Invitro Studies on the potential protective effects of *Dietes bicolor* plant against carbon tetrachloride-induced hepatotoxicity

A thesis submitted for the partial fulfillment of the requirements of Masters' degree in pharmaceutical sciences (Pharmacology and Toxicology).

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Table of Contents

List of figures:

List of tables:

List of Abbreviations:

ROS	Reactive oxygen species
DNA	Deoxyribonucleic acid
FR	Free radicals
$OH^{\bullet}$	Hydroxyl anion
LPO	Lipid peroxidation
MDA	Malondialdehyde
TNF	Tumor necrosis factor
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
CXCR4	CXC chemokine receptor 4
COX-2	Cyclooxygenase-2
RNA	Ribonucleic acid
8-OHdG	8-hydroxydeoxyguanosine
PI3K	Phosphoinositide 3-kinase
H_2O_2	Hydrogen peroxide
c-Myc	Cellular avian myelocytomatosis virus oncogene
Cdk	Cyclin dependent kinase
NF-κB	Nuclear factor kappa B
ICAM-1	Intercellular adhesion protein-1
MMP	Matrix metalloproteinase
VEGF	Vascular Endothelial growth factor
FGF	Fibroblast growth factor
PDGF	Platelet-derived growth factor
DISC	Death-inducing signaling complex
Apaf-1	Apoptotic protease activating factor-1
CARD	Caspase recruitment domain
Nrf2	Nuclear factor erythroid 2- related factor 2
Keap1	Kelch-like ECH-associated protein-1
ARE	Antioxidant response element
CAT	Catalase enzyme

SOD	Superoxide dismutase enzyme
GSH-Px	Glutathione peroxidase
GSH	Reduced glutathione
4-HNE	4-hydroxynonenal
HCV	Hepatitis C virus
GST	Glutathione S-transferase
IFN	Interferon
ATP	Adenosine triphosphate
ALD	Alcoholic liver disease
RNS	Reactive nitrogen species
GSSG	Oxidized glutathione
HBV	Hepatitis B virus
TGF-β	Transforming growth factor- β
HSC	Hepatic stellate cells
HCC	Hepatocellular carcinoma
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
FFAs	Free fatty acids
CCl_4	Carbon tetrachloride
IUPAC	International union of pure and applied chemistry
OECD	Organization for economic co-operation and development
PBS	Phosphate-buffered saline
DMSO	Dimethylsulfoxide
ELISA	Enzyme-linked immunosorbent assay
PGE_2	Prostaglandin E2
pNpp	*Para*-nitrophenylphosphate
pNA	*Para*-nitroaniline

Introduction

1. Redox stress, Inflammation, and Cancer

Reactive oxygen species (ROS) are involved in a wide spectrum of diseases, including chronic inflammation, and a wide variety of cancers. Chronic inflammation is induced by biological, chemical, and physical factors and is associated with an increased risk of several human diseases including cancer **(Bartsch and Nair, 2006; Schetter *et al.*, 2010)**.

For example, inflammatory bowel diseases such as Crohn disease and ulcerative colitis are associated with increased risk of colon adenocarcinoma **(Ekbom *et al.*, 1990a; 1990b; Gillen *et al.*, 1994)**. Similarly, pancreatitis and esophagitis, both induced by tobacco and alcohol, may transform normal tissue into pancreatic or esophageal cancer if the antioxidant system is not sufficiently effective **(Garcia-Monzon *et al.*, 2000; Murphy *et al.*, 2005)**.

During inflammation, mast cells and leukocytes are recruited to the site of damage. This results in a “respiratory burst” due to an increased uptake of oxygen, and thus, induces an increased release and accumulation of ROS at the site of damage **(Coussens and Werb, 2002; Hussain *et al.*, 2003)**.

If two free radicals (FR) meet, they can join their unpaired electrons to form a covalent bond; the product is a non-radical. However, when a radical reacts with a non-radical, a new radical results, and a chain reaction can occur **(Halliwell and Gutteridge, 1999)**. Since most biological molecules are non-radicals, the generation of reactive radicals such as $OH^{\bullet}$ *in vivo* often initiates chain reactions.

For example, their attack upon fatty acid side chains in membranes and lipoproteins can initiate the chain reaction of lipid peroxidation, resulting in the production of a plethora of lipid peroxidation (LPO) products, many of them reactive toward protein and DNA **(Halliwell and Gutteridge, 1999)**. One of the most abundant carbonyl products of lipid peroxidation is malondialdehyde (MDA), which is also generated as a side-product of prostaglandin biosynthesis **(Golding *et al.*, 1989; Marnett, 2002)**.

Moreover, inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines, and chemokines, which act by further recruiting inflammatory cells to the site of damage, and thus producing more reactive species (**Figs. 1 and 2**).

