00	5.000	2.887	20.58	45.42	28	38
	Total	12	47.50	18.672	5.390	35.64	59.36	28	80
Day 8 after IR	NC	3	79.00	2.646	1.528	72.43	85.57	76	81
	EC	3	38.33	1.528	.882	34.54	42.13	37	40
	PRT	3	60.33	8.505	4.910	39.21	81.46	52	69
	PST	3	41.00	8.000	4.619	21.13	60.87	33	49
	Total	12	54.67	17.900	5.167	43.29	66.04	33	81

Descriptive table for GSH 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	69.33	2.517	1.453	63.08	75.58	67	72
	EC	3	66.67	3.512	2.028	57.94	75.39	63	70
	PRT	3	66.33	1.528	.882	62.54	70.13	65	68
	PST	3	70.33	1.528	.882	66.54	74.13	69	72
	Total	12	68.17	2.725	.787	66.44	69.90	63	72
Day 1 after IR	NC	3	68.67	2.082	1.202	63.50	73.84	67	71
	EC	3	33.67	2.517	1.453	27.42	39.92	31	36
	PRT	3	48.33	.577	.333	46.90	49.77	48	49
	PST	3	31.67	4.509	2.603	20.47	42.87	27	36
	Total	12	45.58	15.641	4.515	35.65	55.52	27	71
Day 8 after IR	NC	3	69.00	1.000	.577	66.52	71.48	68	70
	EC	3	26.67	2.517	1.453	20.42	32.92	24	29
	PRT	3	59.67	3.512	2.028	50.94	68.39	56	63
	PST	3	46.00	13.000	7.506	13.71	78.29	33	59
	Total	12	50.33	17.634	5.091	39.13	61.54	24	70

Descriptive table for GSH 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	69.33	2.517	1.453	63.08	75.58	67	72
	EC	3	68.00	2.000	1.155	63.03	72.97	66	70
	PRT	3	62.00	1.000	.577	59.52	64.48	61	63
	PST	3	65.00	2.000	1.155	60.03	69.97	63	67
	Total	12	66.08	3.397	.981	63.93	68.24	61	72
Day 1 after IR	NC	3	68.67	2.082	1.202	63.50	73.84	67	71
	EC	3	32.33	2.082	1.202	27.16	37.50	30	34
	PRT	3	55.67	2.517	1.453	49.42	61.92	53	58
	PST	3	31.00	5.000	2.887	18.58	43.42	26	36
	Total	12	46.92	16.860	4.867	36.20	57.63	26	71
Day 8 after IR	NC	3	69.00	1.000	.577	66.52	71.48	68	70
	EC	3	29.67	2.887	1.667	22.50	36.84	28	33
	PRT	3	59.67	1.528	.882	55.87	63.46	58	61
	PST	3	44.67	4.509	2.603	33.47	55.87	40	49
	Total	12	50.75	15.801	4.561	40.71	60.79	28	70

Descriptive table GSH 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	69.33	2.517	1.453	63.08	75.58	67	72
	EC	3	65.00	1.000	.577	62.52	67.48	64	66
	PRT	3	68.00	1.000	.577	65.52	70.48	67	69
	PST	3	73.67	2.517	1.453	67.42	79.92	71	76
	Total	12	69.00	3.643	1.052	66.69	71.31	64	76
Day 1 after IR	NC	3	68.67	2.082	1.202	63.50	73.84	67	71
	EC	3	39.33	.577	.333	37.90	40.77	39	40
	PRT	3	56.67	7.506	4.333	38.02	75.31	49	64
	PST	3	40.33	1.528	.882	36.54	44.13	39	42
	Total	12	51.25	13.171	3.802	42.88	59.62	39	71
Day 8 after IR	NC	3	69.00	1.000	.577	66.52	71.48	68	70
	EC	3	35.67	2.517	1.453	29.42	41.92	33	38
	PRT	3	60.00	7.000	4.041	42.61	77.39	53	67
	PST	3	42.33	4.041	2.333	32.29	52.37	38	46
	Total	12	51.75	14.410	4.160	42.59	60.91	33	70

Descriptive table for SOD 2Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	78.00	2.000	1.155	73.03	82.97	76	80
	EC	3	74.67	8.505	4.910	53.54	95.79	66	83
	PRT	3	76.00	4.000	2.309	66.06	85.94	72	80
	PST	3	82.00	1.000	.577	79.52	84.48	81	83
	Total	12	77.67	5.033	1.453	74.47	80.86	66	83
Day 1 after IR	NC	3	80.67	2.517	1.453	74.42	86.92	78	83
	EC	3	36.00	1.000	.577	33.52	38.48	35	37
	PRT	3	57.67	8.505	4.910	36.54	78.79	49	66
	PST	3	37.33	5.508	3.180	23.65	51.01	31	41
	Total	12	52.92	19.505	5.631	40.52	65.31	31	83
Day 8 after IR	NC	3	79.00	1.000	.577	76.52	81.48	78	80
	EC	3	30.00	2.000	1.155	25.03	34.97	28	32
	PRT	3	39.67	3.512	2.028	30.94	48.39	36	43
	PST	3	61.00	8.000	4.619	41.13	80.87	53	69
	Total	12	52.42	20.224	5.838	39.57	65.27	28	80

Descriptive table for GSH 4Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	78.00	2.000	1.155	73.03	82.97	76	80
	EC	3	74.33	4.726	2.728	62.59	86.07	69	78
	PRT	3	65.67	1.528	.882	61.87	69.46	64	67
	PST	3	76.33	3.512	2.028	67.61	85.06	73	80
	Total	12	73.58	5.664	1.635	69.98	77.18	64	80
Day 1 after IR	NC	3	80.67	2.517	1.453	74.42	86.92	78	83
	EC	3	32.33	5.686	3.283	18.21	46.46	26	37
	PRT	3	47.00	5.000	2.887	34.58	59.42	42	52
	PST	3	35.67	2.517	1.453	29.42	41.92	33	38
	Total	12	48.92	20.286	5.856	36.03	61.81	26	83
Day 8 after IR	NC	3	79.00	1.000	.577	76.52	81.48	78	80
	EC	3	29.33	4.041	2.333	19.29	39.37	25	33
	PRT	3	50.67	3.512	2.028	41.94	59.39	47	54
	PST	3	40.67	6.506	3.756	24.50	56.83	34	47
	Total	12	49.92	19.566	5.648	37.49	62.35	25	80

Descriptive table for SOD 6Gy

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Before IR	NC	3	78.00	2.000	1.155	73.03	82.97	76	80
	EC	3	72.00	2.646	1.528	65.43	78.57	69	74
	PRT	3	70.67	6.658	3.844	54.13	87.21	65	78
	PST	3	77.00	2.000	1.155	72.03	81.97	75	79
	Total	12	74.42	4.641	1.340	71.47	77.37	65	80
Day 1 after IR	NC	3	80.00	3.606	2.082	71.04	88.96	76	83
	EC	3	29.00	5.292	3.055	15.86	42.14	23	33
	PRT	3	53.00	5.000	2.887	40.58	65.42	48	58
	PST	3	30.00	4.000	2.309	20.06	39.94	26	34
	Total	12	48.00	22.087	6.376	33.97	62.03	23	83
Day 8 after IR	NC	3	79.00	1.000	.577	76.52	81.48	78	80
	EC	3	34.00	2.000	1.155	29.03	38.97	32	36
	PRT	3	63.67	2.517	1.453	57.42	69.92	61	66
	PST	3	50.33	7.371	4.256	32.02	68.64	42	56
	Total	12	56.75	17.674	5.102	45.52	67.98	32	80

