Effectiveness of Grape Seed Extract on Gamma Radiation-Induced Hazards in Albino Rats

Thesis
Submitted to the Faculty of Science
Ain Shams University, Cairo, Egypt
For the Award of the Ph.Degree

By

Afrag Shawki Abd El Azime

Radiation Biology Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo

Supervisors

Prof. Dr. Nefissa H. Meky

Professor of Physiology
Faculty of Science, Ain Shams University, Cairo

Prof. Dr. Helen N. Saada

Professor of Physiological Chemistry
National Center for Radiation Research and Technology,
Atomic Energy Authority, Cairo

Prof. Dr. Ussama Z. Said

Professor of Physiological Chemistry
National Center for Radiation Research and Technology,
Atomic Energy Authority, Cairo

(2008)

فاعلية مستخلص بذرة العنب ضد الأضرار التى يسببها التعرض للإشعاع الجامى فى الجرذان البيضاء

رسالة مقدمة من

إفراج شوقى عبد العظيم

للحصول على درجة الدكتوراة فى العلوم فى فسيولوجيا الحيوان

تحت أشـراف

الأستاذ الدكتور/ نفيسة حسين مكى

أستاذ الفسيولوجى - قسم علم الحيوان

كلية العلوم - جامعة عين شمس

الأستاذ الدكتور/ هيلين نقولا سعادة

أستاذ الكيمياء الفسيولوجية - قسم البيولوجيا الإشعاعية

المركز القومي لبحوث وتكنولوجيا الإشعاع

الأستاذ الدكتور/ أسـامة زكريا سعيد

أستاذ الكيمياء الفسيولوجية - قسم البيولوجيا الإشعاعية

المركز القومي لبحوث وتكنولوجيا الإشعاع

كلية العلوم- جامعة عين شمس

٢٠٠٨

TABLE OF CONTENTS

CHAPTER 3

3. MATERIALS AND METHODS

CHAPTER 4

4. RESULTS

CHAPTER 5

5. DISCUSSION

Acknowledgement

I wish to express my deep gratitude and appreciation to **Prof. Dr. Nefissa H. Mekey**, Prof. of Physiology, Zoology Department Faculty of Science, Ain Shams University for sponsoring the thesis, for kind encouragement throughout the period of investigation and for valuable advices.

I am sincerely grateful to **Prof. Dr. Helen N. Saada,** Prof. of physiological chemistry, Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), for suggesting the subject, great scientific help, valuable guidance and indispensable helps given through all the stages of this work.

I would like to express my gratitude to **Prof. Dr. Ussama Z. Said,** Professor of physiological chemistry, Radiation Biology Department, (NCRRT), for planning the subject, his great scientific help, valuable guidance during the practical work and handwriting of this manuscript.

My great thank to my colleagues in the Radiation Biology Department (NCRRT), particularly to my colleagues in the unit of physiological chemistry, for their valuable kind assistances in completing this work.

ABSTRACT

Effectiveness of Grape Seed Extract on Gamma Radiation-Induced Hazards in Albino Rats

Key words: Gamma radiation, Grape seed Extract, Oxidative stress, Enzymes.

The present study was designed to determine the possible protective effect of grape seed extract (GSE), rich in proanthocyanidins against gamma radiation-induced oxidative stress associated to serum metabolic disorders in the liver, heart and pancreas tissues of rats. Male albino rats received GSE (100 mg/day/Kg body weight), by gavages, for 14 successive days before whole body exposure to 5Gy gamma radiation (shot dose). Animals were sacrificed 1, 14, and 28 days post radiation exposure.

The results showed that in the irradiated group, tissues superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities were decreased significantly, while thiobarbituric acid reactive substances (TBARS) content was increased, which was in parallel with significant increases in the activity of serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate and alanine aminotransferase (AST and ALT). Hyperglycemia, hyperinsulinemia,

hyperlipidemia, decreases in red blood cells count (RBCs) count and hemoglobin (Hb) content were also observed in irradiated rats.

In the GSE-treated irradiated group, significant increases of SOD, CAT, and GSH-Px activities with significant reduction of TBARS levels were observed in cardiac, liver, and pancreas tissues, in parallel to significant decreases in the activity of serum LDH, CPK, AST, and ALT compared with their corresponding values in the irradiated group. Moreover, serum glucose and insulin contents, RBCs count and Hb content were significantly improved in the GSE-treated irradiated rats. Furthermore, the marked increase in serum triglycerides and total cholesterol observed in irradiated rats, along with elevated LDL-C and decreased HDL-C levels were significantly improved in GSE treated rats.

In conclusion, the present data demonstrate that GSE through its free radical scavenging and antioxidant properties attenuates ionizing radiation-induced oxidative injury suggesting that it may be a potential dietary supplement to minimize the side effects of radiotherapy.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The presence of free radicals in biological materials was discovered about 50 years ago **(Drogue, 2002),** and their importance in cellular injury and the aging process has attracted increasing attention over the past 20 years **(Shimosa, 2005**). Today, there is a large body of evidence indicating that body are constantly exposed to free radicals created by electromagnetic radiation from the environment, both natural (e.g., radon, cosmic radiation) and man-made, and by internal cellular metabolism. Even patients in hospital intensive care units are exposed to excessive free radicals from drugs **(Dalton *et al.,* 1999).**

Free radicals are highly reactive molecules, which destroy tissue by oxidizing cell membrane lipids and damaging the body's genetic materials **(Kuhn, 2003).** Paradoxically, free radicals can also be produced by many cells as a protective mechanism, for example neutrophils produce free radicals to attack and destroy pathogens, while the liver uses free radicals for detoxification **(Lunec *et al.,* 2002)**. Moreover, low levels of free radicals are also vital for many cell signaling events and are essential for proper cell function. In humans, superoxide anion ($O_2^{\bullet -}$) is the most commonly produced free radical.

To protect against oxidative damage, organisms have developed a variety of antioxidant defenses. For example: $O_2^{\bullet -}$

undergoes dismutation by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). The majority of the H_2O_2 is broken down to oxygen and water by the antioxidant enzyme catalase (CAT). In addition to catalase, glutathione peroxidase (GSH-Px) can also break down H_2O_2 and any peroxides that form on lipids within the body **(Gutteridge and Mitchell, 1999).**

Excess free radicals can result from a variety of conditions such as tissue damage and hypoxia (limiting oxygen levels), overexposure to environmental factors (tobacco smoke, ultraviolet radiation, and pollutants), a lack of antioxidants, or destruction of free radical scavengers. When the production of damaging free radicals exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs. The resultant cellular injury caused by oxidative stress has been linked to over 200 clinical disorders, many of which are atherosclerosis, cancer, aging and several other conditions, including inflammatory diseases **(Kohen and Nyska, 2002).** Experimental studies have demonstrated that exposures to ionizing radiation induce oxidative stress in different tissues **(Saada *et al.,* 2003; Said *et al.,* 2004).**

When tissues are exposed to ionizing radiation, most of the energy taken up is absorbed by the cell water; largely because there is more water than any other molecule, thus creating two radicals: a hydrogen radical ($^{\bullet}H$), and a hydroxyl radical ($^{\bullet}OH$).

The latter can attack and damage almost every molecule found in living cells. Reactions of $^{\bullet}OH$ include its ability to interact with DNA, that have a number of possible chemical fates and to attack the fatty acid side chains of the membrane phospholipids which result in cell membrane damage **(Halliwell and Gutteridge, 2006).**

In order to overcome the potential harmful effect of free radicals and to reduce the damage by oxidants, a variety of synthetic antioxidants have been examined. However, the uses of synthetic compounds are restricted because of their toxic or carcinogenic effects (**Pokorny, 1991**). Natural antioxidants, particularly those containing phenolic components, are of considerable interest as dietary supplements or food preservatives (**Halliwell, 1995**).

Flavonoids are a class of water-soluble pigments. They constitute a large group of low molecular weight polyphenolic phytochemicals found in plants. Over 5000 naturally occurring flavonoids have been characterized and classified according to their chemical structure. Berries, grapes and cherries are recognized as fruits with a high content of antioxidants. The antioxidant properties of these fruits are believed to be due to a high content of flavonoids mainly the proanthocyanidins. Similarly, concentrated juices with high content of phenolic compounds exhibit antioxidant effects **(García *et al.*, 2004).**

Proanthocyanidins, sometimes referred as “condensed tannins” are responsible for astringency in many foods and medicinal herbs. These flavonoids occur naturally in black and green teas **(Kris and Keen, 2002),** chocolate and cacao (**Wan *et al.*, 2001),** red rice **(Oki *et al.*, 2002),** and many fruits: blueberries, blackberries, strawberries, elderberries, and other red/blue/purple colored plant parts **(Gu *et al.*, 2002).**

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols, from the plant raw material used. A great deal of research effort is being devoted to testing the putative beneficial effects of grape polyphenols extracted from grape seeds and widely used as nutrition supplements.

Grape *(Vitis vinifera*) is one of the world's largest fruit crops and grape seed is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars, mineral salts, etc.

Grape seed extract (GSE) is a natural extract from the seeds of *Vitis vinifera,* rich in flavonoids, mainly flavan-3-ols and proanthocyanidins **(Ferreira and Li, 2000).** These flavonoids have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties

against oxygen free radicals and oxidative stress **(Bagchi *et al.*, 2000).**

Experimental studies have shown that GSE possesses free radicals scavenging and chelation abilities **(Ariga, 2004).** Its protection against free radicals and free radical-induced lipid peroxidation and DNA damage was reported to be greater than vitamins C, E, and beta-carotene **(Bagchi *et al.*, 2000).** In addition, *in vitro* and *in vivo* experiments have demonstrated that GSE is highly bioavailable, rapidly absorbed, and distributed to various organs **(Tsang *et al.*, 2005**).

Antioxidants play an important role in providing protection of humans against infection and degenerative diseases **(Gupta *et al.*, 2004).** Due to increasing interest in flavonoids as dietary antioxidant supplements and a growing understanding of their potential health benefits, the objective of the present study was to assess the influence of GSE on gamma radiation-induced hazards.

AIM OF THE WORK

The present study has been carried out to elucidate the relationship between radiation-induced oxidative stress in cardiac, liver and pancreas tissues of albino rats and changes in some serum metabolites after total body exposure to gamma radiation at 5 Gy applied as one shot dose, and to evaluate the role of grape seed extract against radiation-induced hazards.

This goal has been achieved by:

1- Determination of the radiation-induced changes in the antioxidant status of cardiac, liver and pancreas tissues by measuring the variations in the activity of the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and changes in the level of thiobarbituric acid-reactive substances; marker of lipid peroxidation.

2- Determination of the radiation-induced changes in some serum enzymatic activities by measuring the changes in the activity of lactate dehydrogenase, creatine phosphokinase, aspartate aminotransferase, and alanine aminotransferase.

3- Determination of radiation-induced changes in some carbohydrate and lipid metabolites by measuring changes in glucose, insulin, triglycerides, total cholesterol, low density

lipoprotein-cholesterol and high density lipoprotein-cholesterol levels in the serum.

4- Determination of radiation-induced changes in red blood cells count and hemoglobin content.

5- Evaluation of the protective role of grape seed extract against radiation hazards by comparing results obtained for irradiated rats with those obtained for grape seed extract-treated irradiated rats.

2- REVIEW OF LITERATURE

Human beings live in a sea of continuous radiation, which is both natural and man-made. Natural radiation comes from cosmic rays, naturally occurring radioactive elements found in the earth's crust (uranium, thorium, etc.), and radioactive decay products such as radon and its subsequent decay products. Artificial sources of radiation include X-ray equipment, nuclear weapons and radioactive medication. Most acute or intermittent excessive exposures to ionizing radiation occur in association with radiation therapy, preparation for organ transplantation, nuclear weapon detonations, nuclear reactor accidents or accidental ingestion of radio-nuclides (**Finch, 1991**).

2.1. General Concept on Radiation

Radiation is defined as energy in transit and comprises electromagnetic rays; such as X-rays or gamma rays and particulate radiation such as neutrons, alpha particles, and heavily charged ions. Radiation affects people by depositing energy in body tissues. The extent of the damage depends upon the total amount of energy absorbed, the time period and dose rate of exposure, and the particular organ(s) exposed **(Yarmonenko, 1988)**.

Radiation may be non-ionizing or ionizing according to energy.

Non ionizing radiation has enough energy to excite molecules and atoms causing them to vibrate faster, which is obvious in a microwave oven where the radiation causes water molecules to vibrate faster creating heat.

Ionizing radiation has more energy than non-ionizing radiation; enough to cause chemical changes by breaking chemical bonds. This effect can cause damage to living tissues. The ionizing radiations of primary concern are alpha and beta particles, gamma and x rays **(Yarmonenko, 1988).**

The types of effects and their probability of occurrence depends on whether the exposure occurs over a large part of a person's lifespan (chronic) or during a very short portion of the lifespan (acute).

Chronic exposure is continuous or intermittent exposure to low levels of radiation over a long period of time. Chronic exposure produces genetic effects, pre cancerous lesions, benign tumors, cataracts, skin changes, and cancer **(Yarmonenko, 1988).**

Acute exposure results from exposure to a large single dose of radiation, or series of doses, for short period of time. Large acute doses can result from accidental or emergency exposures or from special medical procedures (radiation therapy). In most cases, a large acute exposure to radiation can

cause both immediate and delayed effects. For humans and other mammals, acute exposure, if large enough, can cause rapid development of radiation sickness, evidenced by gastrointestinal disorders, bacterial infections, hemorrhage, anemia, loss of body fluids, and electrolyte imbalance. Delayed biological effects can include cataracts, temporary sterility, cancer, and genetic effects. Extremely high levels of acute radiation exposure can result in death within few hours, days or weeks **(Yarmonenko, 1988).**

2.2. Biological Effect of Radiation

Radiation interacts with matter by direct and indirect processes. Both processes lead to molecular damage, which is then translated to biochemical damage **(Pinon et al., 1991**).

Direct effect: Radiation energy breaks some chemical bonds in vital molecules such as proteins, lipids, carbohydrate, and nucleic acids.

Indirect effect: Because human tissues contain 80% water, the major radiation damage is due to aqueous free radicals. Ionizing radiation interacts with water molecules to form a free electron and an ionized water molecule in a process termed radiolysis. The end products of the radiolysis of water without oxygen are: hydrogen radical ($^{\bullet}H$), hydroxyl radical ($^{\bullet}OH$), hydrogen ion (^{+}H), and hydroxyl ion (^{-}OH), in which: $^{\bullet}H$, $^{\bullet}OH$ are highly reactive. In the presence of oxygen hydroperoxy radical

($^{\bullet}HO_2$), hydroperoxy ion ($^{-}HO_2$) and hydrogen peroxide (H_2O_2) are formed. These chemical entities are powerful oxidizing agents with longer half lives. They may diffuse even further from the initial sites of ionization (**Ward, 1990)**. Thus, the biological material and living systems irradiated in the presence of oxygen are more susceptible to injury than in the absence of oxygen. This response is known as the oxygen effect (**Walden and Farzaneh, 1990**).

2.3. Free Radicals and Reactive Oxygen Species

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals (**Goyns, 2002**).

One chemical element frequently involved in free radical formation is oxygen. Molecular oxygen can accept a total of four electrons, one at a time, and the corresponding number of protons to generate two molecules of water. During this process, different oxygen radicals are successively formed as intermediate products, including superoxide ($O_2^{\bullet -}$); peroxide ($O_2^{=}$), which

normally exists in cells as H_2O_2; and $^{\bullet}OH$. Superoxide can react with itself to produce H_2O_2. Superoxide, peroxide, and the $^{\bullet}OH$ are considered reactive oxygen species.

