Ain Shams University
Faculty of Science

Hesperidin as a Radioprotector Against Hepatocellular and DNA Damage Induced by γ-Irradiation in Rats: Biochemical, Histopathological and Molecular Studies

Thesis Submitted to the Department of Zoology, Faculty of Science Ain Shams University

by

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For the Award of the Degree of Doctor of Philosophy of Science in Zoology (Physiology)

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Approval sheet

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List of Abbreviations

$^1O_2$	**Singlet oxygen**
ALT	**Alanine aminotransferase**
ALP	**Alkaline phosphatase**
AST	**Aspartate aminotransferase**
CDNB	**1-chloro-2,4-dinitrobenzene**
cDNA	**Complementary deoxyribonucleic acid (DNA molecule produced using RNA as template)**
CAT	**Catalase**
CSCs	**Cancer stem cells**
Ct	**Cycle threshold**
Da	**Dalton (atomic mass unit)**
DMF	**Dose modifying factor**
DMSO	**Dimethylsulfoxide**
DNA	**Deoxyribonucleic acid**
DSBs	**Double strand breaks**
F.D	**Fractionated dose**
GGT	**Serum γ-glutamyl transferase**
g	**Gram**
GPx	**Glutathione Peroxidase**
GR	**Glutathione Reductase**
GSH	**Reduced Glutathione**
GSSG	**Glutathione disulfide**
GST	**Glutathione S-transferase**
h	**Hour**
H&E	**Haematoxylin– Eosin**
HBSS	**Hank's balanced salt solution buffer with supplements (for cutting slices)**
HGF	**Hepatocytes growth factor**
HCC	**Hepatocellular carcinoma**
HES	**Hesperidin**
4HNE	**4-hydroxynonenal**
i.g.	**Intragastric.**
i.p.	**Intraperitonial**
i.v.	**Intravenous**
LMPA	**Low Melting Point Agarose**
ml	**Milliliter**
MDA	**Malondialdehyde**
mRNA	**Messenger ribonucleic acid**
MAPK	**mitogen-activated protein kinase**
NADPH	**Nicotinamide adenine dinucleotide phosphate**

NBT	**Nitroblue tetrazolium**
NOS	**inducible nitric oxide oxidase**
NPSH	**Nonprotein sulfhydryl compound**
PBS	**Phosphate-buffered saline (0.15 M phosphate buffer saline)**
PCR	**Polymerase chain reaction**
PUFAs	**polyunsaturated fatty acids**
OD	**Optical density**
ROI	**Reactive oxygen intermediates**
rpm	**Rotation per minute**
SAR	**Structure–activity relationships**
SCGE	**The single cell gel electrophoresis**
S.D	**Single dose**
SD	**Standard deviation**
SOD	**Superoxide dismutase**
TBA	**Thiobarbituric acid**
TBARS	**Thiobarbituric acid reactive substances**
µg	**Microgram**
µl	**Micro Liter**

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Introduction

Radiation can directly interact with the critical target molecules in the cell, causing ionization and excitation that leads to biochemical damage **(Cai *et al.*, 2010)**. Ionization can cause breakage of covalent bonds in DNA molecules, leading to a range of effects that include strand breaks, base loss and cross linking **(Barker *et al.*, 2005)**. The fate of cell repair by itself is dependent on the type of damage caused by the different types of irradiation. When a direct hit results in complete break in the DNA, the cell either dies immediately or ultimately **(Dowd and Tilson, 1999)**. However, mammals have a huge number of cells and the cells that die are replaced by new ones through mitosis. When these new cells are exposed to high doses of radiation, the system of replacing cells weakens and deleterious effects of radiation are seen **(Ahmed, 2005).**