APPENDIX C: Multiple comparison table

Multiple Comparisons for ALP 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	4.333	2.357	.324	-3.21	11.88
		PRT	6.000	2.357	.126	-1.55	13.55
		PST	8.000*	2.357	.038	.45	15.55
	EC	NC	-4.333	2.357	.324	-11.88	3.21
		PRT	1.667	2.357	.892	-5.88	9.21
		PST	3.667	2.357	.452	-3.88	11.21
	PRT	NC	-6.000	2.357	.126	-13.55	1.55
		EC	-1.667	2.357	.892	-9.21	5.88
		PST	2.000	2.357	.830	-5.55	9.55
	PST	NC	-8.000*	2.357	.038	-15.55	-.45
		EC	-3.667	2.357	.452	-11.21	3.88
		PRT	-2.000	2.357	.830	-9.55	5.55
Day 1 after IR	NC	EC	-22.000	6.884	.050	-44.04	.04
		PRT	-9.333	6.884	.557	-31.38	12.71
		PST	-28.333*	6.884	.014	-50.38	-6.29
	EC	NC	22.000	6.884	.050	-.04	44.04
		PRT	12.667	6.884	.323	-9.38	34.71
		PST	-6.333	6.884	.795	-28.38	15.71
	PRT	NC	9.333	6.884	.557	-12.71	31.38
		EC	-12.667	6.884	.323	-34.71	9.38
		PST	-19.000	6.884	.093	-41.04	3.04
	PST	NC	28.333*	6.884	.014	6.29	50.38
		EC	6.333	6.884	.795	-15.71	28.38
		PRT	19.000	6.884	.093	-3.04	41.04
Day 8 after IR	NC	EC	-28.000*	3.764	.000	-40.05	-15.95
		PRT	-3.000	3.764	.854	-15.05	9.05
		PST	-9.000	3.764	.156	-21.05	3.05
	EC	NC	28.000*	3.764	.000	15.95	40.05
		PRT	25.000*	3.764	.001	12.95	37.05
		PST	19.000*	3.764	.004	6.95	31.05
	PRT	NC	3.000	3.764	.854	-9.05	15.05
		EC	-25.000*	3.764	.001	-37.05	-12.95
		PST	-6.000	3.764	.433	-18.05	6.05
	PST	NC	9.000	3.764	.156	-3.05	21.05
		EC	-19.000*	3.764	.004	-31.05	-6.95
		PRT	6.000	3.764	.433	-6.05	18.05

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for ALP 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	3.333	1.716	.284	-2.16	8.83
		PRT	-3.000	1.716	.362	-8.50	2.50
		PST	2.333	1.716	.555	-3.16	7.83
	EC	NC	-3.333	1.716	.284	-8.83	2.16
		PRT	-6.333*	1.716	.025	-11.83	-.84
		PST	-1.000	1.716	.935	-6.50	4.50
	PRT	NC	3.000	1.716	.362	-2.50	8.50
		EC	6.333*	1.716	.025	.84	11.83
		PST	5.333	1.716	.057	-.16	10.83
	PST	NC	-2.333	1.716	.555	-7.83	3.16
		EC	1.000	1.716	.935	-4.50	6.50
		PRT	-5.333	1.716	.057	-10.83	.16
Day 1 after IR	NC	EC	-37.667*	2.963	.000	-47.15	-28.18
		PRT	-9.333	2.963	.054	-18.82	.15
		PST	-34.667*	2.963	.000	-44.15	-25.18
	EC	NC	37.667*	2.963	.000	28.18	47.15
		PRT	28.333*	2.963	.000	18.85	37.82
		PST	3.000	2.963	.747	-6.49	12.49
	PRT	NC	9.333	2.963	.054	-.15	18.82
		EC	-28.333*	2.963	.000	-37.82	-18.85
		PST	-25.333*	2.963	.000	-34.82	-15.85
	PST	NC	34.667*	2.963	.000	25.18	44.15
		EC	-3.000	2.963	.747	-12.49	6.49
		PRT	25.333*	2.963	.000	15.85	34.82
Day 8 after IR	NC	EC	-35.000*	3.606	.000	-46.55	-23.45
		PRT	2.000	3.606	.943	-9.55	13.55
		PST	-4.000	3.606	.694	-15.55	7.55
	EC	NC	35.000*	3.606	.000	23.45	46.55
		PRT	37.000*	3.606	.000	25.45	48.55
		PST	31.000*	3.606	.000	19.45	42.55
	PRT	NC	-2.000	3.606	.943	-13.55	9.55
		EC	-37.000*	3.606	.000	-48.55	-25.45
		PST	-6.000	3.606	.399	-17.55	5.55
	PST	NC	4.000	3.606	.694	-7.55	15.55
		EC	-31.000*	3.606	.000	-42.55	-19.45
		PRT	6.000	3.606	.399	-5.55	17.55

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for ALP 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-2.667	1.732	.460	-8.21	2.88
		PRT	6.667*	1.732	.020	1.12	12.21
		PST	6.333*	1.732	.027	.79	11.88
	EC	NC	2.667	1.732	.460	-2.88	8.21
		PRT	9.333*	1.732	.003	3.79	14.88
		PST	9.000*	1.732	.004	3.45	14.55
	PRT	NC	-6.667*	1.732	.020	-12.21	-1.12
		EC	-9.333*	1.732	.003	-14.88	-3.79
		PST	-.333	1.732	.997	-5.88	5.21
	PST	NC	-6.333*	1.732	.027	-11.88	-.79
		EC	-9.000*	1.732	.004	-14.55	-3.45
		PRT	.333	1.732	.997	-5.21	5.88
Day 1 after IR	NC	EC	-37.667*	3.712	.000	-49.55	-25.78
		PRT	-11.000	3.712	.070	-22.89	.89
		PST	-19.667*	3.712	.003	-31.55	-7.78
	EC	NC	37.667*	3.712	.000	25.78	49.55
		PRT	26.667*	3.712	.000	14.78	38.55
		PST	18.000*	3.712	.006	6.11	29.89
	PRT	NC	11.000	3.712	.070	-.89	22.89
		SC	-26.667*	3.712	.000	-38.55	-14.78
		PST	-8.667	3.712	.169	-20.55	3.22
	PST	NC	19.667*	3.712	.003	7.78	31.55
		EC	-18.000*	3.712	.006	-29.89	-6.11
		PRT	8.667	3.712	.169	-3.22	20.55
Day 8 after IR	NC	EC	-41.000*	1.546	.000	-45.95	-36.05
		PRT	-4.333	1.546	.088	-9.28	.62
		PST	-13.000*	1.546	.000	-17.95	-8.05
	EC	NC	41.000*	1.546	.000	36.05	45.95
		PRT	36.667*	1.546	.000	31.72	41.62
		PST	28.000*	1.546	.000	23.05	32.95
	PRT	NC	4.333	1.546	.088	-.62	9.28
		EC	-36.667*	1.546	.000	-41.62	-31.72
		PST	-8.667*	1.546	.002	-13.62	-3.72
	PST	NC	13.000*	1.546	.000	8.05	17.95
		EC	-28.000*	1.546	.000	-32.95	-23.05
		PRT	8.667*	1.546	.002	3.72	13.62