Many transition metal ions e.g. iron (Fe^{2+}) and (Fe^{3+}) and copper (Cu^{+}) and (Cu^{2+}) are remarkably good promoters of free radical reactions. Fe^{2+} ions reduce H_2O_2 to produce $^{\bullet}OH$, which is also produced by reaction of H_2O_2 with copper ions **(Halliwell and Gutteridge, 1998).**

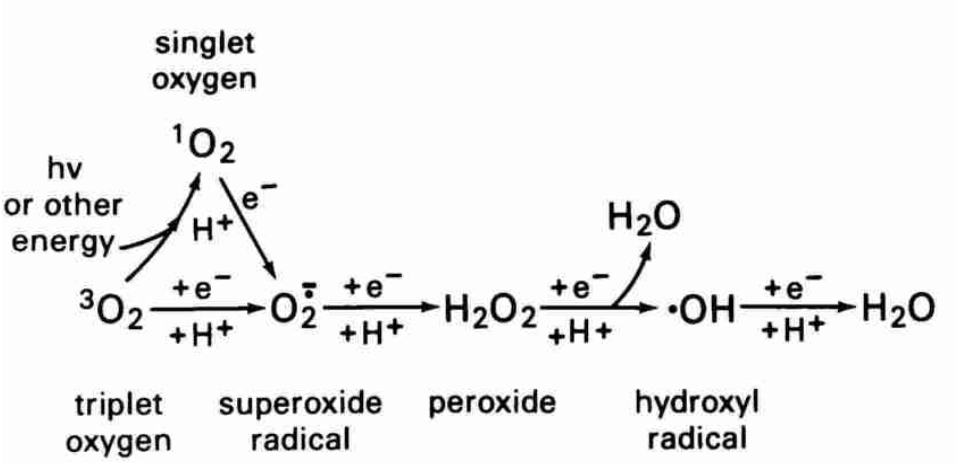


Figure 1: Reduction of molecular oxygen to superoxide, and of peroxide to hydroxyl radical

Reactive oxygen species (ROS) is a term collectively describing free radicals and other non-radical reactive oxygen derivatives. For example $^{\bullet}OH$ is considered a free radical while H_2O_2 is a non-radical ROS. Because ROS are unstable and rapidly react with additional electrons and protons, most of them are converted to water before they can damage cells. It has been estimated that only about 2 to 3 % of the oxygen consumed by

the respiratory chain is converted to ROS (**Chance *et al.,* 1979**). Free radicals and their precursors may have endogenous or exogenous sources.

Endogenous sources of free radicals: The major source of ROS production in the cell is the mitochondrial respiratory chain, which utilizes approximately 80 to 90% of the oxygen a person consumes. Mitochondria; the intracellular power-houses, which produce the universal energy molecule, adenosine triphosphate (ATP), normally consume oxygen in this process and convert it to water. However, unwanted by-products such as $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ are inevitably produced, due to incomplete reduction of oxygen. It has been estimated that more than 20 billion molecules of oxidants per day are produced by each cell during normal metabolism **(Ames *et al.,* 1993).**

Another major source of ROS, especially in the liver, is a group of enzymes called the cytochrome P450 mixed–function oxidases that prevent damage by toxic foreign chemicals like drugs and pesticides **(Ames *et al.,* 1993).** Furthermore, some evidence suggests that ROS, especially H_2O_2, may be important in signal transduction mechanisms in cells and thus may be an integral component of cellular physiology and metabolism (**Lander, 1997).** Activated phagocytic cells, monocytes, neutrophils, eosinophils, and macrophages of all types produce nitric oxide radical ($NO^{\bullet}$), $O_2^{\bullet-}$, and H_2O_2 to destroy parasites,

bacteria and viruses. Consequently, chronic infections result in prolonged phagocytic activity and increased exposure of body tissues to the oxidants. Peroxisomes produce H_2O_2 as a byproduct of the degradation of fatty acids and other molecules **(Ames *et al.*, 1993).**

ROS are produced also by a variety of oxidative enzymes present in cells, such as xanthine oxidase (XO). Under normal physiological conditions, it is present as xanthine dehydrogenase (XDH) and removes hydrogen from xanthine or hypoxanthine and attaches it to nicotinamine adenine dinucleotides (NAD), thereby generating nicotinamine adenine dinucleotides hydrogen (NADH). However, under certain conditions, such as the disruption of blood flow to a tissue, XDH is converted to XO.

Exogenous sources of free radicals: Besides the reactive oxygen species generation that occurs naturally in the body, humans are constantly exposed to environmental free radicals. Exogenous sources of free radicals include air pollution, of which industrial waste and cigarette smoke are major contributors. Radiation and trace metals, notably lead, mercury, iron and copper, are also major sources of free radical generation **(Ames *et al.*, 1993).**

There are also certain medications used for cancer treatment, where the toxicity of these medications against tumor

cells (as well as normal body cells) results from the fact that the compounds are modified by cellular enzymes to an unstable intermediate, which then reacts with molecular oxygen to produce the original product plus a superoxide radical. Thus, a vicious cycle of chemical reactions involving these compounds continually produces ROS **(Hahn *et al.*, 2000)**.

Ionizing radiation has become an integral part of modern medicine. It is used for diagnostic as well as therapeutic purposes. Radiation therapy is one of the most important and popular tools for cancer treatment, where ROS react with cellular macromolecules such as DNA, proteins, lipids, etc. and cause dysfunction and mortality. However, these reactions take place in tumor as well as normal cells when exposed to radiation (**Tominaga *et al.*, 2004**).

2.4. Antioxidants

Antioxidants can be described as substances capable of counteracting the damaging effects of oxidation in body tissues. Antioxidants can be endogenous (produced by the body) or exogenous (obtained through the diet). Endogenous antioxidants include enzymes, coenzymes and sulfur-containing compounds. Exogenous antioxidants include vitamins C and E, bioflavonoids and carotenes.

Antioxidants are divided in general into two classes based on mechanisms of action:

1. Chain-breaking antioxidants, such as Vitamin E and beta-carotene, “break the chain” of free radical formation by donating an electron to stabilize an existing free radical. In general, they act by reacting with peroxyl radicals.
2. Preventive antioxidants are enzymes that scavenge initiating radicals before they start an oxidation chain.

Chain-breaking antioxidants are found in the blood and the fluids of the extra-cellular space, where preventive antioxidant enzymes are absent or present in very small quantities **(McDermott, 2000).** These small-molecule antioxidants include both water and lipid-soluble varieties. The lipid-soluble antioxidants are located in the cellular membranes and lipoproteins, whereas the water-soluble antioxidants are present in the aqueous environments, such as fluids inside cells and in the blood.

Preventive antioxidant enzymes inside the cell are an important defense against free radicals. The main enzymatic “scavengers” responsible for the prevention of ROS formation and oxidation are superoxide dismutase, catalase, and glutathione peroxidase.

Superoxide dismutase (SOD) is found in virtually every oxygen-based organism, and its major function is to catalyze the dismutation of $O_2^{\bullet -}$ to H_2O_2.This reaction is generally considered to be the body's primary antioxidant defense because it prevents further generation of free radicals. In humans, the highest levels of SOD are found in the liver, adrenal gland, kidney, and spleen **(Halliwell, 1996).**

Catalase (CAT) is an iron–containing enzyme found primarily in the small membrane–enclosed cell components called peroxisomes; it serves to detoxify H_2O_2 and various other molecules. One way that CAT eliminates H_2O_2 is by catalyzing a reaction between two H_2O_2 molecules, resulting in the formation of H_2O and O_2. In addition, CAT can promote the interaction of H_2O_2 with compounds that can serve as hydrogen donors so that the H_2O_2 can be converted to one molecule of H_2O, and the reduced donor becomes oxidized (a process sometimes called the peroxidatic activity of catalase) **(Victor *et al.*, 2006).** The liver, kidney, and red blood cells possess high levels of catalase.

Glutathione peroxidase (GSH-Px) system consists of several components, including the enzymes GSH-Px and GSH-reductase and the co-factors reduced glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH). Together, these molecules effectively remove H_2O_2 with formation of oxidized glutathione (GSSG). GSH is an

essential component of this system and serves as a cofactor for an enzyme called glutathione transferase, which helps remove certain drugs and chemicals as well as other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (e.g., $^{\bullet}OH$) to detoxify them, as well as performing other critical activities in the cell **(Victor *et al.*, 2006).**

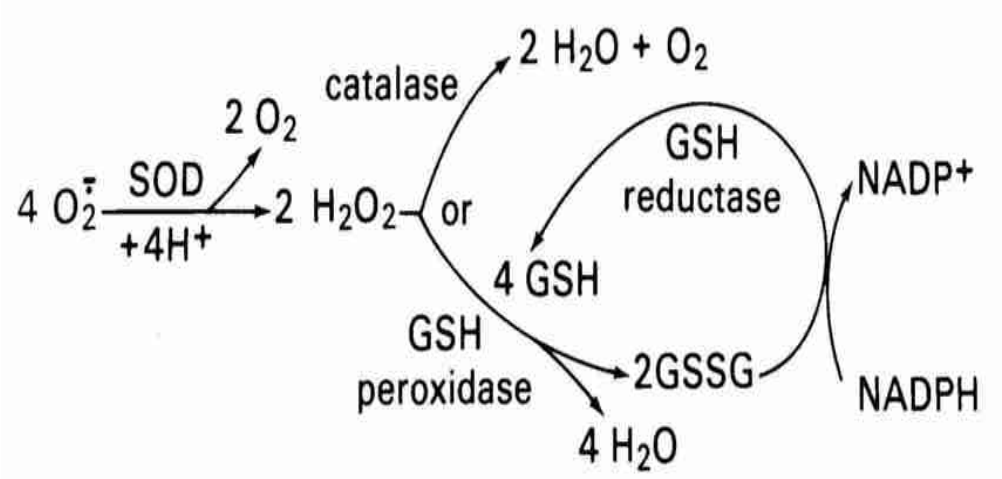


Figure 2: Superoxide dismutase (SOD), catalase, and the GSH peroxidase/GSSG reductase system.

In addition to GSH, numerous other non-enzymatic antioxidants are present in the cells, most prominently vitamin E (α–tocopherol) and vitamin C (ascorbate). Vitamin E is a major antioxidant found in the lipid phase of membranes and acts as a powerful terminator of lipid peroxidation. During the reaction between vitamin E and a lipid radical, the vitamin E radical is formed from which vitamin E can be regenerated in a reaction involving GSH and ascorbate (**Nanji and Hiller 1997**).

2.5. Oxidative Stress

Under certain conditions, such as acute or chronic radiation exposure, ROS production is enhanced and/or the level or activity of antioxidants is reduced. The resulting state which is characterized by a disturbance in the balance between ROS production on one hand and ROS removal and repair of damaged complex molecules (such as proteins or DNA) on the other is called oxidative stress (**Halliwell, 1999**).

Many processes and factors are involved in causing radiation-induced oxidative stress, including:

1- Changes in the NAD^+/NADH ratio in the cell and these changes lead to formation of NADH, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened oxygen use and ROS formation (**Osiewacz, 2002**).

2- Production of ROS during radiation exposure, which through its interactions with proteins and lipids also can lead to radical formation and cell damage (**de Groot, 1994)**.

3- Damage to a mitochondrion accumulates over time making it less efficient at producing adenosine triphosphate (ATP) and increasing the release of free radicals, resulting in even more damage. Eventually, the mitochondria of a cell

cannot meet cellular energetic needs resulting in mitochondrial and/or cellular death (**Rose *et al.,* 2002**).

4- Effects on cell structure (e.g., the membranes) and function caused by free radicals interactions with either membrane components (i.e., phospholipids) or enzymes and other protein components of the cells **(Fridovich, 1997).**

5- Radiation–induced oxygen deficiency (i.e., hypoxia) in tissues, especially in certain areas of the liver lobules (i.e., the pericentral region), where extra oxygen is required to metabolize reactive oxygen species **(Halliwell, 1999).**

6- Radiation effects on the immune system, which lead to altered production of certain signaling molecules called cytokines, which in turn lead to the activation of an array of biochemical processes **(Rosen *et al.,* 1995).**

7- Radiation –induced increases in the levels of free iron in the cell (i.e., iron that is not bound to various proteins), which can promote ROS generation **(Halliwell, 1999).**

8- Effects on antioxidant enzymes and chemicals, particularly glutathione **(Cadenas and Davies, 2000).**

9- Conversion of the XDH into a form called XO, which can generate ROS **(Srivastava *et al.,* 2002).**

ROS are toxic to cells because they can react with most cellular macromolecules, including proteins, lipids, and DNA. It

has been implicated in the pathogenesis of a number of diseases and clinical conditions. These include atherosclerosis, cancer, adult respiratory distress syndrome, Alzheimer's and Parkinson's diseases, ischaemia-reperfusion injury of various organs, chemical and radiation-induced injury, diabetes, etc (**Lakshmi *et al.*, 2005**).

The deep complicated structure of the animal tissues and the metabolic relationship between the different chemical components present such as proteins, fats, carbohydrates, hormones, enzymes as well as their intermediates and by-products represent a serious problem in understanding the action of ionizing radiation on individual metabolic process of these compounds. So, the interference between the cell constituents, the sensitivity of the organ itself as well as the susceptibility of the cell constituents toward radiation levels, greatly differ and are considered dose and time dependent **(Yarmonenko, 1988)**.

Proteins perform numerous crucial functions in the cell, primarily in the form of enzymes that mediate most biochemical reactions required for cellular functions. Proteins are made up of approximately 20 different building blocks called amino acids, which differ in their sensitivity to interactions with ROS. For example, the amino acids cysteine, methionine, and histidine are especially sensitive to attack and oxidation by the hydroxyl radical. Accordingly, enzymes in which these amino acids are

located at positions that are critical to the enzyme's activity will become inactivated by the interaction with ROS (**Toykuni, 1999**).

Alternatively, the ROS–induced oxidation of proteins can lead to changes in the proteins' three dimensional structure as well as to fragmentation, aggregation, or cross–linking of the proteins. Finally, protein oxidation often will make the marked protein more susceptible to degradation by cellular systems responsible for eliminating damaged proteins from the cell (**Toykuni, 1999**).

Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria. Consequently, damage to the phospholipids will compromise the viability of the cells. The complete degradation (i.e., peroxidation) of lipids is a hallmark of oxidative damage **(Saada *et al.*, 2001)**.

The polyunsaturated fatty acids (PUFA) present in the membranes' phospholipids are particularly sensitive to attack by $^{\bullet}OH$ and other oxidants. A single $^{\bullet}OH$ can result in the peroxidation of many PUFA molecules because the reactions involved in this process are part of a cyclic chain reaction. In addition to damaging cells by destroying membranes, lipid peroxidation can result in the formation of reactive products that

themselves can react with and damage proteins and DNA (**Cederaum, 2001**).

DNA is the cell's genetic material, and any permanent damage to the DNA can result in changes (i.e., mutations) in the proteins encoded in the DNA, which may lead to malfunctions or complete inactivation of the affected proteins. Thus it is essential for the viability of individual cells or even the entire organism that the DNA remain intact. ROS are a major source of DNA damage, causing strand breaks, removal of nucleotides, and a variety of modifications of the organic bases of the nucleotides (**Goyns, 2002**).

2.6. Effect of Gamma Irradiation on

2.6.1. Antioxidants Status

Exposure to ionizing radiation produces significant alterations in the oxidant activity in different tissues, and causes overproduction of ROS leading to oxidative damage of the lipids, proteins and DNA. The oxidation of PUFA in membranes induced by ROS is called lipid peroxidation which has been shown to increase in irradiated tissues **(Sadani and Nadkarni, 1997)**. Lipid peroxidation is believed to be an important cause of destruction and damage to cell membranes and has been shown to be a contributing factor to the development of oxygen radicals-mediated tissue damage. Commonly measured parameter of lipid

damage after ionizing radiation exposure is thiobarbituric acid reactive substances (TBARS) **(Samuni *et al.*, 1997)**. However, organisms have protective systems against ROS, like endogenous antioxidant enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) constitute primary enzymatic defense system **(Portakal *et al.*, 2000).**

Radiation represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation, or by indirect evidence of reduced antioxidant reserve, like SOD and CAT, in animal models (**Palanivel *et al.*, 1998**). The results of extensive studies showed that exposure to ionizing radiation result in significant alterations of antioxidant enzymes which depends upon the dose of radiation exposure, the time of biochemical analysis, and the tissue analyzed.

Saada *et al.*, (1999) reported that whole body gamma irradiation of rats with a fractionated dose of 8 Gy (4 x 2 Gy weekly) produces significant increase of blood GSH content, GSH-Px, and GSH-reductase activities 1hr and 1day post-irradiation followed by a significant decrease on the 3rd day. Experimental studies demonstrated also that 1Gy delivered every other day to reach 9 Gy produces significant increases of blood and lung SOD and CAT activities and GSH content parallel to an increase in TBARS one day after the last dose of radiation (**Saada *et al.*, 2001**).

