THE EFFECT OF SOME HERBAL EXTRACTS ON LIVER CELLS

(BIOLOGICAL STUDY)

Submitted By

Eman Mohamed Ali Shaban

B.Sc. of Science (Chemistry), Faculty of Science, Tanta University, 1995

Diploma in Biochemistry, Faculty of Science, Alexandria University, 1999

A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Master Degree
In
Environmental Sciences

Department of Environmental Basic Sciences
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APPROVAL SHEET

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ABSTRACT

Usnic acid (UA), a natural botanical product, is a constituent of some dietary supplements used for weight loss. It has been associated with clinical hepatotoxicity leading to liver failure in humans. The present study was undertaken for toxicity evaluations of (+)UA on HepG2 cell line in culture. The cells were treated with the vehicle control and (+)UA at concentrations of 0–100 µM for 24 h at 37°C in 5% CO_2 incubator. Following the treatment period, the cells were evaluated by biochemical endpoints of toxicity that included MTT activity, LDH release, liver function tests and alpha-fetoprotein as a tumor marker. (+)UA exposure resulted in increased cytotoxicity and mitochondrial dysfunction in HepG2 cells. compared with the controls, low non-toxic concentrations of UA separately showed no effect on the cells as determined by the biochemical endpoints compared with higher concentrations ($P<0.001$). The findings in this study demonstrated the toxicity of the (+) (UA) to human hepatoblastoma HepG2 cells, suggesting an oxidative mechanism of action.

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List of abbreviation

$\bar{x}$	Arithmetic mean
ADME	Absorption, Distribution, Metabolism,
AFLD	Alcoholic Fatty liver Disease (alcoh
AFP	Alfa-FetoProtein
ALP	Alkaline phosphatase
AST	Aspartate Transaminase
ATF6	Activating Transcription factor-6
ATP	Adenosine Triphosphate
BC	Bile Canalicular.
CCl4	carbon Tetrachloride
CD14	cluster of Differentiation 14
CES	Carboxylesterase
DEMSO	Dimethyl Sulfoxide
DILI	drug-induced liver toxicity
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ER	Endoplasmic Reticulum
FBS	Fetal bovine serum
GOT	Glutamate-Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
GST	Glutathione S Transferase
HaCaT	Non transformed human keratinocyte cel
HCC	Hepatocellular carcinoma.
HEC-50	Endometrial carcinoma cell culture
HEPG2	Human hepatoblastoma cells
HPLC	High Performance liquid Chromatogr
IC50	The concentration of an inhibitor th
IRE1a	Inositol-requiring enzyme 1 alph
Keap1-Nrf2-AR	Keap1-nuclear factor erythroid-related
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharides
MASRI	Faculty of Medicine Research Institut
MD2	Lymphocyte antigen 96...
MDH	malate dehydrogenase
NAD(P)H dehyd	rogenase (nicotinamide adenine dinucleot
NAFLD	Non-alcoholic fatty
NK	Natural killer
NQO1	NAD(P)H quinone oxid
OD	Optical Density
P	Propability

List of abbreviation

P450	Cytochromes P450 (CYPs
PERK	Protein kinase RNA–like
PFIC	Progressive Familial
RLUs	relative light Units
RNA	Ribonucleic Acid
SD	Standard Deviation
TCM	Traditional Chinese Medi
TLR4	Toll-like Receptor 4
UPR	unfolded Protein Response
WIF-B	Hepatoma-derived hybrid cell lin
γ-GT	Gamma-Glutamyl Transpepti

INTRODUCTION

1. Introduction

The liver is the primary organ involved in xenobiotic metabolism. Because of its high level of metabolic activity and exposure to blood-borne agents, the liver is a major target organ of many chemicals, drugs and microbial pathogens. Thus, hepatotoxicity is a serious safety concern related to food additives, food contaminants, dietary supplements and food-borne microbial pathogens (***Treinen-Molsen, 2001***).

Development of alternative *in vitro* assays is necessary for rapid, cost-effective and high-throughput toxicological screening and characterization of compounds to complement and/or supplement costly and time-consuming *in vivo* animal tests. Human cell cultures and toxicogenomics are sensitive tools for such high-throughput toxicity testing. They have the potential to eliminate the need for interspecies extrapolation, to increase efficiencies in testing and to reduce the use of animals when used in combination with traditional biochemical endpoints ***(Meek and Doull, 2009)***.

Human hepatoblastoma HepG2 cells have been well characterized and are widely used as an *in vitro* model ***(Fang and Beland, 2009)***. These cells are highly differentiated and display many genotypic and phenotypic features of normal liver cells. They preserve many of the cellular functions found in normal hepatocytes ***(Roe et al., 1993)*** and can be grown indefinitely for long-term studies. These cells have been used in many toxicity studies for the

screening of hepatotoxic compounds ***(Jennen et al., 2010; O'Brien et al., 2006)***. Compared with primary hepatocytes, they have low levels of phase I cytochrome P450 enzymes, but they have normal levels of phase II enzymes (***Westerink and Schoonen, 2007***). HepG2 cells have been used to classify 70% of compounds with known toxicity as cytotoxic. The cytotoxicity of compounds is determined in HepG2 cells with 80% sensitivity and 90% specificity (***O'Brien et al., 2006***). HepG2 cells have been used to determine genotoxic and nongenotoxic carcinogens. These studies demonstrate that, despite known limitations, HepG2 cells represent a valuable *in vitro* model for hepatotoxicity studies (***Jennen et al. 2010***).

Aim of the study:

The objective of the study reported here was to evaluate the metabolism and hepatotoxic potential of usnic acid in human hepatoblastoma HepG2 cells using different biochemical endpoints.