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for ALT 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-.333	3.528	1.000	-11.63	10.96
		PRT	1.667	3.528	.963	-9.63	12.96
		PST	3.333	3.528	.783	-7.96	14.63
	EC	NC	.333	3.528	1.000	-10.96	11.63
		PRT	2.000	3.528	.939	-9.30	13.30
		PST	3.667	3.528	.733	-7.63	14.96
	PRT	NC	-1.667	3.528	.963	-12.96	9.63
		EC	-2.000	3.528	.939	-13.30	9.30
		PST	1.667	3.528	.963	-9.63	12.96
	PST	NC	-3.333	3.528	.783	-14.63	7.96
		EC	-3.667	3.528	.733	-14.96	7.63
		PRT	-1.667	3.528	.963	-12.96	9.63
Day 1 after IR	NC	EC	-7.333	3.779	.285	-19.43	4.77
		PRT	-.667	3.779	.998	-12.77	11.43
		PST	-4.667	3.779	.624	-16.77	7.43
	EC	NC	7.333	3.779	.285	-4.77	19.43
		PRT	6.667	3.779	.355	-5.43	18.77
		PST	2.667	3.779	.892	-9.43	14.77
	PRT	NC	.667	3.779	.998	-11.43	12.77
		SC	-6.667	3.779	.355	-18.77	5.43
		PST	-4.000	3.779	.722	-16.10	8.10
	PST	NC	4.667	3.779	.624	-7.43	16.77
		EC	-2.667	3.779	.892	-14.77	9.43
		PRT	4.000	3.779	.722	-8.10	16.10
Day 8 after IR	NC	EC	-25.000*	2.273	.000	-32.28	-17.72
		PRT	10.000*	2.273	.010	2.72	17.28
		PST	9.000*	2.273	.018	1.72	16.28
	EC	NC	25.000*	2.273	.000	17.72	32.28
		PRT	35.000*	2.273	.000	27.72	42.28
		PST	34.000*	2.273	.000	26.72	41.28
	PRT	NC	-10.000*	2.273	.010	-17.28	-2.72
		EC	-35.000*	2.273	.000	-42.28	-27.72
		PST	-1.000	2.273	.970	-8.28	6.28
	PST	NC	-9.000*	2.273	.018	-16.28	-1.72
		EC	-34.000*	2.273	.000	-41.28	-26.72
		PRT	1.000	2.273	.970	-6.28	8.28

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for ALT 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	0.000	1.546	1.000	-4.95	4.95
		PRT	-1.667	1.546	.711	-6.62	3.28
		PST	-1.667	1.546	.711	-6.62	3.28
	EC	NC	0.000	1.546	1.000	-4.95	4.95
		PRT	-1.667	1.546	.711	-6.62	3.28
		PST	-1.667	1.546	.711	-6.62	3.28
	PRT	NC	1.667	1.546	.711	-3.28	6.62
		EC	1.667	1.546	.711	-3.28	6.62
		PST	0.000	1.546	1.000	-4.95	4.95
	PST	NC	1.667	1.546	.711	-3.28	6.62
		EC	1.667	1.546	.711	-3.28	6.62
		PRT	0.000	1.546	1.000	-4.95	4.95
Day 1 after IR	NC	EC	-9.333*	2.603	.029	-17.67	-1.00
		PRT	-4.667	2.603	.343	-13.00	3.67
		PST	-7.667	2.603	.072	-16.00	.67
	EC	NC	9.333*	2.603	.029	1.00	17.67
		PRT	4.667	2.603	.343	-3.67	13.00
		PST	1.667	2.603	.916	-6.67	10.00
	PRT	NC	4.667	2.603	.343	-3.67	13.00
		EC	-4.667	2.603	.343	-13.00	3.67
		PST	-3.000	2.603	.670	-11.34	5.34
	PST	NC	7.667	2.603	.072	-.67	16.00
		EC	-1.667	2.603	.916	-10.00	6.67
		PRT	3.000	2.603	.670	-5.34	11.34
Day 8 after IR	NC	EC	-13.000*	2.297	.002	-20.36	-5.64
		PRT	5.333	2.297	.172	-2.02	12.69
		PST	-1.667	2.297	.884	-9.02	5.69
	EC	NC	13.000*	2.297	.002	5.64	20.36
		PRT	18.333*	2.297	.000	10.98	25.69
		PST	11.333*	2.297	.005	3.98	18.69
	PRT	NC	-5.333	2.297	.172	-12.69	2.02
		EC	-18.333*	2.297	.000	-25.69	-10.98
		PST	-7.000	2.297	.062	-14.36	.36
	PST	NC	1.667	2.297	.884	-5.69	9.02
		EC	-11.333*	2.297	.005	-18.69	-3.98
		PRT	7.000	2.297	.062	-.36	14.36

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for ALT 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	0.000	1.856	1.000	-5.94	5.94
		PRT	7.667*	1.856	.014	1.72	13.61
		PST	5.667	1.856	.062	-.28	11.61
	EC	NC	0.000	1.856	1.000	-5.94	5.94
		PRT	7.667*	1.856	.014	1.72	13.61
		PST	5.667	1.856	.062	-.28	11.61
	PRT	NC	-7.667*	1.856	.014	-13.61	-1.72
		EC	-7.667*	1.856	.014	-13.61	-1.72
		PST	-2.000	1.856	.712	-7.94	3.94
	PST	NC	-5.667	1.856	.062	-11.61	.28
		EC	-5.667	1.856	.062	-11.61	.28
		PRT	2.000	1.856	.712	-3.94	7.94
Day 1 after IR	NC	EC	-33.000*	2.415	.000	-40.73	-25.27
		PRT	-7.000	2.415	.077	-14.73	.73
		PST	-30.667*	2.415	.000	-38.40	-22.93
	EC	NC	33.000*	2.415	.000	25.27	40.73
		PRT	26.000*	2.415	.000	18.27	33.73
		PST	2.333	2.415	.772	-5.40	10.07
	PRT	NC	7.000	2.415	.077	-.73	14.73
		EC	-26.000*	2.415	.000	-33.73	-18.27
		PST	-23.667*	2.415	.000	-31.40	-15.93
	PST	NC	30.667*	2.415	.000	22.93	38.40
		EC	-2.333	2.415	.772	-10.07	5.40
		PRT	23.667*	2.415	.000	15.93	31.40
Day 8 after IR	NC	EC	-32.667*	5.050	.001	-48.84	-16.50
		PRT	-4.333	5.050	.826	-20.50	11.84
		PST	-8.667	5.050	.376	-24.84	7.50
	EC	NC	32.667*	5.050	.001	16.50	48.84
		PRT	28.333*	5.050	.002	12.16	44.50
		PST	24.000*	5.050	.006	7.83	40.17
	PRT	NC	4.333	5.050	.826	-11.84	20.50
		EC	-28.333*	5.050	.002	-44.50	-12.16
		PST	-4.333	5.050	.826	-20.50	11.84
	PST	NC	8.667	5.050	.376	-7.50	24.84
		EC	-24.000*	5.050	.006	-40.17	-7.83
		PRT	4.333	5.050	.826	-11.84	20.50