Saada *et al.*, (2003) reported that whole body gamma irradiation of rats with 7 Gy induces significant decrease in liver and kidney SOD and CAT activities 3, 7, 10 days post-irradiation. On the contrary lung SOD and CAT activities were increased. TBARS levels showed significant increases in all the tissues**. Kalpana and Menon, (2004)** reported that in irradiated rats the activities of SOD, CAT and GSH-Px were decreased in the blood and liver tissues.

Said (2004) showed that 5 Gy whole body gamma irradiation results in significant decreases of brain SOD and GSH-Px activities associated with an increase in TBARS content 1, 7 and 14 days post-irradiation. Also, whole body gamma irradiation of rats with 6 Gy produces an increase in liver and plasma TBARS and a decrease in GSH content 1, and 2 weeks post-irradiation **(Said *et al.*, 2005).** Furthermore, gamma-radiation at different doses (1, 2 and 4Gy) was found to significantly increase TBARS level whereas the levels of GSH and antioxidant enzymes SOD, CAT, and GSH-Px in lymphocytes were significantly decreased. The maximum damage to lymphocytes was observed at 4Gy irradiation (**Srinivasan *et al.*, 2006).**

El-Missiry *et al.*, (2007) reported that in irradiated rats subjected to two doses of 2 and 4Gy from Cesium-137 source, the TBARS, protein carbonyl and CAT activity were

significantly increased in the liver, 5 days after irradiation. **Prabhakar *et al.,* (2007)** reported that there is a depletion of SOD and CAT in the livers of irradiated mice.

2.6.2. Red Blood Cells and Hemoglobin

Red blood cells (RBCs) are important because they carry O_2 to all parts of the body. Oxygen is required for energy and to keep the body functioning properly. When the number of RBCs falls below a certain number, O_2 is in short supply.

An important part of the RBCs is hemoglobin, the part that carries O_2 throughout the body. Therefore, when the level of hemoglobin is low, O_2 levels are decreased and the body has to work harder in order to compensate. The end result is that the body will show signs of being very tired. Free radicals attacks RBCs and actually interrupts the process of cell development that takes place deep inside the bones. When this happens, the number of RBCs circulating throughout the body drops lower and lower (the amount of O_2 available to the lungs and other organs is less and less) and the body begins to feel more tired, more fatigued. Eventually, if left untreated, the number of RBCs remains low and results in anemia **(Groopman and Itri, 1999).**

Experimental studies have shown that exposure to gamma radiation induced oxidative damage and hemolysis of RBCs (**Krokosz**, **2003).** Furthermore, **Mieczyslaw *et al.,* (2004)** reported that radiation provoked RBCs membrane damage,

hemoglobin oxidation and denaturation. **Puchała *et al.*, (2004)** postulated that $^{\bullet}OH$ play a crucial role in the induction of the processes for radiation-induced RBCs membrane damage, hemoglobin oxidation and denaturation. Lysis of RBCs and oxidized hemoglobin and increased amount of TBARS in RBCs were also detected after exposure to radiation **(Misra *et al.*, 2005).**

2.6.3. Glucose and Insulin

Carbohydrate metabolism suffers remarkable disturbances in irradiated animals. Literature regarding radiation-induced changes on glucose showed that whole body gamma irradiation of rats at doses below 10 Gy produces at first a hyperglycemic state followed by a hypoglycemic state till the animal death **(Navratil *et al.*, 1998).**

Hassan *et al.*, (1996) observed an increase in blood glucose level on the 14^{th} day after whole body gamma irradiation of rats with 5 Gy and a decrease on the 45^{th} day. **Mahdy *et al.*, (1996)** reported that whole body gamma irradiation of rats with a fractionated dose of 7.5 Gy (3 x 2.5 Gy twice weekly) produces significant increase in blood glucose level 7 and 14 days post-irradiation followed by a significant decrease at 21 days.

Azab *et al.*, (1999) observed significant increase of blood glucose 7, and 14 days after whole body gamma irradiation of rats with a fractionated dose of 8 Gy (4 x 2 Gy weekly) followed

by a significant decrease on the 30th day. **Saada *et al.*, (2003**) recorded that whole body gamma irradiation of rats with one shot dose of 7 Gy induced significant increase in blood glucose and insulin levels 7 and 21 days after irradiation

2.6.4. Lipids

Lipids in the blood are essential, yet they become a healthy threat when the blood contains too much of them. So, blood lipids must be maintained at the proper level and ratios **(Mehta, 2003).**

Whole body gamma irradiation of rats with a single dose of 7.5 Gy, resulted in significant increase in plasma triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and significant decrease in high density lipoprotein-cholesterol (HDL-C) 1 hr, 3 and 7 days post-irradiation (**Saada *et al.*, 2001).** Also, **Said *et al.*, (2005)** reported that whole body gamma irradiation of rats with 6 Gy produces an increase in plasma TC 1hr, 1, and 2 weeks post-irradiation and significant increases in the plasma content of TG, TC and LDL-C from the 1st up to the 4th week post irradiation while the content of HDL-C showed significant decreases.

Said *et al.*, (2006) showed a significant increases in the serum content of TG, TC and LDL-C while the content of HDL-C showed significant decreases 7 days post whole body gamma irradiation (7 Gy; single shot dose). Also, significant increases in

TG, LDL-C, HDL-C, TC were observed in irradiated rats with a single dose of gamma irradiation (6 Gy) in liver and lung tissues **(Mansour, 2006)** and in sera (**El-Missiry *et al.*, 2007**).

2.6.5. Metabolic Enzymes Activity

Damaging effects of ROS include oxidative attack on vital cell constituents. Several soluble enzymes of blood serum have been considered as indicators of the tissue damage.

Transaminases: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important and critical enzymes in the biological processes; their activities are related with the maintenance of amino acid homeostasis and might be an indicator of mitochondrial injury. ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. If the liver is injured, the liver cells spill the enzymes into blood, raising their levels in the blood and signaling the liver damage.

Exposure of rats to gamma irradiation 5 Gy (single dose) caused a significant increase in the liver activities of ALT and AST 1, 2, 3 and 4 weeks post-irradiation (**Said *et al.*, 2002).** While, **Azab *et al.*, (2004)** observed that rats exposed to fractionated whole body gamma irradiation (2Gy every other day up to 8 Gy) showed a significant increase in the activity of AST and significant decrease in the activity of ALT.

Lactate dehydrogenase (LDH) is responsible for metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in the activity of LDH enzyme leads to biochemical impairment and lesions of the tissue and cellular function (**Khan *et al.*, 2001**). Elevated LDH levels can be caused by a number of conditions, including heart failure, hypothyroidism, anemia, and lung or liver injury. **Abbady *et al.*, (1999)** reported that **w**hole body gamma irradiation of rats with a fractionated dose of 8 Gy (4 x 2 Gy weekly) produced significant increase of serum LDH activity 7, 14 and 30 days post irradiation. **Azab *et al.*, (2003)** found also that whole body gamma irradiation of rats with a shot dose of 6 Gy produces remarkable increases of serum LDH activity 1, 7, and 14 days post exposure. Rats exposed to 1 Gy whole body gamma irradiation exhibited a significant increase in serum activities of ALT and LDH **(Said and Hanafy, 2006).**

Creatine phosphokinase (CPK) plays an important role in cellular energy metabolism in vertebrates **(Saksa *et al.*, 1996).** It is specifically located at places of energy demand and energy production and plays an important role in the energetic of calcium (Ca^{2+}) homeostasis and mitochondrial membrane stability**.** Elevation of serum CPK is an indication of muscle damage **(Holger *et al.*, 2005)**.

Sanchez *et al.*, (2002) found a release of enzyme activities of LDH, CPK, AST, and ALT to blood plasma in mice exposed to oxidative stress. **Sridharan and Shyamaladevi, (2002)** reported that whole body exposure of rats to γ-rays (3.5 Gy) caused increases in liver lipid peroxides which might cause the leakage of the cytosolic enzymes AST, ALT, LDH, and CPK into the blood stream.

Whole body gamma irradiation induced significant increases in serum AST, CPK and LDH activities **(Said and Azab, 2006; Sirmali *et al.*, 2007)**. Also, significant increases in ALT and AST activities were observed in rats exposed to oxidative stress **(Subir *et al.*, 2007)**.

2.7. Protection Against Reactive Oxygen Species Toxicity

Sustained oxidative stress from a heavy cumulative burden of oxidants may deplete the body's antioxidant reserves to a point of "distress," beyond which the individual's antioxidant defenses are overwhelmed. The resultant negative antioxidant balance begins to compromise life functions **(Kidd, 1991).** Experimental studies have shown that the extent of damage caused by free radicals might be modified through three dietary intervention strategies: (a) caloric restriction and thus a depression in free radicals arising due to normal metabolism; (b) minimizing the

intake of components that increase free radicals such as polyunsaturated fats; and (c) supplementation with one or more anti-oxidants **(van den Berg *et al.,* 2001)**. Flavonoids are a ubiquitous group of polyphenolic substances with potent antioxidant activity which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers.

2.8. Grape Seed Extract (GSE)

Grape seed extract is a natural extract from the seeds of *Vitis vinifera* (Linn.) (Family: Vitaceae). *Vitis vinifera* also called as common grape or wine grape or European grape is one of the fruit crops most widely grown throughout the world **(Winkler *et al.,* 1997).** Recently the safety of food supplements has received great attention from the general public. Safety evaluations of GSE have been carried out by *in vitro* and *in vivo* studies **(Erexson, 2003).**

Grape seeds are waste products containing lipid, protein, carbohydrates, and 5-8% polyphenols depending on the variety. The polyphenols in grape seeds are mainly flavonoids that range from the monomeric flavanols **(Waterhouse and Walzem, 1998)** to oligomers with 7 or more flavanol units: the proanthocyanidins **(Shi *et al.,* 2003).** Although proantho-cyanidins are found in grape, cocoa, chocolate, apple and berries, the most abundant source is grape seeds **(Ayako *et al.,* 2004)**.

GSE has been reported to show various beneficial properties such as anticarcinogenic, cardioprotective, hepatoprotective effects via its potent antioxidative activities. It was claimed to penetrate the blood brain barrier and protect the brain and central nervous system from free radicals damage **(Bagchi *et al.*, 2003).** It has been used also for medical therapy for vascular disorders, such as venous insufficiency, and microvascular problems including capillary fragility and retinopathies. GSE proanthocyanidins are also used as food additives and nutritional supplements in many countries **(Ayako *et al.*, 2004)**.

Extensive research demonstrated that GSE rich in proanthocyanidins reduces the signs/symptoms of chronic age-related disorders **(Preuss *et al.*, 2002),** prevent the development of cataract formation **(Yamakoshi *et al.*, 2002),** improve vision in irradiated rats (**Said *et al.*, 2005)** and promote wound healing **(Sen *et al.*, 2002).** It was shown to be useful in the amelioration of chronic pancreatitis **(Banerjee and Bagchi, 2001),** and to protect rat's liver from oxidative damage **(Dulundu *et al.*, 2007).**

GSE was reported to promote bone formation **(Ishikawa *et al.*, 2005)**, and to exert neuro-protective action **(Balu *et al.*, 2006**). Daily administration of the seed extract of *Vitis vinifera* at doses of 100, 200 and 300 mg/kg body weight for 7 consecutive days prior to induction of stress (animals were forced to swim)

reversed the stress induced biochemical changes in a dose dependent manner **(Sreemantula *et al.,* 2005)**.

Moreno *et al.,* (2003) reported that GSE rich in bioactive photochemical might be useful as a treatment to limit dietary fat absorption and the accumulation of fat in adipose tissue. It reduces energy intake and could, therefore, play a significant role in body-weight management **(Vogels and Nijs, 2004)**.

GSE was reported to possess high anti-tumor-promoting activity and can be a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents used in treatment of cancer **(Bagchi *et al.,* 2002).** *In vitro* and *in vivo* studies demonstrated that GSE could be used as chemo-preventive agents against breast cancer by suppressing in situ estrogen biosynthesis (**Eng *et al.,* 2003)**. Experimental studies in cell culture and in nude mice showed that GSE inhibits advanced human prostate cancer growth (**Dhanalakshmi *et al.,* 2003).** In another study, **Singh *et al.,* (2004)** observed that mice fed with 100 and 200 mg/kg/day (5 days/week) doses of GSE for 7 weeks showed in vivo anticancer efficacy against human prostate cancer. **Said and Hanafy, (2006)** attributed the anti-tumor properties of GSE to its antioxidant activities.

A human clinical trial conducted on hypercholesterolemic subjects showed that GSE supplementation of 100 mg /kg body weight/day decreased the amount of ROS, reduced TBARS

formation in the heart **(Bagchi *et al.,* 2003)** and protect total GSH pool **(Ray *et al.,* 2004)**.

GSE was reported to be cardio-protective **(Fitzpatrick *et al.,* 2002; Shao *et al.,* 2003**) through its effects on hypertension **(Al-Awwadi *et al.,* 2005).** It was shown also to enhance the resistance of LDL-C to oxidative modification, a contributing factor to the development of atherosclerosis **(Shafiee *et al.,* 2003)** and to prevent cardiac cell apoptosis via the induction of endogenous antioxidant enzymes (**Du *et al.,* 2007).** In addition, GSE possesses anti-inflammatory properties and effect on platelet release of reactive oxygen intermediates **(Vitseva *et al.,* 2005).** It was reported also to strongly decreased nitric oxide radical and ROS production and nitric oxide synthase expression by lipopolysaccharides-stimulated macrophages **(Houde *et al.,* 2006**).

3- MATERIAL AND METHODS

3.1. Experimental Animals

Male Swiss Albino rats (100-120 g), purchased from the Egyptian Organization for Biological Products and Vaccines, were used for the different investigations carried out in this work.

Animals were housed in specially designed cages and maintained in conditions of good ventilation, normal temperatures and humidity ranges and kept under observation for one week prior to experimentation. Animals were fed on a standard diet containing all the necessary elements (proteins, fats, carbohydrates, vitamins, salts and minerals). Food and water were available *ad libitum* throughout the experimental periods.

All animal studies were conducted in accordance with criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

3.2. Radiation Facility

Irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source of radiation was a Gamma Cell-40 (Cesium 137), which ensured a homogeneous distribution of irradiation. The dose rate was 0.61 Gy/minute during the experimental

periods. Animals were whole body exposed to 5 Gy delivered as a single shot dose.

3.3. Grape Seed Extract (GSE) Treatment

GSE from *Vitis vinifera* was obtained as NATROL-ACTIVIN from USA. The product is supplied as tablets of 50 mg. Tablets were dissolved in water and animals received by gavages the equivalent of 100 mg/Kg body weight/day **(Said et *al.*, 2005)**

3.4. Experimental Design

Experimental animals: 72 rats were randomly divided into 4 equal groups.

Control group: rats received 0.5ml distilled water, by gavages, for 14 consecutive days.

GSE-treated group: rats received GSE (100 mg/Kg body weight/day), by gavages, for 14 consecutive days.

Irradiated group: rats were whole body exposed to gamma radiation at 5 Gy applied as one shot dose.

GSE-treated irradiated group: rats received GSE during 14 consecutive days before whole body exposure to gamma radiation at 5 Gy.

Experimental technique: 6 rats from each group were sacrificed 1, 14, and 28 days post-irradiation. Blood was

collected by heart puncture and centrifugated at 3000 r p m for 15 minutes to separate the serum. Liver, heart and pancreas tissues were quickly removed. One gram of each tissue was homogenized in normal 0.9% saline for biochemical analysis.

3.5. Biochemical Analysis

3.5.1. Determination of Superoxide Dismutase Activity

Measurement of the superoxide dismutase (SOD) activity was done based on the method of **Minami and Yoshikawa, (1979)**. The assay relies on the ability of the enzyme to inhibit the phenazine methosulfate mediated reduction of nitroblue tetrazolium (NBT) dye. The increase in absorbance at 560 nm due to the formation of reduced NBT was recorded in a spectrophotometer.

One unit of SOD activity is defined as the amount of the enzyme causing half the maximum inhibition of NBT reduction. The activity was expressed as U/g fresh tissue.