For example, the aberrant expression of inflammatory cytokines (tumor necrosis factor (TNF), interleukin-1 (IL-1),

interleukin-6 (IL-6) and chemokines (IL-8, CXC chemokine receptor 4 (CXCR4)) as well as alterations in the expression of specific microRNAs and the further induction of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) enzymes, have been reported to play a role in oxidative stress-induced inflammation **(Hussain and Harris, 2007)**.

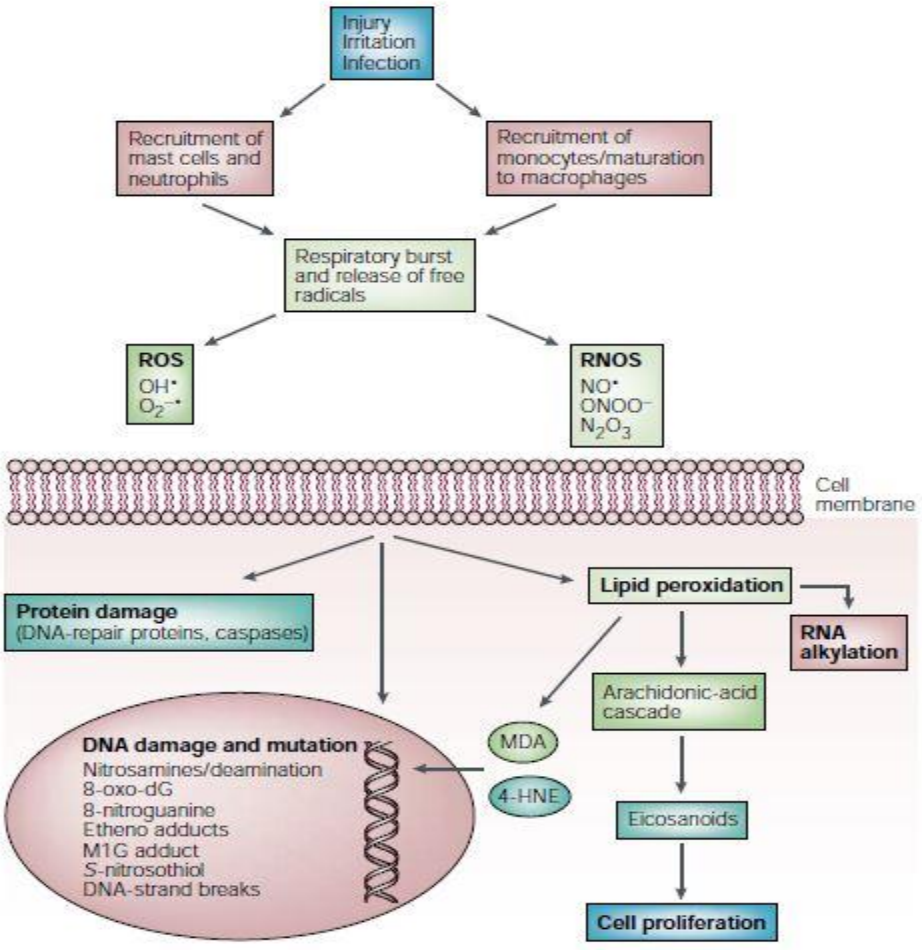

Figure 1: Impact of free radicals released at sites of inflammation on cellular molecules. Adopted from **Hussain *et al.* (2003)**.

This sustained inflammatory/oxidative environment leads to a vicious circle that can damage healthy neighboring epithelial and stromal cells, and over a long period of time may lead to carcinogenesis **(Federico *et al.*, 2007)**.

Cancer is a multistage process defined by at least three stages: initiation, promotion, and progression. Oxidative stress interacts with all three stages of this process **(Schulte-Hermann *et al.*, 1990; Ames and Gold, 1992; Mantovani, 2005)**.

After an inflammatory stimulus, initiation of carcinogenesis mediated by ROS may be direct (oxidation, nitration, and halogenation of nuclear DNA, RNA, and lipids) or mediated by the signaling pathways activated by ROS (**Figs. 1 and 2**). The hydroxyl radical ($OH^{\bullet}$)-derived DNA damage includes the generation of 8-hydroxyguanosine, the hydrolysis product of which is 8-hydroxydeoxyguanosine (8-OHdG), the most widely used fingerprint of radical attack on DNA **(Wiseman and Halliwell, 1996; Marnett, 2000) (Fig. 1)**.

In the promotion stage, ROS can contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second-messenger systems, resulting in an increase in cell proliferation, metastasis and angiogenesis or a decrease in apoptosis of the initiated cell population **(Storz, 2005)**.