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for AST 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	1.000	2.108	.963	-5.75	7.75
		PRT	4.333	2.108	.246	-2.42	11.08
		PST	6.333	2.108	.066	-.42	13.08
	EC	NC	-1.000	2.108	.963	-7.75	5.75
		PRT	3.333	2.108	.439	-3.42	10.08
		PST	5.333	2.108	.129	-1.42	12.08
	PRT	NC	-4.333	2.108	.246	-11.08	2.42
		EC	-3.333	2.108	.439	-10.08	3.42
		PST	2.000	2.108	.781	-4.75	8.75
	PST	NC	-6.333	2.108	.066	-13.08	.42
		EC	-5.333	2.108	.129	-12.08	1.42
		PRT	-2.000	2.108	.781	-8.75	4.75
Day 1 after IR	NC	EC	-27.000*	2.667	.000	-35.54	-18.46
		PRT	4.333	2.667	.418	-4.21	12.87
		PST	-24.333*	2.667	.000	-32.87	-15.79
	EC	NC	27.000*	2.667	.000	18.46	35.54
		PRT	31.333*	2.667	.000	22.79	39.87
		PST	2.667	2.667	.754	-5.87	11.21
	PRT	NC	-4.333	2.667	.418	-12.87	4.21
		EC	-31.333*	2.667	.000	-39.87	-22.79
		PST	-28.667*	2.667	.000	-37.21	-20.13
	PST	NC	24.333*	2.667	.000	15.79	32.87
		SC	-2.667	2.667	.754	-11.21	5.87
		PRT	28.667*	2.667	.000	20.13	37.21
Day 8 after IR	NC	EC	-30.000*	1.929	.000	-36.18	-23.82
		PRT	3.000	1.929	.452	-3.18	9.18
		PST	-12.333*	1.929	.001	-18.51	-6.16
	EC	NC	30.000*	1.929	.000	23.82	36.18
		PRT	33.000*	1.929	.000	26.82	39.18
		PST	17.667*	1.929	.000	11.49	23.84
	PRT	NC	-3.000	1.929	.452	-9.18	3.18
		EC	-33.000*	1.929	.000	-39.18	-26.82
		PST	-15.333*	1.929	.000	-21.51	-9.16
	PST	NC	12.333*	1.929	.001	6.16	18.51
		EC	-17.667*	1.929	.000	-23.84	-11.49
		PRT	15.333*	1.929	.000	9.16	21.51

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for AST 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-.333	2.896	.999	-9.61	8.94
		PRT	-.333	2.896	.999	-9.61	8.94
		PST	-.333	2.896	.999	-9.61	8.94
	EC	NC	.333	2.896	.999	-8.94	9.61
		PRT	0.000	2.896	1.000	-9.28	9.28
		PST	0.000	2.896	1.000	-9.28	9.28
	PRT	NC	.333	2.896	.999	-8.94	9.61
		EC	0.000	2.896	1.000	-9.28	9.28
		PST	0.000	2.896	1.000	-9.28	9.28
	PST	NC	.333	2.896	.999	-8.94	9.61
		EC	0.000	2.896	1.000	-9.28	9.28
		PRT	0.000	2.896	1.000	-9.28	9.28
Day 1 after IR	NC	EC	-23.667*	4.223	.002	-37.19	-10.14
		PRT	-5.667	4.223	.565	-19.19	7.86
		PST	-22.333*	4.223	.003	-35.86	-8.81
	EC	NC	23.667*	4.223	.002	10.14	37.19
		PRT	18.000*	4.223	.012	4.48	31.52
		PST	1.333	4.223	.988	-12.19	14.86
	PRT	NC	5.667	4.223	.565	-7.86	19.19
		EC	-18.000*	4.223	.012	-31.52	-4.48
		PST	-16.667*	4.223	.018	-30.19	-3.14
	PST	NC	22.333*	4.223	.003	8.81	35.86
		EC	-1.333	4.223	.988	-14.86	12.19
		PRT	16.667*	4.223	.018	3.14	30.19
Day 8 after IR	NC	EC	-30.000*	2.321	.000	-37.43	-22.57
		PRT	-.667	2.321	.991	-8.10	6.77
		PST	-11.000*	2.321	.006	-18.43	-3.57
	EC	NC	30.000*	2.321	.000	22.57	37.43
		PRT	29.333*	2.321	.000	21.90	36.77
		PST	19.000*	2.321	.000	11.57	26.43
	PRT	NC	.667	2.321	.991	-6.77	8.10
		EC	-29.333*	2.321	.000	-36.77	-21.90
		PST	-10.333*	2.321	.009	-17.77	-2.90
	PST	NC	11.000*	2.321	.006	3.57	18.43
		EC	-19.000*	2.321	.000	-26.43	-11.57
		PRT	10.333*	2.321	.009	2.90	17.77

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for AST 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-1.667	4.035	.975	-14.59	11.25
		PRT	4.333	4.035	.714	-8.59	17.25
		PST	6.000	4.035	.487	-6.92	18.92
	EC	NC	1.667	4.035	.975	-11.25	14.59
		PRT	6.000	4.035	.487	-6.92	18.92
		PST	7.667	4.035	.300	-5.25	20.59
	PRT	NC	-4.333	4.035	.714	-17.25	8.59
		EC	-6.000	4.035	.487	-18.92	6.92
		PST	1.667	4.035	.975	-11.25	14.59
	PST	NC	-6.000	4.035	.487	-18.92	6.92
		EC	-7.667	4.035	.300	-20.59	5.25
		PRT	-1.667	4.035	.975	-14.59	11.25
Day 1 after IR	NC	EC	-19.667*	2.483	.000	-27.62	-11.71
		PRT	-9.333*	2.483	.023	-17.29	-1.38
		PST	-21.333*	2.483	.000	-29.29	-13.38
	EC	NC	19.667*	2.483	.000	11.71	27.62
		PRT	10.333*	2.483	.013	2.38	18.29
		PST	-1.667	2.483	.905	-9.62	6.29
	PRT	NC	9.333*	2.483	.023	1.38	17.29
		EC	-10.333*	2.483	.013	-18.29	-2.38
		PST	-12.000*	2.483	.006	-19.95	-4.05
	PST	NC	21.333*	2.483	.000	13.38	29.29
		EC	1.667	2.483	.905	-6.29	9.62
		PRT	12.000*	2.483	.006	4.05	19.95
Day 8 after IR	NC	EC	-30.000*	2.134	.000	-36.84	-23.16
		PRT	-2.333	2.134	.703	-9.17	4.50
		PST	-11.000*	2.134	.004	-17.84	-4.16
	EC	NC	30.000*	2.134	.000	23.16	36.84
		PRT	27.667*	2.134	.000	20.83	34.50
		PST	19.000*	2.134	.000	12.16	25.84
	PRT	NC	2.333	2.134	.703	-4.50	9.17
		EC	-27.667*	2.134	.000	-34.50	-20.83
		PST	-8.667*	2.134	.015	-15.50	-1.83
	PST	NC	11.000*	2.134	.004	4.16	17.84
		EC	-19.000*	2.134	.000	-25.84	-12.16
		PRT	8.667*	2.134	.015	1.83	15.50

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for MDA 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-2.000	1.599	.615	-7.12	3.12
		PRT	.333	1.599	.997	-4.79	5.45
		PST	1.000	1.599	.921	-4.12	6.12
	EC	NC	2.000	1.599	.615	-3.12	7.12
		PRT	2.333	1.599	.501	-2.79	7.45
		PST	3.000	1.599	.309	-2.12	8.12
	PRT	NC	-.333	1.599	.997	-5.45	4.79
		EC	-2.333	1.599	.501	-7.45	2.79
		PST	.667	1.599	.974	-4.45	5.79
	PST	NC	-1.000	1.599	.921	-6.12	4.12
		EC	-3.000	1.599	.309	-8.12	2.12
		PRT	-.667	1.599	.974	-5.79	4.45
Day 1 after IR	NC	EC	-24.000*	1.972	.000	-30.32	-17.68
		PRT	-11.667*	1.972	.002	-17.98	-5.35
		PST	-30.000*	1.972	.000	-36.32	-23.68
	EC	NC	24.000*	1.972	.000	17.68	30.32
		PRT	12.333*	1.972	.001	6.02	18.65
		PST	-6.000	1.972	.063	-12.32	.32
	PRT	NC	11.667*	1.972	.002	5.35	17.98
		EC	-12.333*	1.972	.001	-18.65	-6.02
		PST	-18.333*	1.972	.000	-24.65	-12.02
	PST	NC	30.000*	1.972	.000	23.68	36.32
		EC	6.000	1.972	.063	-.32	12.32
		PRT	18.333*	1.972	.000	12.02	24.65
Day 8 after IR	NC	EC	-31.000*	4.859	.001	-46.56	-15.44
		PRT	-3.667	4.859	.872	-19.23	11.89
		PST	-23.667*	4.859	.005	-39.23	-8.11
	EC	NC	31.000*	4.859	.001	15.44	46.56
		PRT	27.333*	4.859	.002	11.77	42.89
		PST	7.333	4.859	.475	-8.23	22.89
	PRT	NC	3.667	4.859	.872	-11.89	19.23
		EC	-27.333*	4.859	.002	-42.89	-11.77
		PST	-20.000*	4.859	.014	-35.56	-4.44
	PST	NC	23.667*	4.859	.005	8.11	39.23
		EC	-7.333	4.859	.475	-22.89	8.23
		PRT	20.000*	4.859	.014	4.44	35.56