3.5.2. Determination of Catalase Activity

The catalase activity (CAT) was determined according to the method of **Aebi, (1983)** where the disappearance of peroxide is followed spectrophotometrically at 240 nm. The method is based on the catalytic function of the enzyme where the enzyme catalyzes the following reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2.$$

One unit of catalase activity is defined as the amount of enzyme required to decompose one micromole of hydrogen peroxide per minute. The activity was expressed as U/g fresh tissue**.**

3.5.3. Determination of Glutathione Peroxidase Activity

Total glutathione peroxidase activity (GSH-Px) was determined according to the method of **Lawrence and Burk, (1976).** The method is based on measuring the oxidation of nicotinamide adenine dinucleotides hydrogen phosphate (NADPH) to nicotinamide adenine dinucleotides phosphate (NADP) at wave length of 340 nm using hydrogen peroxide as the substrate.

One unit is defined as the amount of enzyme catalyzing the oxidation of one nmol NADPH/min. The activity was expressed as U/g fresh tissue**.**

3.5.4. Determination of Thiobarbituric Acid Reactive Substances Level

The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to **Yoshioka *et al.*, (1979)**. The method is based on the determination of malondialdehyde (MDA) an end product of lipid peroxidation, which can react with thiobarbituric acid in

acidic medium to yield a pink colored trimethine complex exhibiting an absorption maximum at 532 nm.

The concentration of TBARS in the sample was calculated as nmole/g fresh tissue.

3.5.5. Determination of Lactate Dehydrogenase Activity

The activity of lactate dehydrogenase activity (LDH) was measured using the method described by **Burtis and Ashwood, (1994).** Lactate dehydrogenase catalyses the inter conversion of pyruvate and lactate with concomitant inter conversion of nicotinamide adenine dinucleotides hydrogen (NADH) and nicotinamide adenine dinucleotides (NAD). The decrease in absorbance due to the oxidation NADH to NAD measured at 340 nm is proportional to the activity of LDH in the sample. The activity was expressed as U/dL serum.

3.5.6. Determination of Creatine Phosphokinase Activity

The determination of creatine phosphokinase (CPK) activity was based on measurement of NADPH produced in a series of reactions **(Meiattini *et al.*, 1978).**

Creatine phosphate + ADP $\xrightarrow{\text{CPK}}$ Creatine +ATP (1)

Adenosine triphosphate (ATP) reacts with glucose in the presence of Mg^{2+} and hexokinase, forming glucose 6-phosphate (G-6P), which is then oxidized by glucose 6-phosphate dehydrogenase with simultaneous reduction of NADP.

Glucose + ATP $\xrightarrow{\text{hexokinase, Mg2+}}$ G-6-P + ADP (2)

G-6-P + NADP $\longrightarrow$ 6-phosphogluconate + $NADPH_2$ (3)

The change in absorbance due to NADP reduction measured at 340 nm is proportional to CPK activity. The activity was expressed as U/dL serum.

3.5.7. Determination of Transaminases Activity

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of **Reitman and Frankel, (1957).**

Alanine aminotransferase (ALT) catalyses the reaction:

Alanine + α-Ketoglutarate → Pyruvate + Glutamate

Aspartate aminotransferase (AST) catalyses the reaction:

Aspartate + α-Ketoglutarate → Oxaloacetate + Glutamate

The formed keto acids (Pyruvate or Oxaloacetate) are coupled with 2, 4 dinitrophenyl hydrazine in alkaline medium. The optical density of the brown colour produced was measured at 505 nm. ALT activity was expressed as mg pyruvate/dLserum /30 min). AST activity was expressed as mg pyruvate/dL serum /60 min).

3.5.8. Determination of Glucose Level

Serum glucose level was determined according to the method of **Tietz, (1986)**. This method depends on the fact that glucose oxidase (GOD) enhances the oxidation of glucose to gluconic acid with the production of an equivalent amount of hydrogen peroxide. In the presence of peroxidase (POD), oxygen from the hydrogen peroxide can be transferred to a suitable acceptor (4-aminoantipyrine), which with phenol gives a red violet chromogen as the following reaction:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{chromogen} + H_2O$$

The intensity of the color is proportional to glucose concentration. The concentration of glucose was expressed as mg/dl serum.

3.5.9. Determination of Insulin Level

Insulin levels were determined by the ELISA technique according to the method of **(Nakagawa *et al.*, 1973)**. In the assay, standards, controls and unknown serum samples are incubated with an anti-insulin antibody in microtitration wells which have been coated with another anti-insulin antibody. After incubation and washing, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of

the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of insulin present. A standards curve of absorbance versus insulin concentration was plot from which the insulin concentrations in the unknowns can be calculated. The concentration was expressed in micro IU/dL serum.

3.5.10. Determination of Serum Lipid Profile

Triglycerides content was measured according to the method of **Stein and Myers, (1995).** The method is based on the hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-amino phenazane and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff. The optical density of the color produced was measured at 550nm. The concentration was expressed as mg/dL serum.

Total cholesterol content was estimated according to the method of **Bartis and Ashood *et al.*, (1994)**. Cholesterol reacts with acetic anhydride and conc. H_2SO_4 yielding a blue green colour. The optical density of the color produced was measured at 546nm. The concentration was expressed as mg/dL serum.

High density lipoprotein cholesterol (HDL-C) content was measured using the method described by **Assmann, (1984).** Low density lipoprotein cholesterol **(**LDL-C and LDL-C**)** and chylomicron fractions are precipitated quantitatively by the addition of phosphotungestic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined according to **Bartis and Ashood *et al.*, (1994)**. The optical density of the color produced was measured at 546nm. The concentration was expressed as mg/dL serum.

Low density lipoprotein cholesterol (LDL-C) concentration was calculated according to Friedewald formula **(Friedewald *et al.*, 1972).** The concentration was expressed as mg/dl serum

$$\text{LDL-C} = \text{Total cholesterol} - \frac{\text{triglycerides}}{5} - \text{HDL-C}$$

3.5.11. Determination of Red Blood Cells (RBC's) count

The number of RBC's was calculated according to the method of **Dacie and Lewis, (1991).** Whole blood was diluted with saline solution. Hemocytometer with Neubour ruling was used. RBC's count was expressed in million per microliter of blood.

3.5.12. Determination of Hemoglobin Content

Determination of hemoglobin (Hb) was based on the conversion of hemoglobin into cyanomethemoglobin under the influence of potassium ferricyanide and potassium cyanide (**Dacie and Lewis, 1991).** Hemoglobin concentration was expressed as g/dl of blood. The reagents used were kits obtained from Stanbio Lab. Inc.

3.6. Statistical analysis

The Statistical Package for the Social Sciences (SPSS/PC) computer program was used for statistical analysis of the results. Data were analyzed using one way analysis of variance (ANOVA). The data were expressed as mean ± standard deviation (SD). Differences were considered significant at $P \leq 0.05$.

4-RESULTS

4.1. Role of Grape Seed Extract (GSE) in the Antioxidant Status of Cardiac Tissues

4.1.1. Cardiac Superoxide Dismutase (SOD) Activity

SOD activity of cardiac tissues in the four animal groups is presented in table (1) and illustrated by figure (1).

In the group of control rats SOD activity ranged from 87 ± 6.9 to 88 ± 7.4 U/g fresh tissue and no significant changes were recorded throughout the period of the experiment (28 days).

In the group of GSE-treated rats no significant changes were recorded in SOD activity throughout the experimental period, compared to control values.

In the group of gamma irradiated rats SOD activity was significantly decreased ($P<0.05$) by -32%, -38% and -40% on the 1^{st}, 14^{th} and 28^{th} day post-irradiation, respectively, compared to the corresponding control values.

In the group of GSE-treated irradiated rats SOD activity showed significant decreases ($P<0.05$) of -13 %, -15% and -18% on the 1^{st}, 14^{th} and 28^{th} day post-irradiation, respectively, compared to the corresponding control values.

The results obtained showed that GSE treatment has significantly diminished the decreases of cardiac SOD activity, compared to their corresponding values in irradiated rats.

5- DISCUSSION

Ionizing radiation is an important environmental risk factor for various diseases such as cardiovascular, neurodegenerative diseases, cancers as well as ageing. Radiation is known to produce various types of reactive oxygen species (ROS) in biological systems, and cause tissue damage due to successive free radical reactions and oxidation of cell constituents **(Devi *et al.,* 2002)**. Excessive oxidation can cause cell death and even moderate oxidation can trigger apoptosis **(Young and Woodside, 2001)**.

To cope with ROS damage, organisms possess comprehensive and integrated endogenous antioxidant enzymatic repair systems, which exist in a number of locations, namely intra-cellular, on the cell membrane and extra-cellular **(Karbownik and Reiter, 2000)**. In addition to these endogenous mechanisms, much attention has been paid to the antioxidant role of dietary components from natural resources which could efficiently quench the elevated oxygen derived free radicals.

5.1. Radiation Effects on Antioxidants Status in Cardiac, Liver and Pancreas Tissues

Antioxidants are central to the redox balance in the body and do not act in isolation, but synergistically with other classes of molecules. They may prevent oxygen radical formation, by

either removing free radical precursors or by inhibiting catalysis, e.g. the enzymes glutathione peroxidase and catalase. Antioxidants may react also with ROS which have already been formed, either to remove or inhibit them, e.g. vitamins C, E and flavonoids which are important non-enzymatic antioxidants, often taken up with food **(Karbownik and Reiter, 2000)**. Ionizing radiation is known to induce imbalance in pro-oxidant/antioxidant status in the cells through an overproduction of ROS **(Bhosle *et al.*, 2005)**.

In the present study, whole body gamma irradiation of rats with 5 Gy (one shot dose) provokes oxidative stress in heart, liver and pancreas tissues, manifested by significant increase in the levels of thiobarbituric acid reactive substances (TBARS) which was concomitant with significant decrease in the activities of the antioxidant enzymes, SOD, CAT, and GSH-Px1, 14, and 28 days post-irradiation in cardiac tissues. The same pattern of changes was recorded in liver and pancreas tissues, except a significant increase of SOD and CAT activities recorded on the 1st day post-irradiation for both tissues.

Radiation- induced lipid peroxidation (LPO) is a marker of oxidative damage and has been linked to loss of fluidity, inactivation of membrane enzymes, increases in permeability to ions, and eventually disruption of cell membrane leading to the

release of cell organelles **(Gupta *et al.,* 1999)**. In the present study, GSE treatment has significantly reduced LPO**.**

In the present study, the increase of LPO in heart, liver and pancreas tissues might be explained on the basis that exposure to ionizing radiation increases the amount of free radicals in the body, the potent hydroxyl radicals ($^{\bullet}OH$) attack the polyunsaturated fatty acids in the phospholipids portion of cell membranes initiating the lipid peroxidation chain reaction **(Rajendra *et al.,* 2003).** LPO has been found to increase with increase in radiation dose in rat tissues **(Rajendra *et al.,* 2005).**

Exposure to ionizing radiation enhances LPO and depletion of certain key endogenous antioxidant enzymes **(Saada *et al.,* 2003)**. The decrease in the activities of SOD, CAT and GSH-Px might be due to their utilization by the enhanced production of ROS **(Rajendra *et al.,* 2003)** or might be attributed to the direct effect of radiation energy that can break some chemical bonds of enzyme molecules, as well as to the indirect effect of radiation whereby the excess of $^{\bullet}OH$ and H_2O_2 resulting from water radiolysis cause oxidation and denaturation of the molecules and partial inactivation of enzyme **(Pigleot *et al.,* 1990)**. Oxidative damage to the enzymes can cause modification of their activities **(Kregel and Zhang, 2007).** Also, the decrease of SOD, CAT and GSH-Px activities in liver, heart and pancreas tissues might be due to their release after cell membrane

destruction by the action of lipid peroxidation **(Saada and Azab 2001)**. However, a decrease in antioxidant enzyme activities might also result from inhibition of enzyme synthesis (**Cristman *et al.*, 1985)**.

The significant increase of SOD and catalase activities recorded on the 1st day post-irradiation in liver and pancreas tissues would indicate a higher exposure of these tissues to the risk of oxidative stress **(John *et al.*, 2001).** The increase of SOD activity is probably a response to the higher superoxide anion generation in these tissues. However, too much SOD may be deleterious to the tissues, because the overproduction of H_2O_2 is not rapidly detoxified **(Kehrer and Smith, 1994).** With respect to pancreas tissues, **Oliveira *et al.*, (1999)** observed that the increase of SOD and catalase activities might be a response towards the toxicity of high glucose levels.

The present study also revealed that GSE pretreatment causes significant decrease in the amount of reactive oxygen species and lipid peroxidation in the heart, liver and pancreas tissues induced by radiation injury. In the same context, GSE provided significant protection against oxidative stress in cardiac **(Ray *et al.*, 2000)** and liver tissues of mice **(Said and Hanafy, 2006)**.

Recently, the study of **Karthikeyan *et al.*, (2007)** indicated that prior administration of GSE resulted in

maintaining the activities of glutathione-dependent antioxidant enzymes, glutathione peroxidase and glutathione transferase, and significantly reduced the lipid peroxidation in isoproterenol-induced tissues injury in rats. Such protection has been primarily attributed to the antioxidant effectiveness of GSE.

5.2. Radiation Effects on Red Blood Cells Count and Hemoglobin Content

Red blood cells are also known as RBCs or erythrocytes. Erythrocytes consist mainly of hemoglobin, a complex molecule containing heme groups whose iron atoms temporarily link to oxygen molecules in the lungs or gills and release them throughout the body. The hematological syndromes are of primary significance in the diagnosis of chronic radiation sickness because the hematopoietic system is highly radiosensitive **(Cockerham and Kelman, 1988)**.

Erythrocytes contain chemical and enzymatic defense systems. Chemical defense systems consist of a pool of antioxidants such as α-tocopherol present in the plasma membrane as well as reduced glutathione and ascorbic acid present in cytoplasm. The enzymatic defense systems are composed by cytosolic enzymes such as methemoglobin reductase, glutathione peroxidase, superoxide dismutase and catalase **(Chiu *et al.*, 1989).** These compounds can scavenge free radicals and also take part in the repair processes of target

molecules by adding hydrogen to organic radicals **(Petkau, 1988)**.

Erythrocytes and leukocytes are particularly susceptible to oxidative damage because of their membranes rich in polyunsaturated fatty acids that can potentiate free radical reactions **(Brown *et al.*, 1996)** or due to the spontaneous generation of superoxide, peroxide and hydroxyl radicals **(Hebbel *et al.*, 1982).** If the RBC is unable to detoxify these ROS, this will probably disturb capillary perfusion and finally, RBCs are lysed or sequestered in the spleen (**Lee and Ducoff, 1994).** So, hematopoietic disorders are one of the most clinical sequels of mammals to ionizing radiations. The hematopoietic symptoms that appear after irradiation include leukocytosis, hyperemia, lymphopenia and hemolysis **(Gridley *et al.*, 2001).**

In the present study, whole body gamma irradiation of rats with 5 Gy (one shot dose) has produced a significant decrease in RBCs count that may be attributed to increased hemorrhage due to the failure of thrombopoiesis **(Hassan *et al.*, 1996).** A decrease in erythrocytes count may result by a drop in their production resulting from radiation-induced damage to the blood-producing cells **(Lass and Sohal, 2000).** However, under the influence of different radiations, the bone marrow was reported to be the most sensitive tissues **(Misra *et al.*, 2005)**. The shortage of red blood

cells (anemia) causes fatigue, weakness, paleness, hemorrhage and difficulty breathing.

Glutathione serves as a co-substrate for glutathione peroxidase in RBC, while glutathione reductase generates GSH via the oxidation of NADPH. The primary source of NADPH in RBC is pentose phosphate shunt, which depends on the activity of glucose-6-phosphate dehydrogenase (G6PD). Thus an alteration of GSH and G6PD activity was reported to increase the vulnerability of RBCs against radiation **(Saada *et al.*, 1999)**.

Misra *et al.*,(2005) observed that RBC membrane constituents', which are responsible for the maintenance of the normal structure and function of membrane, were damaged by γ-radiation probably via the inhibition of membrane enzymes like adenosine triphosphatase (ATPase) and acetyl cholinesterase and G6PD, in addition to alteration in GSH content and enhancement of lipid peroxidation. Thus, the combined effects of radiation induced biochemical consequences challenged the integrity of the RBC and thus hemolysis took place. Haemolysis could be considered as a special example of the interphase death of non dividing cells **(Szweda-Lewandowska *et al.*, 2003)**.