The reactive oxygen species (ROS) play a critical role in cell damage by causing DNA strand breaks, lipid peroxidation, protein modification and also by modulating a variety of cellular transduction pathways **(Bacsi *et al.*, 2005; Well *et al.*, 2005).** This will result in physical and chemical damage to tissues if the ROS are not scavenged. In this regard, it is worth mentioning that ROS such as O_2^{--} and H_2O_2 are consistently produced during normal cellular aerobic metabolism. Cells are well equipped to defend themselves against ROS, with continuous reproduction of antioxidant enzymes

and non-enzymatic molecules **(Jagetia *et al.*, 2003)**. These antioxidant systems consist of low molecular weight antioxidants like glutathione (GSH) and various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Humans are constantly exposed to IR both from natural sources like cosmic rays, radioisotopes found in the earth crust and also from artificial sources such as IR applications. IR are also widely used in treating cancers of various tissues where it is used to kill or arrest the growth of tumor cells. Currently, more than one-half of all cancer patients are treated with radiation therapy. Radiotherapy can cause tissue injury to normal tissues in long-term cancer survivors in spite of its therapeutic value **(Meister, 2005).**

The cellular damage caused by IR is mainly mediated through free radicals and ROS **(Weiss and Landauer, 2003)**. The interaction of IR with intracellular water, results in its radiolysis and generation of primary water radical species that may react with oxygen producing secondary radicals (H_2O_2 and $O_2^{\bullet -}$), which are highly reactive and could attack DNA, pivotal protein molecules and biological membranes, leading to cell death (**Pandey and Mishra, 1999; Mates and Sanchez-Jimenez, 2000).**

An "oxidative stress" is the consequence of an imbalance between pro-oxidant and antioxidant processes and the result of a disturbance of the "cellular redox homeostasis" **(Meloni and Nicolay, 2003)**. Radiation-induced free radicals impair the antioxidative defense mechanism, leading to several pathophysiological impacts including DNA damage and increased membrane lipid peroxidation (LPO) with the damage of the membrane-bound enzymes. The damage of these enzymes causes alterations in cell transduction pathways and sever disconcert in the induction of cell regulatory signals (**Feng *et al*., 2012**)

The increase in LPO could be due to significant reduction in the activities of enzymatic antioxidants such as CAT, SOD, GPX and GST as well as non- enzymatic antioxidants such as GSH. Superoxide radical is converted to H_2O_2 by SOD. Furthermore, H_2O_2 is transferred to molecular oxygen and water by CAT and GPX. Therefore, SOD, CAT, GPX and GST constitute the principal components of the antioxidant system and their deficiencies leads to oxidative stress **(El-Bahr, 2014)**.

Since free radicals play a major role in the initiation and progression of radiation induced toxicity, the use of antioxidants might offer protection against radiation induced damage. Consequently, much research has been focused on the potential use

of flavonoids as free radical scavengers to prevent oxidative damage **(Van Acker *et al*., 1996)**.

A wide variety of natural plant products are non-toxic with noticeable therapeutic benefits and have been exploited since ancient times for curing ailments. About 60 % of the new drugs developed over the past 25 years owe their origin to natural sources **(Newman and Cragg, 2007; Gupta, 2010)**. Several plant products have been screened for their radioprotective potential in various *in vitro* and *in vivo* studies. These plants are rich sources of polyphenols which include anthocyanins, flavonoids, stilbenes, tannins, lignins, etc. **(Manach *et al*., 2005)**.

Hesperidin (Hesperetin-7-rhamnoglucoside), a flavanone-type flavonoid, is abundant in citrus fruits **(Wilmsen *et al*., 2005)**. The peel and the membranous parts of these fruits have the highest hesperidin concentrations. Hesperidin is comprised of the flavanone hesperitin and the disaccharide rutinose. Hesperidin has been reported to exert a wide range of pharmacological effects **(Tommasini *et al*., 2005)** which includes antioxidant, anti-allergic, anti-inflammatory, hypolipidemic and anti-carcinogenic actions **(Emim *et al*., 1994).**

In young immature oranges, hesperidin accounts for up to 14% of the fresh weight of the fruit **(Tanaka *et al*., 1997a)**. Hesperidin is