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for MDA 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-1.667	1.130	.493	-5.29	1.95
		PRT	-4.667*	1.130	.014	-8.29	-1.05
		PST	-1.000	1.130	.813	-4.62	2.62
	EC	NC	1.667	1.130	.493	-1.95	5.29
		PRT	-3.000	1.130	.108	-6.62	.62
		PST	.667	1.130	.932	-2.95	4.29
	PRT	NC	4.667*	1.130	.014	1.05	8.29
		EC	3.000	1.130	.108	-.62	6.62
		PST	3.667*	1.130	.047	.05	7.29
	PST	NC	1.000	1.130	.813	-2.62	4.62
		EC	-.667	1.130	.932	-4.29	2.95
		PRT	-3.667*	1.130	.047	-7.29	-.05
Day 1 after IR	NC	EC	-42.667*	2.789	.000	-51.60	-33.74
		PRT	-26.667*	2.789	.000	-35.60	-17.74
		PST	-40.000*	2.789	.000	-48.93	-31.07
	EC	NC	42.667*	2.789	.000	33.74	51.60
		PRT	16.000*	2.789	.002	7.07	24.93
		PST	2.667	2.789	.777	-6.26	11.60
	PRT	NC	26.667*	2.789	.000	17.74	35.60
		EC	-16.000*	2.789	.002	-24.93	-7.07
		PST	-13.333*	2.789	.006	-22.26	-4.40
	PST	NC	40.000*	2.789	.000	31.07	48.93
		EC	-2.667	2.789	.777	-11.60	6.26
		PRT	13.333*	2.789	.006	4.40	22.26
Day 8 after IR	NC	EC	-46.667*	6.355	.000	-67.02	-26.31
		PRT	-8.667	6.355	.553	-29.02	11.69
		PST	-11.667	6.355	.325	-32.02	8.69
	EC	NC	46.667*	6.355	.000	26.31	67.02
		PRT	38.000*	6.355	.001	17.65	58.35
		PST	35.000*	6.355	.003	14.65	55.35
	PRT	NC	8.667	6.355	.553	-11.69	29.02
		EC	-38.000*	6.355	.001	-58.35	-17.65
		PST	-3.000	6.355	.963	-23.35	17.35
	PST	NC	11.667	6.355	.325	-8.69	32.02
		EC	-35.000*	6.355	.003	-55.35	-14.65
		PRT	3.000	6.355	.963	-17.35	23.35

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for MDA 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	-4.333	1.491	.076	-9.11	.44
		PRT	-2.000	1.491	.565	-6.77	2.77
		PST	4.000	1.491	.104	-.77	8.77
	EC	NC	4.333	1.491	.076	-.44	9.11
		PRT	2.333	1.491	.447	-2.44	7.11
		PST	8.333*	1.491	.002	3.56	13.11
	PRT	NC	2.000	1.491	.565	-2.77	6.77
		EC	-2.333	1.491	.447	-7.11	2.44
		PST	6.000*	1.491	.016	1.23	10.77
	PST	NC	-4.000	1.491	.104	-8.77	.77
		EC	-8.333*	1.491	.002	-13.11	-3.56
		PRT	-6.000*	1.491	.016	-10.77	-1.23
Day 1 after IR	NC	EC	-26.667*	2.828	.000	-35.72	-17.61
		PRT	-1.667	2.828	.933	-10.72	7.39
		PST	-27.333*	2.828	.000	-36.39	-18.28
	EC	NC	26.667*	2.828	.000	17.61	35.72
		PRT	25.000*	2.828	.000	15.94	34.06
		PST	-.667	2.828	.995	-9.72	8.39
	PRT	NC	1.667	2.828	.933	-7.39	10.72
		EC	-25.000*	2.828	.000	-34.06	-15.94
		PST	-25.667*	2.828	.000	-34.72	-16.61
	PST	NC	27.333*	2.828	.000	18.28	36.39
		EC	.667	2.828	.995	-8.39	9.72
		PRT	25.667*	2.828	.000	16.61	34.72
Day 8 after IR	NC	EC	-34.000*	3.127	.000	-44.01	-23.99
		PRT	1.667	3.127	.949	-8.35	11.68
		PST	-22.333*	3.127	.000	-32.35	-12.32
	EC	NC	34.000*	3.127	.000	23.99	44.01
		PRT	35.667*	3.127	.000	25.65	45.68
		PST	11.667*	3.127	.024	1.65	21.68
	PRT	NC	-1.667	3.127	.949	-11.68	8.35
		EC	-35.667*	3.127	.000	-45.68	-25.65
		PST	-24.000*	3.127	.000	-34.01	-13.99
	PST	NC	22.333*	3.127	.000	12.32	32.35
		EC	-11.667*	3.127	.024	-21.68	-1.65
		PRT	24.000*	3.127	.000	13.99	34.01

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for CAT 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	2.333	1.856	.612	-3.61	8.28
		PRT	0.000	1.856	1.000	-5.94	5.94
		PST	2.333	1.856	.612	-3.61	8.28
	EC	NC	-2.333	1.856	.612	-8.28	3.61
		PRT	-2.333	1.856	.612	-8.28	3.61
		PST	0.000	1.856	1.000	-5.94	5.94
	PRT	NC	0.000	1.856	1.000	-5.94	5.94
		EC	2.333	1.856	.612	-3.61	8.28
		PST	2.333	1.856	.612	-3.61	8.28
	PST	NC	-2.333	1.856	.612	-8.28	3.61
		EC	0.000	1.856	1.000	-5.94	5.94
		PRT	-2.333	1.856	.612	-8.28	3.61
Day 1 after IR	NC	EC	40.333*	2.309	.000	32.94	47.73
		PRT	21.333*	2.309	.000	13.94	28.73
		PST	42.667*	2.309	.000	35.27	50.06
	EC	NC	-40.333*	2.309	.000	-47.73	-32.94
		PRT	-19.000*	2.309	.000	-26.40	-11.60
		PST	2.333	2.309	.748	-5.06	9.73
	PRT	NC	-21.333*	2.309	.000	-28.73	-13.94
		EC	19.000*	2.309	.000	11.60	26.40
		PST	21.333*	2.309	.000	13.94	28.73
	PST	NC	-42.667*	2.309	.000	-50.06	-35.27
		EC	-2.333	2.309	.748	-9.73	5.06
		PRT	-21.333*	2.309	.000	-28.73	-13.94
Day 8 after IR	NC	EC	39.333*	2.728	.000	30.60	48.07
		PRT	28.000*	2.728	.000	19.26	36.74
		PST	26.333*	2.728	.000	17.60	35.07
	EC	NC	-39.333*	2.728	.000	-48.07	-30.60
		PRT	-11.333*	2.728	.014	-20.07	-2.60
		PST	-13.000*	2.728	.006	-21.74	-4.26
	PRT	NC	-28.000*	2.728	.000	-36.74	-19.26
		EC	11.333*	2.728	.014	2.60	20.07
		PST	-1.667	2.728	.926	-10.40	7.07
	PST	NC	-26.333*	2.728	.000	-35.07	-17.60
		EC	13.000*	2.728	.006	4.26	21.74
		PRT	1.667	2.728	.926	-7.07	10.40