Furthermore, radiation oxidizes sulfhydryl groups situated on the protein surface (SH groups) in the erythrocyte membrane, leading to potassium and sodium accumulation, and as a

consequence of ion imbalance, cells swell and hemolysis occur **(Weinmann *et al.,* 2004).**

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells. Radiation-induced damage to hemoglobin has been considered as one of the most important mechanisms triggering radiation sickness **(Nunia *et al.,* 2006).** In the present study, the significant decrease of Hb content might be linked to the decrease of RBCs count, erythrocytes consist mainly of haemoglobin. Furthermore, ROS induced hemoglobin oxidation and denaturation **(Mieczyslaw *et al.,* 2004)**. Exposure to γ-radiation produces a decrease in hemoglobin binding to erythrocyte membrane **(Dreval and Sichevskaia, 2000)**.

Radiation-induced membrane damage, hemoglobin oxidation and loss of reduced glutathione. On the basis of these observations one could suggest that under the conditions of irradiation, the depletion of not only of GSH but also of other antioxidants can take place at RBCS membrane **(Anita *et al.,* 2006)**. In addition, the processes of lipid and protein destruction in RBCs membrane which is the main intracellular erythrocyte target for radiation and these can lead to hemoglobin oxidation **(Koufen *et al.,* 2000).**

In the present study rats pretreated with GSE demonstrated marked protection against the radiation-induce haemolysis. GSE

natural antioxidant, which suppressed lipid peroxidation and protected membrane proteins against degradation induced by peroxyl radicals, also effectively delayed free radicals-induced hemolysis **(Cheng-Gang *et al.,* 2001).**

5.3. Radiation Effects on Serum Enzymes Activity

Unlike free radicals, lipid peroxides are long lived and can therefore spread from their sites of origin and attack targets distant from the initial radical event by circulating in blood **(Esterbauer *et al.,* 1996).** Plasma low density lipoproteins are susceptible to lipid peroxidation and the oxidized lipoproteins are capable of inhibiting cell functions and can even mediate direct cytotoxicity **(Diaz *et al.,* 1997).**

In the present study, whole body gamma irradiation of rats with 5 Gy induced significant increases in the activity of serum LDH, CPK, AST and ALT on the 1^{st}, 14^{th} and 28^{th} day post-irradiation. According to the results obtained in the present study, the increase of serum enzyme activities is probably the consequence of radiation-induced oxidative stress in the different tissues causing rupture of cell membranes and release of their contents to the blood.

Oxidative stress is the major etiopathological factor in radiation-induced heart toxicity. AST, LDH and CPK have been found to increase in the plasma due to apoptosis and were

considered common characteristics of cardiotoxicity **(Pispirigos and Chrysanthopoulos, 2001).**

Heart tissues are particularly vulnerable to ROS due to a relatively low amount of endogenous antioxidant **(Banerjee *et al.,* 2002)**. Whole body gamma irradiation of rat results in loss of cardiac muscles and accumulation of inflammatory cells surrounded by edema **(Said *et al.,* 2004)**. In the present study, the significant increase in serum LDH, CPK, and AST might therefore results from alterations in dynamic permeability of membranes due to peroxidation. In several organs, cell membrane damage is followed by the release of a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basis for clinical diagnosis **(Gaw *et al.,* 2001).** Alterations in LDH may be attributed to severe damage to heart tissue due to myocardial necrosis and pathological changes in the heart **(Said and Azab, 2006**).

Furthermore, according to the results obtained in the present study, the increase in serum enzymes activity might be due to their release from injured liver cells resulting from radiation induced significant alteration in the architecture of liver tissues associated to an increase in the levels of lipid peroxides **(Said *et al.,* 2003).** The decrease in RBC's count, might also contribute to the increase in serum enzymes activity resulting from their release of red blood cells. The destruction of red blood

cells is characterized by increased lactate dehydrogenase **(Gurreet *et al.*, 2004)**.

Animals pretreated with GSE demonstrated marked protection against the radiation-induce damage. Such protection was manifested by the normalization of cardiac necrosis markers, AST, LDH and CPK activity indicating the cytoprotective property of GSE. In support of our findings, **Hung *et al.*, (2004)** reported that polyphenolic compounds of red grapes offered protection against ischemic reperfusion injury in rat heart.

5.4. Radiation Effects on Serum Glucose and Insulin

It is well known that carbohydrates play a significant role in oxidative processes *in vivo*. Hyperglycemia is associated with an increased oxidative stress status **(Odetti *et al.*, 1999)**. Hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose autooxidation and nonenzymatic glycation processes **(Domínguez *et al.*, 1998).**

The present results showed that whole body gamma irradiation at 5 Gy provoked a significant increase of glucose and insulin levels, 1, 14 and 28 days post-irradiation.

In the present study, the significant increase of serum glucose levels might result from the diminished utilization of glucose by irradiated tissues **(Ahlersova *et al.*, 1988)** or to the indirect effect of radiation exposure which accelerated the

process of gluconeogenesis **(Kilberg and Neuhaus (1976).** The increase of glucose might also reflect impairment in glucose transport **(Dumont *et al.*, 1996).**

Elevated glucose levels in the blood, causes the sugar to chemically react with proteins of the blood vessel walls and form glycosylated proteins that subsequently alters the configuration of proteins. Glycation eventually produce highly complex cross-linked molecules called advanced glycosylation end products (AGEs) that often possess reactive carbonyl (CO) groups **(Thornalley, 2005)**. AGEs and carbonylated aggregates can become cytotoxic and have been associated with a large number of disorders, including Parkinson disease, Alzheimer disease, heart disease and cancer (**Thomas *et al.*, 2005**).

The present results showed that whole body gamma irradiation produced significant increase in insulin levels during the experimental period. Hyperglycemia can lead to multiple complications and, at least in part, to the induction of muscular and adipocyte insulin resistance **(Marfella *et al.*, 2001)**. Insulin resistance may stem from the fact that ROS affect various components of the insulin-signaling cascade, as well as the gene regulation of the glucose transporters **(Burdon, 1995).** Furthermore, radiation is toxic to pancreatic β-cells **(Mazunder *et al.*, 2005)** and oxidative stress cause abnormalities of insulin secretion and actions **(Khamaisi *et al.*, 2001).**

In the present work, the increase of serum insulin level might be attributed to radiation-induced oxidative stress in the pancreas resulting in cell membrane damage and release of insulin. **Harber, (2000)** observed that insulin secretion from β-cells is principally regulated by glucose levels. Increased uptake of glucose by pancreatic β–cells leads to a concomitant increase in metabolism, leading to an elevation in the ATP/ADP ratio. This in turn leads to an inhibition of ATP-sensitive K^+ channel. The net result is a depolarization of the cell leading to Ca^{2+} influx and insulin secretion.

On the other side, oxidative stress contributes, at least in part, to the development of insulin resistance **(Shimosawa, 2005),** characterized by a decline in skeletal muscle glucose utilization and/or an excessive hepatic glucose production **(Bitar *et al.,* 2005).** As consequences of insulin resistance the pancreas continues to produce insulin causing hyperinsulinemia.

In the present study animals pretreated with GSE demonstrated marked protection against the radiation-induce increases in glucose and insulin levels. It has been reported that GSE improve insulin sensitivity or reduce insulin resistance **(Grassi *et al.,* 2005),** whereas reactive oxygen species have a causal role in insulin resistance **(Houstis *et al.,* 2006).** Also, GSE inhibit formation of glycosylated proteins and can help those with eye diseases such as macular degeneration, retinitis,

glaucoma, and myopia (**Osakabe *et al.*, 2004)**. It also protects the tissues from oxidative stress possibly by virtue of its anti-oxidative activity through augmentation of antioxidant enzymes and decreasing the amount of pro-oxidants **(Said *et al.*, 2005).**

5.5. Radiation Effects on Serum Lipid Profile

Lipids in the blood, primarily triglycerides and cholesterol are essential. Yet they become a healthy threat and a factor of reduced longevity when the blood contains too much of them. Hyperlipidemia plays a pivotal role in heart damage, because hyperlipidemia leads to an increase in cardiac peroxynitrite formation and a decrease in the bioavailability of nitric acid, which contributes to the deterioration of cardiac performance and may lead to further cardiac pathologies **(Onody *et al.*, 2003).** So, blood lipids must be maintained at the proper levels and ratios **(Mehta *et al.*, 2003).**

In the present study, whole body gamma irradiation of rats with 5 Gy (one shot dose) produced significant increases of triglycerides (TG), total cholesterol (TC), and low density lipoprotein-cholesterol (LDL-C) along with significant decreases of high density lipoprotein-cholesterol (HDL-C), 1, 14, and 28 days post-irradiation.

Radiation induced dyslipidemic profiles that were associated with increasing oxidative stress. The hyperlipidemic state observed in the present study could be explained on the basis that

radiation can act directly on the metabolic capacity of the liver and intestine which synthesize lipoproteins. **Chrysohoou *et al.,* (2004)** observed that total serum phospholipids, their fractions and cholesterol were significantly changed after radiation exposure. These changes were referred to hepatic damage and to severe anemia (observed in the present results). Furthermore, some serum lipid polyunsaturated fatty acids were significantly altered, since these alterations are a sign of lipid oxidation **(Feurgard *et al.,* 1998)**. The increase in serum TG levels occurring after irradiation might result from inhibition of lipoprotein lipase **(Sedlakova *et al.,* 1986).**

Free radicals impair liver function and can be a major cause of hormone imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of TG and LDL **(Lamarche *et al.,* 1997).** However, hyperlipidemia might result from radiation induces injury to cellular membranes **(Saada *et al.,* 2001)**; consequently fats are released in the circulation, by intestinal mucosa, adipose tissues, and liver. In addition, radiation induced liver injury**,** will affect lipid metabolism and serum lipid profile **(Said *et al.,* 2003).** Thus, the increase in serum cholesterol level due to irradiation may be originated from the migration of tissue cholesterol via the blood circulation or/and the decrease in

utilization of cholesterol for synthesis of higher substances **(Said *et al.,* 2004)**.

In the present study, alteration in the serum lipid profile was associated to high serum glucose and insulin levels all over the experimental period. **McEwen and Stellar, (1993)** observed that hyperglycemia and too much insulin promote LDL-C rise and HDL-C drop. Furthermore, LDL-C particles rich in polyunsaturated fatty acids are vulnerable to oxidation and may have substantial importance on dyslipidemic profile **(Bradamante *et al.,* 2000).**

Experimental studies have demonstrated that insulin resistance constitutes a major pathogenic importance in dyslipidemia **(Bitar *et al.,* 2005)** and may be one of the driving factors behind elevated TG levels **(Schwarz *et al.,* 2003).** Insulin decrease lipolysis by inhibiting the activity of lipases in adipose tissue **(Saravanan *et al.,* 2002***)***.** In the present study, the elevated level of TC might result from increased synthesis in the liver **(Abbady *et al.,* 2000),** as an early reaction necessary for the restoration of biomembranes **(Kolomijtseva, 1986).** Elevated serum LDL-C levels might result from radiation-induced damage to the receptors on the surface of many cells in the body that prevents the ingestion of LDL-C by endocytosis **(Huang *et al.,* 1995).** In the study pretreatment with GSE showed a significant effect on all lipid parameters in the treatment group rats when

compared to irradiated rats. This protective action might be due to the effective quenching of free radicals by GSE. Grape seed extract inhibiting oxidation of LDL, and possesses hypolipidemic effect (**Karthikeyan *et al.*, 2007).**

5.6. Protective Effect of Grape Seed Extract (GSE)

Epidemiological studies have shown that diets rich in plant foods significantly reduce the incidence and mortality rates of degenerative diseases caused by oxidative stress **(Tibble *et al.*, 2000)**. This protective effect has been attributed to the fact that such foods may provide an optimal mix of phytochemicals **(Ames *et al.*, 1993).** Antioxidant vitamins, carotenoids, and especially the polyphenols found in fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods **(Prior, 2003).** For this reason, several international organizations have recommended increasing the consumption of fruits and vegetables to five or more daily servings, in order to provide a desirable intake of antioxidants and to improve human health (**World Health Organization 1990; World Cancer Research Foundation and American Institute for Cancer Research 1997**).

Among the most common dietary sources of natural antioxidants, grapes is rich in polyphenolic compounds, particularly flavonoids, which are valued for their role in anticarcinogenic, antiatherogenic, anti-inflammatory,

antimicrobial and antioxidant activities **(Moure *et al.*, 2001)**. Grape seed extract (GSE) is a natural extract from the seeds of *Vitis vinifera* (Linn.) (Family: Vitaceae). Chemical identification studies showed that GSE contains a wide variety of flavonoids that range from the monomeric flavan-3-ols; (+)-catechin, (-)-epicatechin, (-)-epicatechin 3-gallate, (-)-epigallocatechin, (-) epigallocatechin 3-gallate, (+)-gallocatechin, and anthocyanidins mainly as cyanidin to dimmers, trimers and oligomers with 7 or more flavanol units: the proanthocyanidins. Proanthocyanidins are so called because when broken apart they yield anthocyanidins mainly cyanidin **(Shi *et al.*, 2003).**

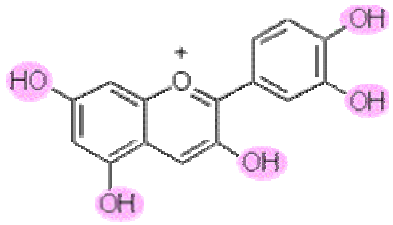
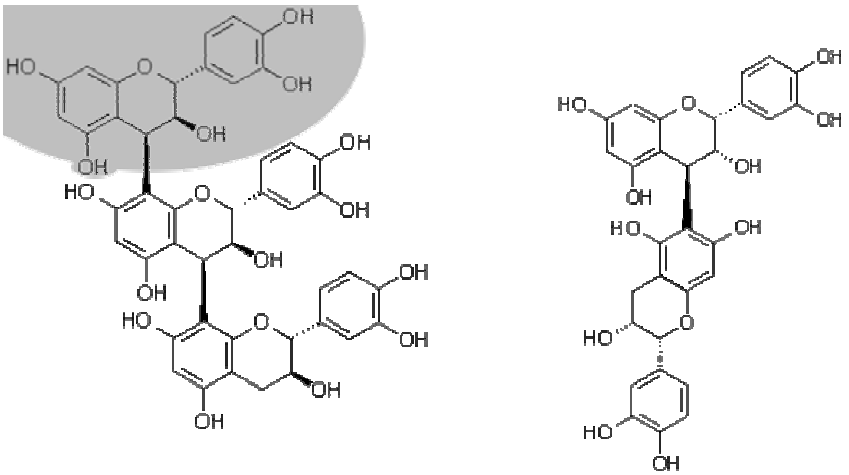


Figure 27 Chemical structure of cyanidin

Experimental investigation has shown that when GSE was orally administered to mice, proanthocyanidins were detected in various organs, but were preferentially bound to the skin, aorta and gastrointestinal mucosa (**Laparra *et al.*, 1977**). Proanthocyanidins are absorbed into the bloodstream in about 20 minutes and can be detected in saliva in less than one hour **(da Silva *et al.*, 1991).** Studies on adult humans revealed that GSE is

highly bioavailable, and after intake of GSE, proanthocyanidins were detected in the serum 2 hrs later **(Sano *et al.*, 2003)**.



a) Proanthocyanidin (3 subunits) b) Proanthocyanidin (2 subunits). The shaded area represents one subunit, which is the flavanol known as *cyanidin.*

Figure 28 Proanthocyanidins

In the present study, experimental animals receiving 100 mg/Kg body weight grape seed extract (GSE), for 14 successive days showed no significant changes in SOD, CAT, GSH-Px activity, and TBARS content of heart, liver and pancreas tissues. Furthermore, there were no significant changes in RBCs count and Hb content. Serum LDH, CK, AST and ALT showed normal activities. Serum glucose, insulin, TG, TC, LDL-C, HDL-C, levels were within normal ranges. The results are consistent with available data indicating that ingestion of 75mg - 300 mg grape polyphenols is safe **(Dubnick and Omaye, 2001)**. Furthermore, **Bentivegna and Whitney, (2002)** reported that clinical pathology and histological examination of different tissues

demonstrated no treatment-related changes of toxicological significance. Meanwhile, dosages of GSE should be optimized to avoid potential harmful pro-oxidant effects **(Shao *et al.*, 2003).**

Experimental studies demonstrated that GSE is effective in minimizing events linked to oxidative stress **(Yamakoshi *et al.*, 2002)** and provides excellent protection against free radical-mediated tissue injury **(Ariga, 2004),** and intestinal mucosa damage **(Flávia *et al.*, 2006).** In the present work, the data obtained showed that rats supplemented with 100 mg GSE/Kg body weight/day for 14 successive days before whole body exposure to 5Gy gamma radiation showed an increased resistance to oxidative challenge. GSE pre-treatment has significantly improved the antioxidant status of heart, liver and pancreas tissues in irradiated rats. This was obvious by an increase in the activities of SOD, CAT and GSH-Px, compared to the corresponding values of irradiated rats.