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for CAT 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	5.333	2.848	.310	-3.79	14.45
		PRT	4.333	2.848	.469	-4.79	13.45
		PST	3.000	2.848	.725	-6.12	12.12
	EC	NC	-5.333	2.848	.310	-14.45	3.79
		PRT	-1.000	2.848	.984	-10.12	8.12
		PST	-2.333	2.848	.844	-11.45	6.79
	PRT	NC	-4.333	2.848	.469	-13.45	4.79
		EC	1.000	2.848	.984	-8.12	10.12
		PST	-1.333	2.848	.964	-10.45	7.79
	PST	NC	-3.000	2.848	.725	-12.12	6.12
		EC	2.333	2.848	.844	-6.79	11.45
		PRT	1.333	2.848	.964	-7.79	10.45
Day 1 after IR	NC	EC	51.000*	3.432	.000	40.01	61.99
		PRT	34.333*	3.432	.000	23.34	45.32
		PST	46.333*	3.432	.000	35.34	57.32
	EC	NC	-51.000*	3.432	.000	-61.99	-40.01
		PRT	-16.667*	3.432	.006	-27.66	-5.68
		PST	-4.667	3.432	.555	-15.66	6.32
	PRT	NC	-34.333*	3.432	.000	-45.32	-23.34
		EC	16.667*	3.432	.006	5.68	27.66
		PST	12.000*	3.432	.033	1.01	22.99
	PST	NC	-46.333*	3.432	.000	-57.32	-35.34
		EC	4.667	3.432	.555	-6.32	15.66
		PRT	-12.000*	3.432	.033	-22.99	-1.01
Day 8 after IR	NC	EC	48.000*	4.836	.000	32.51	63.49
		PRT	29.333*	4.836	.001	13.85	44.82
		PST	35.000*	4.836	.000	19.51	50.49
	EC	NC	-48.000*	4.836	.000	-63.49	-32.51
		PRT	-18.667*	4.836	.020	-34.15	-3.18
		PST	-13.000	4.836	.103	-28.49	2.49
	PRT	NC	-29.333*	4.836	.001	-44.82	-13.85
		EC	18.667*	4.836	.020	3.18	34.15
		PST	5.667	4.836	.659	-9.82	21.15
	PST	NC	-35.000*	4.836	.000	-50.49	-19.51
		EC	13.000	4.836	.103	-2.49	28.49
		PRT	-5.667	4.836	.659	-21.15	9.82

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for CAT 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	4.667	5.233	.809	-12.09	21.43
		PRT	3.000	5.233	.937	-13.76	19.76
		PST	4.000	5.233	.868	-12.76	20.76
	EC	NC	-4.667	5.233	.809	-21.43	12.09
		PRT	-1.667	5.233	.988	-18.43	15.09
		PST	-.667	5.233	.999	-17.43	16.09
	PRT	NC	-3.000	5.233	.937	-19.76	13.76
		EC	1.667	5.233	.988	-15.09	18.43
		PST	1.000	5.233	.997	-15.76	17.76
	PST	NC	-4.000	5.233	.868	-20.76	12.76
		EC	.667	5.233	.999	-16.09	17.43
		PRT	-1.000	5.233	.997	-17.76	15.76
Day 1 after IR	NC	EC	42.333*	2.667	.000	33.79	50.87
		PRT	31.667*	2.667	.000	23.13	40.21
		PST	44.000*	2.667	.000	35.46	52.54
	EC	NC	-42.333*	2.667	.000	-50.87	-33.79
		PRT	-10.667*	2.667	.017	-19.21	-2.13
		PST	1.667	2.667	.921	-6.87	10.21
	PRT	NC	-31.667*	2.667	.000	-40.21	-23.13
		EC	10.667*	2.667	.017	2.13	19.21
		PST	12.333*	2.667	.007	3.79	20.87
	PST	NC	-44.000*	2.667	.000	-52.54	-35.46
		EC	-1.667	2.667	.921	-10.21	6.87
		PRT	-12.333*	2.667	.007	-20.87	-3.79
Day 8 after IR	NC	EC	40.667*	4.927	.000	24.89	56.45
		PRT	18.667*	4.927	.022	2.89	34.45
		PST	38.000*	4.927	.000	22.22	53.78
	EC	NC	-40.667*	4.927	.000	-56.45	-24.89
		PRT	-22.000*	4.927	.009	-37.78	-6.22
		PST	-2.667	4.927	.946	-18.45	13.11
	PRT	NC	-18.667*	4.927	.022	-34.45	-2.89
		EC	22.000*	4.927	.009	6.22	37.78
		PST	19.333*	4.927	.018	3.55	35.11
	PST	NC	-38.000*	4.927	.000	-53.78	-22.22
		EC	2.667	4.927	.946	-13.11	18.45
		PRT	-19.333*	4.927	.018	-35.11	-3.55

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for GSH 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	2.667	1.972	.559	-3.65	8.98
		PRT	3.000	1.972	.469	-3.32	9.32
		PST	-1.000	1.972	.955	-7.32	5.32
	EC	NC	-2.667	1.972	.559	-8.98	3.65
		PRT	.333	1.972	.998	-5.98	6.65
		PST	-3.667	1.972	.316	-9.98	2.65
	PRT	NC	-3.000	1.972	.469	-9.32	3.32
		EC	-.333	1.972	.998	-6.65	5.98
		PST	-4.000	1.972	.255	-10.32	2.32
	PST	NC	1.000	1.972	.955	-5.32	7.32
		EC	3.667	1.972	.316	-2.65	9.98
		PRT	4.000	1.972	.255	-2.32	10.32
Day 1 after IR	NC	EC	35.000*	2.285	.000	27.68	42.32
		PRT	20.333*	2.285	.000	13.02	27.65
		PST	37.000*	2.285	.000	29.68	44.32
	EC	NC	-35.000*	2.285	.000	-42.32	-27.68
		PRT	-14.667*	2.285	.001	-21.98	-7.35
		PST	2.000	2.285	.818	-5.32	9.32
	PRT	NC	-20.333*	2.285	.000	-27.65	-13.02
		EC	14.667*	2.285	.001	7.35	21.98
		PST	16.667*	2.285	.000	9.35	23.98
	PST	NC	-37.000*	2.285	.000	-44.32	-29.68
		EC	-2.000	2.285	.818	-9.32	5.32
		PRT	-16.667*	2.285	.000	-23.98	-9.35
Day 8 after IR	NC	EC	42.333*	5.608	.000	24.38	60.29
		PRT	9.333	5.608	.399	-8.62	27.29
		PST	23.000*	5.608	.015	5.04	40.96
	EC	NC	-42.333*	5.608	.000	-60.29	-24.38
		PRT	-33.000*	5.608	.002	-50.96	-15.04
		PST	-19.333*	5.608	.035	-37.29	-1.38
	PRT	NC	-9.333	5.608	.399	-27.29	8.62
		EC	33.000*	5.608	.002	15.04	50.96
		PST	13.667	5.608	.147	-4.29	31.62
	PST	NC	-23.000*	5.608	.015	-40.96	-5.04
		EC	19.333*	5.608	.035	1.38	37.29
		PRT	-13.667	5.608	.147	-31.62	4.29