Furthermore, GSE treatment has significantly minimized the formation of lipid peroxidation products obvious by a lower level of TBARS when compared to their corresponding values in irradiated rats. The results are consistent with **Hüseyin *et al.*, (2007)** who reported that GSE enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation when administered to rats before whole-body irradiation. Furthermore, experimental studies have demonstrated

that GSE protects against oxidative stress by doubling the intracellular synthesis of anti-oxidative enzymes **(Puiggros *et al.*, 2005),** and improving glutathione synthesis **(Valls-Belles *et al.*, 2006).**

In the present study, the protective effect of GSE against radiation-induced oxidative tissue damage was observed since the 1st day post-irradiation indicating that the lipophilicity of GSE phenolic compounds enhances their rapid cellular uptake and their localization in lipid compartments **(Sestili et al. 2002)**, thereby, scavenging radiation-induced hydroxyl radicals **(Sreemantula et al. 2005),** and reducing lipid peroxidation in cell membranes. Furthermore, the significant improvement of radiation-induced oxidative damage may be attributed to the role of proanthocyanidins in scavenging peroxyl radicals **(Hwang *et al.*, 2004)**, and nitric oxide radicals **(Yoshimura *et al.*, 2003).** Furthermore, GSE inhibits lipoxygenase and cyclo-oxygenase activities, thereby preventing lipid oxidation **(Murray and Pizzorno, 1999).** Moreover, *in vitro* experimental results have demonstrated proanthocyanidins have the ability to inhibit the activity of xanthine oxidase, a major generator of free radicals **(Murray and Pizzorno, 1999)**.

However, in addition to its radical scavenging activity, proanthocyanins possess an ideal structural chemistry to chelate free iron molecules **(Bagchi *et al.*,1998),** supporting the role of

polyphenols as preventative antioxidants in terms of inhibiting transition-metal catalyzed free radical formation **(Rice-Evans et al. 1997)**. In this regard, it has been shown that cell death induced by radiation depends on a cellular source if iron. Peroxides react with ferrous iron to produce more potent oxidants, the hydroxyl and t-butyl alkoxyl radicals، respectively, by Fenton chemistry **(Farber *et al.,* 1990).** Thus, iron chelation will decrease the toxicity of oxygen to cells and may contribute to the radio-protective activity of GSE **(Sestili *et al.,* 2002)**.

The results obtained in the present study showed that oral supplementation of GSE before irradiation has significantly improved RBCs count and Hb content, which might be attributed to the free radical scavenging activity of GSE leading to the preservation of RBCs membrane and its content of Hb. Furthermore, the significant amelioration of serum LDH, CPK, AST and ALT activities recorded in GSE-treated irradiated rats might be attributed to its antioxidant properties, which is substantiated by the remarkable reduction in the levels of TBARs in cardiac and liver tissues, and enhanced antioxidant activity of SOD, CAT and GSH-Px in the different tissues, compared to their corresponding values in irradiated rats. So by protecting cellular membrane from radiation-induced injury, it will prevent the release of intracellular enzymes.

In the present study, oral supplementation of GSE has significantly ameliorated the radiation-induced hyperglycemia, and hyperinsulinemia, which is consistent with the effects of GSE on insulin resistance **(Al-Awwadi *et al.*, 2005).** However, the blood glucose-lowering activity of GSE has been defined for epicatechin, catechin, and epicatechin gallate. These monomeric forms act through different mechanisms: epicatechin induces pancreatic ß-cell regeneration **(Kim *et al.*, 2003**); catechin inhibits intestinal glucose absorption **(Shimizu *et al.*, 2000**), and epicatechin gallate increases hepatic glycogen synthesis **(Waltner-Law *et al.*, 2002**).

Furthermore, GSE possesses cholesterol-lowering effect **(Tebib *et al.*, 1994**) and in vitro studies showed that it stimulates lipolysis **(Vogels *et al.*, 2004).** In the present study, the increase of TG, TC, LDL-C, as well as the decrease of HDL-C was less marked in the serum of GSE-treated irradiated rats when compared to the corresponding values of irradiated rats.

According to the results obtained in the present study, it could be concluded that GSE, by enhancing antioxidant activities and decreasing lipid peroxidation, it protects cellular membrane from oxidative damage, preserve the integrity of tissue functions and minimize metabolic disorders induced by ionizing irradiation. Hence GSE administration prior to radiation therapy may be useful to cancer patients to prevent normal cell damage.

SUMMARY

Grapes and grape byproducts contain large amounts of phenolic compounds; mostly flavonoids at high concentrations. The present study was designed to investigate the effect of aqueous extract of grape seed (GSE) on gamma radiation-induced oxidative stress in liver, heart and pancreas tissues of rats in parallel to serum metabolic disturbances.

Rats received GSE (100 mg/day/Kg body weight) in 0.5ml distilled water, by gavages, for 14 successive days before whole body exposure to 5Gy γ-radiation (shot dose). A total of 72 rats were divided into 4 equal groups.

1. **Control group**: rats received 0.5ml distilled water, by gavages, for 14 consecutive days.

2. **GSE-treated group**: rats received GSE (100mg/Kg body weight/day) for 14 consecutive days.

3. **Irradiated group**: rats were whole body exposed to 5Gy gamma irradiation applied as one shot dose.

4. **GSE-treated irradiated group**: rats received GSE during 14 consecutive days, by gavages, before whole body exposure to gamma irradiation at 5 Gy.

1-Radiation-Induced Alteration in Antioxidant Status of Cardiac, Liver and Pancreas tissues

The results obtained in the present study showed that whole body gamma irradiation of rats with 5 Gy provoke oxidative stress in heart, liver and pancreas tissues. Significant ($p \leq 0.05$) increase in the levels of thiobarbituric acid reactive substances (TBARS) concomitant with significant decrease in the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were recorded 1, 14 and 28 days post-irradiation in cardiac tissues. The same pattern of changes was recorded in liver and pancreas tissues, except a significant increase of SOD and CAT activities recorded on the 1st day post-irradiation for both tissues, compared to control values.

2-Radiation-Induced Alteration in Serum Enzyme Activities

The results in the present study showed that whole body exposure of rats to 5 Gy induced significant increases in the activity of serum lactate dehydrogenase (LDH), creatine phophokinase (CPK), aspartate and alanine amino transferase (AST and ALT) 1, 14, and 28 days post-irradiation, compared to control values.

3-Radiation-Induced Alteration in Red Blood Cells (RBCs) Count and Hemoglobin Content

Whole body exposure of rats to 5 Gy induced a significant decrease of hemoglobin (Hb) content associated to a significant decrease in RBCs count 1, 14 and 28 days post-irradiation, compared to corresponding control values.

4-Radiation-Induced Alteration in Serum Glucose and Insulin Levels

Whole body exposure of rats to 5 Gy induced significant ($p \leq 0.05$) increase in insulin and glucose levels 1, 14 and 28 days post-irradiation, compared to corresponding control values.

5-Radiation-Induced Alteration in Serum Lipid Profile

Whole body exposure of rats to 5 Gy results in hyperlipidemic state obvious by significant increase of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), along with significant decrease of high density lipoprotein-cholesterol (HDL-C) 1, 14, and 28 days post irradiation as compared with control values.

6-Protective Effect of Grape Seed Extract (GSE)

The results in the present study showed that experimental animals receiving 100 mg/Kg body weight GSE for 14 successive days showed no significant changes in SOD, CAT, GSH-Px activities, and TBARS contents of heart, liver and pancreas tissues. Furthermore, there were no significant changes in RBCs count and Hb content. Serum LDH, CPK, AST and ALT showed normal activities. Serum glucose, insulin, TG, TC, LDL-C, HDL-C, levels were within normal ranges.

Experimental animals receiving 100 mg/Kg body weight GSE for 14 successive days before whole body exposure of rats to 5 Gy of ionizing gamma radiations showed an increased resistance to oxidative challenge. The results showed that GSE enhances antioxidant defenses and specific metabolic activities of rat heart, liver, and pancreas. This was obvious by an increase in the activities of SOD, CAT and GSH-Px, compared to the corresponding values of irradiated rats. Furthermore, GSE treatment has significantly minimized the formation of lipid peroxidation products obvious by a lower level of TBARS when compared to their corresponding values in irradiated rats. Significant amelioration in the antioxidant status of cardiac and liver tissues was substantiated by the significant amelioration of serum LDH, CPK, AST and ALT activities.

The results in the present study showed that treatment with GSE has significantly protected against radiation-induced decrease of RBCs and Hb content. GSE treatment has also ameliorated the radiation-induced hyperglycemia and hyperinsulinemia. Furthermore, the marked increase in serum TG and TC observed in irradiated rats, along with elevated LDL-C and decreased HDL-C levels were significantly improved in GSE treated rats

According to the results obtained it could be concluded that GSE might improve metabolic functions and attenuates radiation-induced oxidative organ injury.

REFERENCES

Abbady MM, Said OZ, Mohamed MA, Saada HN and Azab KhSh (1999): Action of prolonged treatment with cod liver oil on certain biochemical changes in rats exposed to fractionated gamma irradiation. Isotope and Rad Res 31: 47-54.

Abbady MM, Zahran AM and Ragab EA (2000): Biochemical studies on rosemary extract as antioxidant in irradiated rats. Egypt J Rad Sci Applic 16(1): 139-151.

Aebi H (1983): Catalase. In: Bergmeyer H U eds. Methods of Enzymatic Analysis 2nd ed.: 273-277 Verlag Chemie Weinheim, Germany.

Ahlersova E, Ahlers I and Molcanova A. (1988): Serum glucose and liver glycogen in gamma irradiated rats. Radiobiol Radiother 29: 156-158.

Al-Awwadi NA, Araiz C, Bornet A, Delbosc S, Cristol JP, Linck N, Azay J, Teissedre PL and Cros G (2005): Extracts enriched in different polyphenolic families normalize increased cardiac NADPH oxidase expression while having differential effects on insulin resistance, hypertension, and cardiac hypertrophy in high-fructose-fed rats. J Agric Food Chem 53(1):151-7.

Ames BN, Shigenaga MK and Hagen TM (1993): Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci (USA) 90: 7915.

Ariga T (2004): The antioxidative function, preventive action on disease and utilization of proanthocyanidins. Biofactors 21(1-4):197-201.

Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H (1984): LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulfate. Clin Chim Acta; 140:77-83.

Ayako S, Shuji I and Keizo U (2004): Grape Seed Extract Prevents H_2O_2-Induced Chromosomal Damage in Human Lymphoblastoid Cells. Biol. Pharm. Bull. 27(9): 1459-1461.

Azab KhSh and Nada ASh (2004): Restitution of energy metabolism in irradiated rats considering Curcumin antioxidant capacity and metal biotransformation. Egypt. J. Sci., Applic. Vol. 37, No, 2, pp. 169-176.

Azab KhSh, El Tahawy NA and Aneas LA (2003): Role of taurine in attenuation of oxidative damages in irradiated rats. The Egypt J of Biochem & Mol Biol 21 (1):173-184.

Azab KhSh, Said UZ, and Abbady MM (1999): The impact of continuous supplementation of vitamin B compounds against radiaiotn injury in rats. J of the Med Res Inst 20 (2): 38-46.

Bagchi D, Bagchi M, Stohs S, Ray SD, Sen CK and Preuss HG (2002): Cellular protection with proanthocyanidins derived from grape seeds. Ann N Y Acad Sci 957:260-70.

Bagchi D, Bagchi M, Stohs Sj, Das DK, Ray SD, Kuszynski CA, Joshi SS and Preuss HG (2000): Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 148(2-3):187-97.

Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J and Stohs SJ (1998): Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. Gen Pharma 30: 771–776.

Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG and Vinson JA (2003): Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. Mutat Res 524: 87-97.

Balu M, Sangeetha P, Haripriya D and Panneerselvam CH (2006): Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neuroscience Letters 383: 295-300.

Banerjee B and Bagchi D (2001): Beneficial effects of a novel IH636 grape seed proanthocyanidin extract in the treatment of chronic pancreatitis. Digestion 63 (3):203-6.

Banerjee SK, Maulik M, Mancahanda SC, Dinda AK, Gupta SK and Maulik SK (2002): Dose dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. Life Sci 70:1509- 1518.

Bentivegna SS and Whitney KM (2002): Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. Food Chem Toxicol 40 (12): 1731-43

Bhosle SM, Huilgol NG and Mishra KP (2005): Enhancement of radiation-induced oxidative stress and cytotoxicity in tumor cells by ellagic acid. Clin Chim Acta 359: 89–100.

Bitar MS, Al-Saleh E and Al-Mulla F (2005): Oxidative stress-mediated alterations in glucose dynamics in a genetic animal model of type II diabetes Life Sci [Epub ahead of print]**.**

Bradamante S, Piccinini F, Barenghi L, Bertelli AA, De Jonge R, Beemster P and De Jong JW (2000): Does resveratrol induce pharmacological preconditioning? Int J Tissue React 22: 1-4.

Brown KM, Morrice PC, Athur JR and Duthie GG (1996): Effects of vitamin E supplementation on erythrocyte antioxidant mechanisms of smoking and non-smoking men. Clin Sci 91: 107.

Burdon RH (1995): Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radical Biol & Med 18:775-794.

Burtis CA and Ashwood ER (1994): In Clinical Chemistry. W.B. Saunders Company, Philadelphia. Page 873.

Cadenas E and Davies KJ (2000): Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29: 222-230**.**

Cederaum AI (2001): Introduction-Serial review: Alcohol, oxidative stress, and cell injury. Free Radical Biology & Medicine 31:1524–1526.

Chance B, Sies H and Boveris A (1979): Hydroperoxide metabolism in mammalian organisms. Physiological Reviews 59:527–605.

Cheng-Gang Z, Nihal S and Graham L (2001): Oxidative insult to human red blood cells induced by free radical initiator AAPH and its inhibition by a commercial antioxidant mixture. Life Sciences 69: 75–86.

Chiu F-C, Barnes EA, Das K, Haley J, Sorcolow P, Macaluso FP and Fant J (1989): Characterization of a novel 66 kd subunit of mammalian neurofilaments. Neuron 2:1435–1445.

Chrysohoou C, Demosthenes B P, Christos PK, John B, Markisia K, Ioannis L and Christodoulos S (2004): Distribution of serum lipids and lipoproteins in patients with β- thalassaemia major; an epidemiological study in young adults from Greece. Lipids Health Dis 3: 3.

Cockerham L and Kelman B (1988): Summary of symposium on acute radiation-induced injury. Fundam Appl Toxicol 11 (4):571-2.

Cristman MF, Morgan RW, Jacobson FS and Ames BN (1985): Positive control for defenses against oxidative

stress and some heat-shock proteins in Salmonella typhymurium. Cell 41: 753-762.

Da Silva J, Rigaud J and Cheynier V (1991): Procyanidin dimers and trimers from grape seeds. *Phytochemistry*; 30:1259-1264.

Dacie SI and Lewis SM (1991): Practical haematology (7th edition) J and A Churchill Ltd. Livingston, London, Melborne and New York.

Dalton TP, Shertzer HG and Puga A (1999): Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 39: 67- 101.

de Groot H (1994): Reactive oxygen species in tissue injury. Hepato–Gastroenterology 41:328–332.

Devi GS, Prasad MH and Saraswathi I (2002): Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. Clin Chem Acta 293:53-62.

Dhanalakshmi S, Agarwal R and Agarwal C (2003): Inhibition of NF-kappaB pathway in grape seed extract-induced apoptotic death of human prostate carcinoma DU145 cells. Int J Oncol 23(3):721-7.

Diaz MN, Frei B, Vita JA and Keaney JF (1997): Antioxidants and atherosclerotic heart disease. N Engl J Med 337:408-16.