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for GSH 4Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	1.333	1.599	.837	-3.79	6.45
		PRT	7.333*	1.599	.008	2.21	12.45
		PST	4.333	1.599	.100	-.79	9.45
	EC	NC	-1.333	1.599	.837	-6.45	3.79
		PRT	6.000*	1.599	.023	.88	11.12
		PST	3.000	1.599	.309	-2.12	8.12
	PRT	NC	-7.333*	1.599	.008	-12.45	-2.21
		SC	-6.000*	1.599	.023	-11.12	-.88
		PST	-3.000	1.599	.309	-8.12	2.12
	PST	NC	-4.333	1.599	.100	-9.45	.79
		SC	-3.000	1.599	.309	-8.12	2.12
		PRT	3.000	1.599	.309	-2.12	8.12
Day 1 after IR	NC	SC	36.333*	2.582	.000	28.06	44.60
		PRT	13.000*	2.582	.004	4.73	21.27
		PST	37.667*	2.582	.000	29.40	45.94
	EC	NC	-36.333*	2.582	.000	-44.60	-28.06
		PRT	-23.333*	2.582	.000	-31.60	-15.06
		PST	1.333	2.582	.953	-6.94	9.60
	PRT	NC	-13.000*	2.582	.004	-21.27	-4.73
		SC	23.333*	2.582	.000	15.06	31.60
		PST	24.667*	2.582	.000	16.40	32.94
	PST	NC	-37.667*	2.582	.000	-45.94	-29.40
		SC	-1.333	2.582	.953	-9.60	6.94
		PRT	-24.667*	2.582	.000	-32.94	-16.40
Day 8 after IR	NC	SC	39.333*	2.309	.000	31.94	46.73
		PRT	9.333*	2.309	.016	1.94	16.73
		PST	24.333*	2.309	.000	16.94	31.73
	EC	NC	-39.333*	2.309	.000	-46.73	-31.94
		PRT	-30.000*	2.309	.000	-37.40	-22.60
		PST	-15.000*	2.309	.001	-22.40	-7.60
	PRT	NC	-9.333*	2.309	.016	-16.73	-1.94
		SC	30.000*	2.309	.000	22.60	37.40
		PST	15.000*	2.309	.001	7.60	22.40
	PST	NC	-24.333*	2.309	.000	-31.73	-16.94
		SC	15.000*	2.309	.001	7.60	22.40
		PRT	-15.000*	2.309	.001	-22.40	-7.60

Multiple Comparison for GSH 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	4.333	1.563	.092	-.67	9.34
		PRT	1.333	1.563	.828	-3.67	6.34
		PST	-4.333	1.563	.092	-9.34	.67
	EC	NC	-4.333	1.563	.092	-9.34	.67
		PRT	-3.000	1.563	.293	-8.01	2.01
		PST	-8.667*	1.563	.002	-13.67	-3.66
	PRT	NC	-1.333	1.563	.828	-6.34	3.67
		EC	3.000	1.563	.293	-2.01	8.01
		PST	-5.667*	1.563	.028	-10.67	-.66
	PST	NC	4.333	1.563	.092	-.67	9.34
		EC	8.667*	1.563	.002	3.66	13.67
		PRT	5.667*	1.563	.028	.66	10.67
Day 1 after IR	NC	EC	29.333*	3.249	.000	18.93	39.74
		PRT	12.000*	3.249	.025	1.60	22.40
		PST	28.333*	3.249	.000	17.93	38.74
	EC	NC	-29.333*	3.249	.000	-39.74	-18.93
		PRT	-17.333*	3.249	.003	-27.74	-6.93
		PST	-1.000	3.249	.989	-11.40	9.40
	PRT	NC	-12.000*	3.249	.025	-22.40	-1.60
		EC	17.333*	3.249	.003	6.93	27.74
		PST	16.333*	3.249	.004	5.93	26.74
	PST	NC	-28.333*	3.249	.000	-38.74	-17.93
		EC	1.000	3.249	.989	-9.40	11.40
		PRT	-16.333*	3.249	.004	-26.74	-5.93
Day 8 after IR	NC	EC	33.333*	3.480	.000	22.19	44.48
		PRT	9.000	3.480	.119	-2.14	20.14
		PST	26.667*	3.480	.000	15.52	37.81
	EC	NC	-33.333*	3.480	.000	-44.48	-22.19
		PRT	-24.333*	3.480	.001	-35.48	-13.19
		PST	-6.667	3.480	.294	-17.81	4.48
	PRT	NC	-9.000	3.480	.119	-20.14	2.14
		EC	24.333*	3.480	.001	13.19	35.48
		PST	17.667*	3.480	.004	6.52	28.81
	PST	NC	-26.667*	3.480	.000	-37.81	-15.52
		EC	6.667	3.480	.294	-4.48	17.81
		PRT	-17.667*	3.480	.004	-28.81	-6.52

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for SOD 2Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	3.333	3.944	.832	-9.30	15.96
		PRT	2.000	3.944	.955	-10.63	14.63
		PST	-4.000	3.944	.746	-16.63	8.63
	EC	NC	-3.333	3.944	.832	-15.96	9.30
		PRT	-1.333	3.944	.986	-13.96	11.30
		PST	-7.333	3.944	.316	-19.96	5.30
	PRT	NC	-2.000	3.944	.955	-14.63	10.63
		EC	1.333	3.944	.986	-11.30	13.96
		PST	-6.000	3.944	.469	-18.63	6.63
	PST	NC	4.000	3.944	.746	-8.63	16.63
		EC	7.333	3.944	.316	-5.30	19.96
		PRT	6.000	3.944	.469	-6.63	18.63
Day 1 after IR	NC	EC	44.667*	4.282	.000	30.96	58.38
		PRT	23.000*	4.282	.003	9.29	36.71
		PST	43.333*	4.282	.000	29.62	57.04
	EC	NC	-44.667*	4.282	.000	-58.38	-30.96
		PRT	-21.667*	4.282	.004	-35.38	-7.96
		PST	-1.333	4.282	.989	-15.04	12.38
	PRT	NC	-23.000*	4.282	.003	-36.71	-9.29
		EC	21.667*	4.282	.004	7.96	35.38
		PST	20.333*	4.282	.006	6.62	34.04
	PST	NC	-43.333*	4.282	.000	-57.04	-29.62
		EC	1.333	4.282	.989	-12.38	15.04
		PRT	-20.333*	4.282	.006	-34.04	-6.62
Day 8 after IR	NC	EC	49.000*	3.682	.000	37.21	60.79
		PRT	39.333*	3.682	.000	27.54	51.12
		PST	18.000*	3.682	.005	6.21	29.79
	EC	NC	-49.000*	3.682	.000	-60.79	-37.21
		PRT	-9.667	3.682	.113	-21.46	2.12
		PST	-31.000*	3.682	.000	-42.79	-19.21
	PRT	NC	-39.333*	3.682	.000	-51.12	-27.54
		EC	9.667	3.682	.113	-2.12	21.46
		PST	-21.333*	3.682	.002	-33.12	-9.54
	PST	NC	-18.000*	3.682	.005	-29.79	-6.21
		EC	31.000*	3.682	.000	19.21	42.79
		PRT	21.333*	3.682	.002	9.54	33.12