Dominguez C, Ruiz E, Gussinye M and Carrascosa A (1998): Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care 21:1736-1742.

Dreval' VI, Sichevskaia LV, Doroshenko AO and Roshal' AD (2000): Radiation induced changes in the structure of erythrocyte membrane proteins. Biofizika 45: 836- 838.

Drogue W (2002): Free radicals in the physiological control of cell function. Physiol Rev 82: 47-95.

Du Y, Guo H and Lou H (2007): Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. J Agric Food Chem. 55(5):1695-701.

Dubnick MA and Omaye ST (2001): Evidence for grape, wine and tea polyphenols as modulators of atherosclerosis and heart disease in humans. J Nutra Func Med Foods 3: 67-93.

Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N and Sener G (2007): Grape seed extract reduces oxidative

stress and fibrosis in experimental biliary obstruction. J Gastroenterol Hepatol. 22(6):885-92.

Dumont AE, Martelli AB, and Mulholland JH (1996): Alterations in tissue reactivity of thoracic duct lymph produced by retention of excess pancreatic secretion. Proc Soc Exp Biol Med 121:1-5.

El-Missiry MA, Fayed TA, El-Sawy MR and El-Sayed AA (2007): Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol Environ Saf 66(2):278-86.

Eng ET, Ye J, Williams D, Phung S, Moore RE, Young MK, Gruntmanis U, Braunstein G and Chen S. (2003): Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds**.** Cancer Res 63(23):8516-22.

Erexson GL (2003): Lack of in vivo clastogenic activity of grape seed and grape skin extracts in a mouse micronucleus assay. Food Chemistry and Toxicology; 41(3):347-350.

Esterbauer H (1996): Estimation of peroxidative-damage: A critical review. Pathol Biol Paris 44: 25–28.

Farber JL, Kyle ME and Coleman JB (1990): Mechanisms of cell injury by activated oxygen species. Lab Invest. 62:670-679.

Ferreira D and Li XC (2000): Oligomeric Proanthocyanidins: Naturally Occurring O-heterocycles. Nat Prod Rep 17: 193-212.

Feurgard C, Bayle D, Guezingar F, Serougne C, Mazur A, Lutton C, Aigueperse J, Gourmelon P and Mathe D (1998): Effects of ionizing radiation (neutrons/ gamma rays) on plasma lipids and lipoproteins in rats. Rad Res 150(1): 43.

Finch SC (1991): Radiation Injury. In: Wilson JD, Braunwald E, Isselbacher EJ. Harrison's principles of internal medicine, 12th ed. New York: McGraw Hill, 2201-2208.

Fitzpatrick DF, Bing B, Maggi DA, Fleming RC and O'Malley RM (2002): Vasodilating procyanidins derived from grape seeds. Ann NY Acad Sci 957:78-89.

Flávia M, Frank S, Pedro D, Flávio R, Frab N, Solange M, Flávia M, Frank S, Pedro D, Flávio RM, Frab N and Solange M (2006): Pycnogenol protects against Ionizing radiation as shown in the intestinal mucosa of rats exposed to X-rays. Phytotherapy Research 20(8**):** 676 – 679.

Fridovich I (1997): Superoxide anion radical, superoxide dismutases, and related matters. Journal of Biological Chemistry 272:18515–18517.

Friedewald WT, Levy RI, and Fredrickson DS (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18(6):499-502.

Garcia-Alons M, Rimbach G, Rivas-Gonzalo JC, De Pascula S (2004): Antioxidant and cellular activities of anthocyanins and their corresponding vitisins. A study in platelets, monocytes, and human endothelial cells. J Agric Food Chem 52: 3378–3384.

Gaw A, Cowan R, O'reilly D, Stewart M and Shepherd J (2001): Bioquímica Clínica. 2nd ed. Rio de Janeiro: Guanabara Koogan.

Goyns MH (2002): Genes, telomeres, and mammalian ageing. Mech Aging Dev 123: 791–799.

Grassi D, Lippi C, Necozione S, Desideri G and Ferri C. (2005): Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. Am J Clin Nutr 81:611– 4.

Gridley DS, Pecaut MJ, Miller GM, Moyers M F and Nelson GA (2001): Dose and dose rate effect of whole body gamma irradiation. II- Hematological variations and cytokines. Vivo 15(3): 209.

Groopman JE and Itri LM (1999): Chemotherapy induced anaemia in adults: incidence and treatment. J Natl Cancer Inst 91: 1616–1634.

Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S and Prior R L (2002): Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. J Agric Food Chem 50:4852-4860.

Gupta LB, Anju P and Najma ZB (2004): Protective effects of sodium orthovanadate in diabetic reticulocytes and ageing red blood cells of Wistar rats. J. Biosci. 29, 1, 73–79.

Gupta A, Rosenberger SF and Bowden GT (1999): Increased ROS levels contribute to elevated transcription factor and MAP kinase activities in malignantly progressed mouse keratinocyte cell lines. Carcinogenesis 11:2063–2073.

Gurreet MD, Patricia A, Cornett MD, Lawrence M, and Tierney JR, (2004): Hemolytic Anemia. Am Fam Physician; 69:2599-2606.

Gutteridge JM and Mitchell J (1999): Redox imbalance in the critically ill. Br Med Bull 55: 49-75.

Hahn UK, Bender RC, Bayne CJ. (2000): Production of reactive oxygen species by hemocytes of Biomphalaria glabrata: carbohydrate-specific stimulation. Dev. Comp. Immunol. 24: 531-541.

Halliwell B (1995): Oxidation of low-density lipoproteins: questions of initiation, propagation, and the effects of antioxidants. Am J Clin Nutr 61:670-7.

Halliwell B (1996): Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritinal antioxidant intake in humans. Free Radic Res 25: 57-74.

Halliwell B (1999): Antioxidant defense mechanisms: From the beginning to the end. Free Radical Research 31:261–272.

Halliwell B and Gutteridge J (1998): Free radicals in biology and medicine. 3rd edition, chapter 9, pp 616, Oxford University Press.

Halliwell B and Gutteridge J. (2006): Free Radicals in Biology and Medicine, Ed 4. Clarendon Press, Oxford.

Harber HA (2000): Review of Physiological Chemistry. Book published by Lange Medical Publications, copyright p-702.

Hassan S, Abu Ghadeer AR, Osman SA and Roushdy M (1996): Possible curative role of the antipsychotic drug "Fluphenazin"against post-irradiation injury in rats. Egypt J Rad Sci Applic **7**(2): 181.

Hebbel RP, Eaton JW, Balasingan M and Steinberg MH (1982): Spontaneous oxygen radical generation by sickle erythrocytes. Journal of Clinical Investigation 79: 1254–1260.

Holger L, Melanie S, Vivienne W and Uwe S (2005): The Creatine Kinase System in Human Skin: Protective Effects of Creatine against Oxidative and UV Damage In Vitro and In Vivo. Journal of Investigative Dermatology 124: 443–452.

Houde V, Grenier D and Chandad F (2006): Protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens. J Periodontol 77(8):1371-9.

Houstis N, Rosen ED and Lander ES (2006): Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 440: 944–8.

Hung KT and Kao CH (2004): Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. J Plant Physiol 161: 43-52.

Hüseyin E, Mustafa C, Turan K and Perihan U (2007): Effect of grape seed extract on lipid peroxidation, antioxidant activity and peripheral blood lymphocytes in rats exposed to x-radiation. Phytotherapy Research 21 (11):1029- 1035**.**

Hwang IK, Yoo KY, Kim DS, Jeong YK, Kim JD, Shin HK, Lim SS, Yoo ID, Kang TC, Kim DW, Moon WK and Won MH. (2004): Neuroprotective effects of grape seed extract on neuronal injury by inhibiting DNA damage in the gerbil hippocampus after transient forebrain ischemia. Life Sci75 (16):1989-2001.

Ishikawa M, Maki K, Tofani I, Kimura K and Kimura M. (2005): Grape seed proanthocyanidins extract promotes bone formation in rats mandibular condyle. Eur J Oral Sci 113(1):47-52.

John S, Kale M, Rathore N and Bhatnager D (2001): Protective effect of vitamin E in dimethoate and malathion

induced oxidative stress in rat erythrocytes. J Nutr Biochem 12: 500-504.

Kalpana C, Menon VP and Pol J (2004): Modulatory effects of curcumin on lipid peroxidation and antioxidant status during nicotine-induced toxicity. Pharmacol 56:581-586.

Karbownik M and Reiter R (2000): Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proceedings of the Society for Experimental Biology and Medicine 225:9-22.

Karthikeyan K, Sarala B and Niranjali D (2007): Efficacy of grape seed proanthocyanidins on serum and heart tissue lipids in rats subjected to isoproterenol-induced myocardial injury. Vascular Phar47: 295–301.

Kehrer JP and Smith CV (1994): Free radicals in biology: sources, reactivities and roles in the etiology of human dieseases. Pages 25–62 in Natural Antioxidants in Human Health and Diseases, B. Frei, ed. Academic Press, San Diego, CA.

Khamaisi M, Kavel O, Rosenstock M, Porat M, Yuli M, Kaiser N and Rudich A (2001): Effect of inhibition of glutathione synthesis on insulin action: *in vivo* and *in vitro*

studies using buthionine sulfoximine. Biochem J 349: 579–586.

Khan KK, He YQ, Domanski TL and Halpert JR (2001): Midazolam oxidation by cytochrome P450 3A4 and active-site mutants: an evaluation of multiple binding sites and of the metabolic pathway that leads to enzyme inactivation. Mol Pharmacol 61**:** 495–506.

Kidd PM (1991): Natural antioxidants first line of defense. PMK Biomedical-Nutritional Consulting 115-142.

Kilberg MS and Neuhaus OW (1976): Role of stimulated amino acid transport in promoting glycogenesis in irradiated rats. Rad Res 66:597.

Kim MJ, Chung JS, Sim SS, Min DS, Rhie DJ, Yoon SH, Hahn SJ, Kim MS and Jo YH (2003): Protective effects of epicatechin against the toxic effects of streptozotocin on rat pancreatic islets: in vivo and in vitro. Pancreas 26:292–299.

Kohen R and Nyska A (2002): Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol; 30: 620-50.

Kolomijtseva IK (1986): On activation of cholesterologenesis under the effect of ionizing radiation on mammalian body. Radiobiologiya 26(1):3-10.

Koufen P, Brdicka D and Stark G (2000): Inverse dose-rate effects at the level of proteins observed in the presence of lipids. Int J Radiat Biol 76: 625–631.

Kregel KC and Zhang HJ (2007): An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 292:18- 36.

Kris EM and Keen CL (2002): Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. Lipid 13:41-9.

Krokosz A (2003): The effect of hypochlorite on human erythrocytes pretreated with X-radiation. Cell Mol Biol Lett 8(1):215-9.

Kuhn MA (2003): Oxygen free radicals and antioxidants. Am J Nurs 103: 58-62.

Lakshmi B, Tilak J, Adhikari S, Devasagayam T and Janardhanan K (2005): Inhibition of lipid peroxidation

induced by γ-radiation and AAPH in rat liver and brain mitochondria by mushrooms. Curr Sci 88: 484-490.

Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ and Despres JP (1997): Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 95:69-75.

Lander HM (1997): An essential role for free radicals and derived species in signal transduction. FASEB J. 11:118–124.

Laparra J, Michaud J and Masqualier J (1977): Pharmacokinetic study of Flavanolic oligomers J Plant Med Phytother 11: 133-142.

Lass A and Sohal R S (2000): Effect of coenzyme Q_{10} and α-tocopherol content of mitochondria on the production of superoxide anion radicals. The FASEB Journal 14:87-94.

Lawrence RA and Burk RF (1976): Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 71(4):952-8.

Lee SW and Ducoff HS (1994): The effects of ionizing radiation on avian erythrocytes. Radiation Research 137(1): 104-110.

Lunec J, Holloway KA, Cooke MS, Faux S, Griffiths HR and Evans MD (2002): Urinary 8-oxo-2′-deoxyguanosine: redox regulation of DNA repair in vivo. Free Radic Biol Med 33: 875-85.

Mahdy AM, Saada HN, El-Naggar AM, Abd El Salam AM and Said UZ (1996): The effect of ascorbic acid and serotonin on some carbohydrate metabolites and enzymatic activities in rats exposed to fractionated gamma irradiation. J Egypt Ger Soc Zool 21 (A): 219-223

Mansour HH (**2006):** Protective role of carnitine ester against radiation-induced oxidative stress in rats. Pharmacological Research 54 (3):165-171.

Marfella R, Lisa Q, Francesco N, Antonio C and Dario G (2001): Acute hyperglycemia induces an oxidative stress in healthy subjects. J Clin Invest 108(4): 635-636.

Mazunder UK, Gupta M and Rajeshwar Y (2005): Antihyperglycaemic effect and antioxidant potential of Phyllantus nuriri (Euphorbiaceae) in streptozotocin induced diabetic rats. European Bulletin of Drug Research 13(1): 15-23.

McDermott JH (2000): Antioxidant nutrients: current dietary recommendations and research update. J Am Pharm Assoc 40: 785-99.

McEwen BS and Stellar E (1993): Stress and the individual. Mechanisms leading to disease. Arch Intern Med. 153(18):2093-101.

Mehta R, Michael H, Laura U, Dragos A, Robert M, Konstantin C and Rajeshwari M (2003): Prevention of N-Methyl-N-Nitrosourea-Induced Mammary Carcino-genesis in Rats by 1α-Hydroxyvitamin D_5. Journal of the National Cancer Institute 92(22): 1836-1840.

Mieczyslaw P, Zofia S and Juegen K (2004): The Influence of radiation quality on radiation-induced hemolysis and hemoglobin oxidation of human erythrocytes. Journal of Radiation Research 45(2): 275-280.

Minami M and Yoshikawa H (1979): A simplified assay method of superoxide dismutase activity for clinical use. Clin Chim Acta 92(3):337-42.

Misra RB, Ray RS and Hans RK (2005): Effect of UVB radiation on human erythrocytes in vitro Toxicology in Vitro 19: 433–438.

Moreno DA, Ilic N, Poulev A, Brasaemle DL, Fried SK and Raskin I (2003): Inhibitory effects of grape seed extract on lipases**.** Nutrition 19(10):876-9.

Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, Núñez MJ and Parajó JC (2001): Natural antioxidants from residual sources. Food Chem 72:145-171.

Murray M and Pizzorno J (1999): In: Murray M, Pizzorno J, eds. The Textbook of Natural Medicine. 2nd ed. London: Churchill Livingston 899-902.

Nakagawa S, Nakagawa H, Sasaki T, Yoshins K, Yu YY, Shinozaki K, Aoki S, Mashimo K (1973): A simple method for the determination of serum free insulin level in insulin-treated patients. Diabetes 22:590-600.

Nanji AA and Hiller S (1997): Apoptosis and necrosis. Alcohol Health & Research World 21:325–330.

Navrátil S, Palíková M and Vajcová V (1998): The Effect of Pure Microcystin LR and Biomass of Blue-green Algae on Blood indices of Carp (Cyprinus carpio L.). Acta vet. Brno 67: 273-279.

Nunia V, Sancheti G and Goya K (2006): Protection of Swiss albino mice against whole-body gamma irradiation by diltiazem. British Journal of Radiology 10:1259 -1262.

Odetti P, Garibaldi S, Noberasco G, Aragno I, Valentini S, Traverso N and Marinari UM (1999): Levels of carbonyl groups in plasma proteins of type 2 diabetes mellitus subjects. Acta Diabetol 36:179-183.

Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S, Suda I, and Sato T (2002): Polymeric procyanidins as radical scavenging components in red-hulled rice. J Agric Food Chem 50:7524- 7529.

Oliveira HR, Curi R and Carpinelli AR (1999): Glucose induces an acute increase of superoxide dismutase activity in incubated rat pancreatic islets Am J Physiol Cell Physiol 276 (2): 507-510.

Onody A, Csonka C, Giricz Z and Ferdinandy P (2003): Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovascular Research 58(3): 663-670.

Osakabe N, Yamagishi M, Natsume M, Yasuda A and Osawa T (2004): Ingestion of proanthocyanidins derived from

cacao inhibits diabetes-induced cataract formation in rats. Exp Biol Med 229:33–9.

Osiewacz HD (2002): Mitochondrial functions and aging. Gene 286: 65-71.