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for SOD 4Gy

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	3.667	2.614	.531	-4.70	12.04
		PRT	12.333*	2.614	.007	3.96	20.70
		PST	1.667	2.614	.917	-6.70	10.04
	EC	NC	-3.667	2.614	.531	-12.04	4.70
		PRT	8.667*	2.614	.043	.30	17.04
		PST	-2.000	2.614	.868	-10.37	6.37
	PRT	NC	-12.333*	2.614	.007	-20.70	-3.96
		EC	-8.667*	2.614	.043	-17.04	-.30
		PST	-10.667*	2.614	.015	-19.04	-2.30
	PST	NC	-1.667	2.614	.917	-10.04	6.70
		EC	2.000	2.614	.868	-6.37	10.37
		PRT	10.667*	2.614	.015	2.30	19.04
Day 1 after IR	NC	EC	48.333*	3.416	.000	37.40	59.27
		PRT	33.667*	3.416	.000	22.73	44.60
		PST	45.000*	3.416	.000	34.06	55.94
	EC	NC	-48.333*	3.416	.000	-59.27	-37.40
		PRT	-14.667*	3.416	.011	-25.60	-3.73
		PST	-3.333	3.416	.767	-14.27	7.60
	PRT	NC	-33.667*	3.416	.000	-44.60	-22.73
		EC	14.667*	3.416	.011	3.73	25.60
		PST	11.333*	3.416	.043	.40	22.27
	PST	NC	-45.000*	3.416	.000	-55.94	-34.06
		EC	3.333	3.416	.767	-7.60	14.27
		PRT	-11.333*	3.416	.043	-22.27	-.40
Day 8 after IR	NC	EC	49.667*	3.464	.000	38.57	60.76
		PRT	28.333*	3.464	.000	17.24	39.43
		PST	38.333*	3.464	.000	27.24	49.43
	EC	NC	-49.667*	3.464	.000	-60.76	-38.57
		PRT	-21.333*	3.464	.001	-32.43	-10.24
		PST	-11.333*	3.464	.045	-22.43	-.24
	PRT	NC	-28.333*	3.464	.000	-39.43	-17.24
		EC	21.333*	3.464	.001	10.24	32.43
		PST	10.000	3.464	.078	-1.09	21.09
	PST	NC	-38.333*	3.464	.000	-49.43	-27.24
		EC	11.333*	3.464	.045	.24	22.43
		PRT	-10.000	3.464	.078	-21.09	1.09

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons for SOD 6Gy

Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Before IR	NC	EC	6.000	3.145	.297	-4.07	16.07
		PRT	7.333	3.145	.170	-2.74	17.40
		PST	1.000	3.145	.988	-9.07	11.07
	EC	NC	-6.000	3.145	.297	-16.07	4.07
		PRT	1.333	3.145	.973	-8.74	11.40
		PST	-5.000	3.145	.435	-15.07	5.07
	PRT	NC	-7.333	3.145	.170	-17.40	2.74
		EC	-1.333	3.145	.973	-11.40	8.74
		PST	-6.333	3.145	.259	-16.40	3.74
	PST	NC	-1.000	3.145	.988	-11.07	9.07
		EC	5.000	3.145	.435	-5.07	15.07
		PRT	6.333	3.145	.259	-3.74	16.40
Day 1 after IR	NC	EC	51.000*	3.697	.000	39.16	62.84
		PRT	27.000*	3.697	.000	15.16	38.84
		PST	50.000*	3.697	.000	38.16	61.84
	EC	NC	-51.000*	3.697	.000	-62.84	-39.16
		PRT	-24.000*	3.697	.001	-35.84	-12.16
		PST	-1.000	3.697	.993	-12.84	10.84
	PRT	NC	-27.000*	3.697	.000	-38.84	-15.16
		EC	24.000*	3.697	.001	12.16	35.84
		PST	23.000*	3.697	.001	11.16	34.84
	PST	NC	-50.000*	3.697	.000	-61.84	-38.16
		EC	1.000	3.697	.993	-10.84	12.84
		PRT	-23.000*	3.697	.001	-34.84	-11.16
Day 8 after IR	NC	EC	45.000*	3.308	.000	34.41	55.59
		PRT	15.333*	3.308	.007	4.74	25.93
		PST	28.667*	3.308	.000	18.07	39.26
	EC	NC	-45.000*	3.308	.000	-55.59	-34.41
		PRT	-29.667*	3.308	.000	-40.26	-19.07
		PST	-16.333*	3.308	.005	-26.93	-5.74
	PRT	NC	-15.333*	3.308	.007	-25.93	-4.74
		EC	29.667*	3.308	.000	19.07	40.26
		PST	13.333*	3.308	.016	2.74	23.93
	PST	NC	-28.667*	3.308	.000	-39.26	-18.07
		EC	16.333*	3.308	.005	5.74	26.93
		PRT	-13.333*	3.308	.016	-23.93	-2.74

*. The mean difference is significant at the 0.05 level.

APPENDIX D:Preparation of chemicals and Reagents for phytochemical analysis

Preparation of chemicals and reagents were done using standard procedures AOAC (1970).

Preparation of 5% (w/v) Ferric chloride solution

5.0 g of ferric chloride was dissolved in 100 ml of distilled water.

Preparation of Ammonium solution

187.5 ml of the stock concentrated ammonium solution was diluted in 31.25 ml of distilled water and then made up to 500 ml with distilled water.

Preparation of 45% (v/v) ethanol

45 ml of absolute ethanol was mixed with 55 ml of distilled water.

Preparation ofAluminium chloride solution

0.5 g of aluminium chloride was dissolved in 100 ml of distilled water.

Preparation ofDilute sulphuric acid

10.9 ml of concentrated sulphuric acid was mixed with 5.0 ml of distilled water and made up to 100 ml.

Preparation of Lead sub-acetate solution

45 ml of 15 % lead acetate (i.e. 15.0 g of lead acetate in 100 ml of distilled water) was dissolved in 20 ml of absolute ethanol and made up to 100 ml with distilled water.

Preparation of Wagner's reagent

2.0 g of iodine crystals and 3.0 g of potassium iodide were dissolved in 40 ml of distilled water and then made up to 100 ml (with distilled water).

Preparation of Mayer's reagent

13.5 g of mercuric chloride was dissolved in 50 ml of distilled water. Also, 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. The two solutions were mixed and the volume made up to 100 ml with distilled water.

Preparation of Dragendorff's reagent

0.85 g of bismuth carbonate was dissolved in 100 ml of glacial acetic acid and 40 ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water. Both solutions were mixed to give a stock solution.

Preparation of 2% (v/v) Hydrochloric acid

2.0 ml of concentrated hydrochloric acid was diluted with some distilled water and made up to 100 ml.

Preparation of 1% (w/v) Picric acid

1.0 g of picric acid was dissolved in 100 ml of distilled water.

Preparation of 0.1% Ferric chloride (w/v)

0.1 g of ferric chloride was dissolved in 100 ml of distilled water.

Preparation of 40% (w/v) Magnesium carbonate

40 g of Magnesium carbonate was dissolved with some distilled water and made up to 100 ml with distilled water.

Preparation of NaOH solution

8.0 g of NaOH pellets were dissolved with distilled water and made up to 100 ml.

Preparation of 50% (v/v) Methanol

50 ml of absolute methanol was mixed with 50 ml of distilled water.

Preparation of 1% (w/v) Aluminium chloride

1 g of Aluminium chloride was dissolved in 100 ml of distilled water.

Preparation of 60% (v/v) H_2SO_4

60 ml of conc. H_2SO_4 was mixed with 40 ml of distilled water.

Preparation of 20% (v/v) H_2SO_4

20 ml of conc. H_2SO_4 was mixed with 80 ml of distilled water.

Preparation of 10% (v/v) acetic acid

10 ml of acetic acid was mixed with 90 ml of distilled water.

Preparation of 2% (w/v) Aluminium chloride

2 g was dissolved with distilled water and made up to 100 ml.

Preparation of 17% (w/v) Na_2CO_3

17 g of Na_2CO_3 was dissolved with distilled water and made up to 100 ml.

Preparation of 75% (w/v) Na_2CO_3

37.5 g of Na_2CO_3 was dissolved with distilled water and made up to 50 ml.

Preparation of 10% (v/v) Folin-Ciocalteau

A quantity, 10 ml of Folin-Ciocalteau was mixed with 90 ml of distilled water.

approved by the University Ethical Committee on the use of Laboratory Animals.