Palanivel R, Ravichandran P and Govindasamy S (1998): Biochemical studies on peroxidation in ammonium para-tungstatetreated experimental diabetic rats. Med. Sci. Res. 26**:** 759–762.

Petkau A (1988): Protection of bone marrow progenitor cells by superoxide dismutase. Molecular and Cellular Biochemistry 84 (2)**:** 133-140.

Pigeolet E, Corbisier P and Houbion A (1990): Glutathione peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech Aging Dev 51: 283–297.

Pinon L, Viguier M, Touzalin A, Maas J and Jegou B (1991): Effects of an acute exposure of rat testis to gamma rays on germ cells, Sertoli and Leydig cell functions. Reproduction, Nutrition, Development 31: 617–623.

Pispirigos K and Chrysanthopoulos K (2001): Evaluation of cardiac subacute toxicity of ciprofloxacin in rats using serum biochemical parameters. Drug Res 51: 582-587.

Pokorný J (1991): Natural antioxidants for food use. Trends Food Sci Technol 2: 223-227.

Portakal O, Ozkaya, O, Erden Inal M, Bozan B, Kosan M and Sayek I (2000): Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin Biochem 33: 279–84.

Prabhakar KR, Veerapur Z, Punit B, Vipan K, Parihar M, Reddy K, Bhagath K, Priyadarsini KI and Unnikrishnan MK (2007): Antioxidant and radioprotective effect of the active fraction of Pilea microphylla (L.) ethanolic extract. Chemico-Biological Interactions 165 22–32.

Preuss HG, Bagchi D and Bagchi M (2002): Protective effects of a novel niacin-bound chromium complex and a grape seed proanthocyanidin extract on advancing age and various aspects of syndrome X. Ann NY Acad Sci 957:250-9.

Prior RL (2003): Fruits and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 78:570-578.

Puchała M, Szweda-Lewandowska Z and Kiefer J (2004): The influence of radiation quality on radiation-induced hemolysis and hemoglobin oxidation of human erythrocytes. J Radiat Res 45(2):275-9.

Puiggros F, **Llopiz N**, **Ardevol A**, **Blade C**, **Arola L and Salvado MJ (2005):** Grape seed procyanidins prevent oxidative injury by modulating the expression of antioxidant enzyme systems. J Agric Food Chem 53(15):6080-6.

Rajendra PN, Menon VP, Vasudev V and Pugalendi KV (2005): Radioprotective effect of sesamol on g-radiation induced DNA damage, lipid peroxidation and antioxidants levels in cultured human lymphocytes. Toxicology 209:225–35.

Rajendra R, Malegaonkar D, Pungaliya P, Marshall H, Rasheed Z, Brownell J, Liu LF, Lutzker S, Saleem A and Rubin EH (2003): Topors functions as an E3 ubiquitin ligase with specific E2 enzymes and ubiquitinates p53. Journal of Biological Chemistry 279: 36440–36444.

Ray S, Bagchi D, Lim PM, Bagchi M, Gross SM, Kothari SC, Preuss HG and Stohs S (2000): Acute and long-term

safety evaluation of a novel IH636 grape seed proanthocyanidin extract. J Res Commun Mol Pathol Pharmacol 109: 165-197.

Ray SD, Lam TS, Rotollo JA, Phadke S, Patel C, Dontabhaktuni A, Mohammad S, Lee H, Strika S, Dobrogowska A, Bruculeri C, Chou A, Patel S, Patel R, Manolas T and Stohs S (2004): Oxidative stress is the master operator of drug and chemically-induced programmed and unprogrammed cell death: Implications of natural antioxidants in vivo. Biofactors 21(1-4):223-32.

Reitman S and Frankel S (1957): Colorimetric method of the determination of serum transaminase. Am J Clin Path 28: 56-60.

Rice-Evans CA, Miller NJ and Paganga G (1997): Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152-159.

Rose G, Passarino G, Carrieri G, Altomare K, Greco V, Bertolini S, Bonafè M, Franceschi C and De Benedictis G (2002): Paradoxes in longevity: sequence analysis of mtDNA haplogroup in centenarians. Eur J Hum Genet 9:701–707.

Rosen GM, Pou S and Ramos CL (1995): Free radicals and phagocytic cells. FASEB Journal 9:200–209.

Saada HN and Azab KhSh (2001): Role of lycopene in recovery of radiation induced injury to mammalian cellular organelles. Pharmazie 56 (3) 239-24.

Saada HN, Azab KhSh and Zahran AM (2001): Post-irradiation effect of broncho-vaxom, OM-85 BV, and its relationship to anti-oxidant activities. Pharmazie 56 (8) 654-656.

Saada HN, Azab KhSh, Said OZ, Mohamed MA and Abbady MM (1999): Role of Ginseng in the control of radiation induced injury in rat erythrocytes. J. Egypt. Ger. Soc. Zool. 27 (A) 9-20.

Saada HN, Said UZ and Mahdy AM (2003): Effectiveness of Aloe Vera on the antioxidant status of different tissues in irradiated rats. Pharmazie 58 (2): 929-931.

Sadani GR and Nadkarni GD (1997): Changes in lipid peroxide levels and the activity of reactive oxygen scavenging systems in thyroid tissue after exposure to radioactive iodine in rats. Thyroid 7 (6):937-41.

Said UZ (2004): Neuroprotective properties of rutin in radiation-induced biochemical disorders in brain of rats. Arab J of Nuclear Sci and Appl 37 (3): 223-231.

Said UZ and Azab Kh Sh (2006): Efficacy of wheat germ oil in modulating radiation-induced heart damage in rats. Egypt J Rad Sci Applic 19 (2): 433-452.

Said UZ and Hanafy NA (2006): Effect of grape seed extract on hepatic function and antioxidant status of mouse bearing-Ehrlich ascites carcinoma and exposed to gamma radiation. Isotope and Rad Res 38(1): 225-240.

Said UZ, Azab KhSh and Soliman SM (2004): Cardioprotective role of garlic (Allium sativum) on oxidative stress induced by gamma radiaiton exposure. Isotope and Rad Res 36(3): 465-479.

Said UZ, Azab KhSh and Soliman SM (2004): Cress oil modulates radiation-induced hormonal, histological, genetic and sperm head abnormalities in albino rats. Isotope and Rad Res 37 (2): 345-360.

Said UZ, Rezk RG and Saada HN (2003): Role of calcium pantothenate in recovery of radiation induced injury to mammalian organs of rats. J Rad Sci and Applic 18 (1): 17-38.

Said UZ, Soliman SM and El Tahawy NA (2002): Possible curative role of thiamine pyrophosphate against radiation induced biochemical and histological changes in albino rats. Egypt J Rad Sci Applic 15 (2): 17-34.

Said UZ, Soliman SM, Azab KhSh and El-Tahawy NA (2005): Oligomeric proanthocyanidins (OPCS) modulating radiation-induced oxidative stress on functional and structural performance of eye in male rats. Isotope and Rad Res 37 (2):395-415.

Saksa R, Ventura C and Aliev ML (1996): Metabolic control and metabolic capacity: two aspects of creatine kinase functioning in the cells. Biochim Biophys Acta 1274**:** 81-88.

Samuni AM, Barenholz Y, Crommelin DJA and Zuidam NJ (1997): Irradiation damage to liposomes differing in composition and their protection by nitroxides. Free Radic Biol Med 23:972–979.

Sánchez O, Arnau A, Pareja M, Poch E, Ramírez I and Soley M (2002): Acute stress-induced tissue injury in mice: differences between emotional and social stress. Cell Stress Chaperones 7(1): 36–46.

Sano A, Yamakoshi J, Tokutake S, Tobe K, Kubota Y and Kikuchi M (2003): Procyanidin B1 is detected in human serum after intake of proanthocyanidin-rich grape seed extract**.** Biosci Biotechnol Biochem 67(5):1140-3.

Saravanan G, Pari L and Venkateswaran S (2002): Effect of cogent db, **an** herbal drug, on plasma insulin and hepatic enzymes of glucose metabolism in experimental diabetes**.** Diabetes Obesity and Metabolism 4(6): 394-398**.**

Schwarz JM, Linfoot P and Dare D (2003): Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. Am J Clin Nutr 77 (1): 43-50.

Sedlakova A, Borikova K and Ahlers A (1986): Change in lipoprotein lipase activity in the adipose tissue and heart of non-lethally X-irradiated rats. Physiol Bohemoslov 35 (5): 400-405.

Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M and Roy S (2002): Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm. Ann NY Acad Sci 957:239-49.

Sestili P, Diamantini G, Bedini A, Cerioni L, Tommasini I, Tarzia G and Cantoni O (2002): Plant-derived phenolic compounds prevent the DNA-single strand breakage and cytotoxicity induced by tert butylhydroperoxide via an iron-chelating mechanism. Biochem J 364:121-128.

Shafiee M, Carbonneau MA, Urban N, Descomps B and Leger CL (2003): Grape and grape seed extract capacities at protecting LDL against oxidation generated by Cu^{2+}, AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extracts or compounds. Free Radic Res 37(5):573-84.

Shao ZH, Becker LB, Vanden Hoek TL, Schumacker PT, Li CQ, Zhao D, Wojcik K, Anderson T, Qin Y, Dey L and Yuan CS (2003): Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. Pharmacol Res 47(6):463-9.

Shi J, Yu J, Pohorly JE and Kakuda Y (2003): Polyphenolics in grape seeds-biochemistry and functionality. J Med Food 6 (4):291-9.

Shimizu M, Kobayashi Y, Suzuki M, Satsu H and Miyamoto Y (2000): Regulation of intestinal glucose transport by tea catechins. Biofactors 13:61–65.

Shimosawa T (2005): Increasing oxidative stress in aging. Nippon Rinsho 63(6):994-9.

Singh RP, Tyagi AK, Dhanalakshmi S, Agarwal R and Agarwal C. (2004): Grape seed extract inhibits advanced human prostate tumor growth and angiogenesis and upregulates insulin-like growth factor binding protein-3. Int J Cancer 108(5):733-40.

Sirmali M, Uz E, Sirmali R, Kilbaş A, Yilmaz HR, Altuntaş I, Naziroğlu M, Delibaş N and Vural H (2007): Protective effects of erdosteine and vitamins C and E combination on ischemia-reperfusion-induced lung oxidative stress and plasma copper and zinc levels in a rat hind limb model. Biol Trace Elem Res 118(1): 43-52.

Sreemantula S, Nammi S, Kolanukonda R, Koppula S and Boini KM (2005): Adaptogenic and nootropic activities of aqueous extract of Vitis vinifera (grape seed): an experimental study in rat model. BMC Complement Altern Med 5 (1): 1-10.

Sridharan S and Shyamaladevi CS (2002): Protective effect of N-acetylcysteine against gamma ray induced damages in rats--biochemical evaluations. Indian J Exp Biol 40(2):181-6.

Srinivasan M, Rajendra Prasad N and Menon VP. (2006): Protective effect of curcumin on gamma-radiation induced DNA damage and lipid peroxidation in cultured human lymphocytes. Mutat Res 611(1-2): 96-103.

Srivastava M, Chandra D and Kale RK (2002): Modulation of radiation-induced changes in the xanthine oxidoreductase system in the livers of mice by its inhibitors. Radiat Res 157(3):290-7.

Stein EA and Myers GL (1995): National Cholesterol Education Program recommendations for triglyceride measurement: executive summary. Clin Chem; 41:1421-1426.

Subir KD, Hiran KR, Sukhes M and Vasudevan DM (2007): oxidative stress in the primary event effects of ethanol consumption in brain. Indian Journal of Clinical Biochemistry 22 (1) 99-104.

Szweda-Lewandowska Z, Krokosz A, Gonciarz M, Zajeczkowska W and Puchala M (2003): Damage to human erythrocytes by radiation-generated HO• radicals: molecular changes in erythrocyte membranes. Free Radic Res 37:1137- 43.

Tebib K, Besançon P and Rounat JM (1994): Dietary Grape Seed Tannins Affect Lipoproteins, Lipoprotein Lipases and Tissue Lipids in Rats Fed Hypercholesterolemic Diets. J Nutr 124: 2451-2457.

Thomas N (2005): Role of oxidative carbonylation in protein quality control and senescence. The EMBO Journal 24: 1311–1317.

Thornalley P (2005): Dicarbonyl intermediates in the Maillard reaction. Ann NY Acad Sci 1043: 111-117.

Tibble JA, Sigthorsson G and Foster R, (2000): Comparison of the intestinal toxicity of celecoxib, a selective COX-2 inhibitor, and indomethacin in the experimental rat. Scand J Gastroenterol 35:802–807.

Tietz NW (1986): Fundamentals of Clinical Chemistry. W B Saunders Co Hardcover 1040 pages, 3rd Edition.

Tominaga H, Kodama S, Matsuda N, Suzuki K and Watanabe M (2004): Involvement of reactive oxygen species (ROS) in the induction of genetic instability by radiation. J Radiat Res 45 (2):181-184.

Toykuni S (1999): Reactive oxygen species–induced molecular damage and its application in pathology. Pathology International 49:91–102.

Tsang C, Higgins S and Duthie GG (2005): The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. Br J Nutr 93(2):233-40.

Valls-Belles V, Torres MC, Muniz P, Beltran S, Martinez-Alvarez JR and Codoner-Franch P (2006): Defatted milled grape seed protects adriamycin-treated hepatocytes against oxidative damage. Eur J Nutr [Epub ahead of print]

Van den Berg R, van Vliet T, Broekmans WM, Cnubben NH, Vaes WH, Roza L, Haenen GR, Bast A and van den Berg H (2001): A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers. J Nutr 131:1714-22.

Victor M, Kenneth J and Milagros R (2006): Recent Progress in Pharmacological Research of Antioxidants in Pathological Conditions: Cardiovascular Health. Recent Patents on Anti-Infective Drug Discovery 1: 17-31.

Vitseva O, Varghese S, Chakrabarti S, Folts JD and Freedman JE (2005): Grape seed and skin extracts inhibit

platelet function and release of reactive oxygen intermediates. J Cardiovasc Pharmacol 46(4):445-51.

Vogels N, Nijs IM and Westerterp-Plantenga MS (2004): The effect of grape-seed extract on 24 h energy intake in humans. Eur J Clin Nutr 2004 58(4):667-73.

Walden TL and Farzaneh NK (1990): Biochemistry of Ionizing Radiation. New York: Raven Press, OCLC: 21337918.

Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M and Granner DK (2002): Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277: 34933–34940.

Wan Y, Vinson JA, Etherton TD, Proch J, Lazarus SA and Kris-Etherton PM (2001): Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. Am J Clin Nutr **74:** 596-602.

Ward JF (1990): The yield of DNA double-strand breaks produced intro-cellular by ionizing radiation: a review. International Journal of Radiation Biology 57: 1141–1150.

Waterhouse AL and Walzem RL (1998): Nutrition of Grape Phenolics. In: Flavonoids in Health and Disease; eds Rice-Evans CA and Packer L. Marcel Dekker Inc New York, NY, 1998; pp. 349-387.

Weinmann S, Richert-Boe K and Glass AG (2004): Prostate cancer screening and mortality: a case-control study (United States). Cancer Causes Control 15 (2): 133-8.

Winkler AJ, Cook JA, Kliewer WM and Lider LA (1997): General viticulture. Berkeley and Los Angels: University of California Press**.**

World Cancer Research Foundation and American Institute for Cancer Research. (1997): Food, nutrition and the prevention of cancer: a global perspective. Washington DC: AICR.

World Health Organization (1990): Diet, nutrition and the prevention of chronic disease. Technical Report series 797. Geneva: World Health Organization.

Yamakoshi J, Saito M, Kataoka S and Tokutake S (2002): Procyanidin-rich extract from grape seeds prevents cataract formation in hereditary cataractous (ICR/f) rats. J Agric Food Chem 50(17):4983-8.

Yarmonenko SP (1988): Radiobiology of Humans and Animals. Mir Publishers, Moscow: 450p.

Yoshimura Y, Nakazawa H and Yamaguchi F (2003): Evaluation of the NO scavenging activity of procyanidin in grape seed by use of the TMA-PTIO/NOC 7 ESR system. J Agric Food Chem. 51(22):6409-12.

Yoshioka T, Kawada K, Shimada T and Mori M (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated- oxygen toxicity in the blood. Am J Obstet Gynecol 135: 372-376.

Young IS and Woodside JV (2001): Antioxidants in health and disease. J Clin Pathol 54: 176