Ain-Shams University
Faculty of Girls
Zoology Department

A Study on the Possible Protective Effect of Ginseng on Hepatic Toxicity of Diclofenac in Rats

A Thesis

Submitted in partial fulfillment for the Degree of Doctorate Philosophy of Science (Ph.D.) in Zoology

By

Sayeda Mahmoud Morsy Omran

Research Assistant,
Developmental Pharmacology Department,
National Organization for Drug Control and Research, Cairo.

Supervised By

Prof. Dr. Mohamed Raouf Hamed.

Professor of Pharmacology & Toxicology
National Organization for Drug Control and Research

Prof. Dr. Samiha Mohamed Abdel-Dayem

Professor of Physiology
Department of Zoology, Faculty of Girls, for Arts, Science and Education,
Ain-Shams University.

Dr. Abd El Rahaman Abass El Garawany

Assoc. Professor of Biochemistry
National Organization for Drug Control and Research

2006

Approval Sheet

Name: Sayeda Mahmoud Morsy Omran

Title: A Study on the possible protective effect of ginseng on hepatic toxicity of diclofenac in rats.

Scientific degree: Ph. D.

Supervisors

Prof. Dr. Mohamed Raouf Hamed.
Professor of Pharmacology & Toxicology
National Organization for Drug Control and Research

Prof. Dr. Samiha Mohamed Abdel-Dayem
Professor of Physiology
Department of Zoology, Faculty of Girls, for Arts,Science and Education.
Ain-Shams University.

Qualifications

Name :**Sayeda Mahmoud Morsy Omran**

Scientific Degree : **M.Sc.**

Department : **Zoology Department**

College : **Faculty of Science**

University : **Cairo University**

Graduation Year : **1998**

ACKNOWLEDGEMENT

First and foremost thanks are due to GOD, the most beneficial and merciful.

I am deeply indebted to Prof. Dr. Mohamed Raouf Hamed, professor of Pharmacology and Toxicology, National Organization for Drug Control and Research (NODCAR), for suggestion this point of research, his continuous encouragement, valuable instruction and judgments as well as continuous supervision of the work.

I would like to express my deepest gratitude to Prof. Dr. Samiha Mohamed Abdel-Dayem, professor of Physiology, Department of Zoology, Faculty for Girls, Ain-Shams University, for her kind supervision, continuous encouragement, valuable assistance and support all over this work.

I wish to express my profound gratitude and sincere appreciation to Prof. Dr. Abd El Rahman Abass El Garawany, Professor of Biochemistry, National Organization for Drug Control and Research (NODCAR), for providing facilities and for his kind help.

I wish to express my profound gratitude and sincere appreciation to the stuff of the Department of Developmental Pharmacology, National Organization for Drug Control and Research (NODCAR), especially for, Assoc. professor.Dr Nahed, Mohamed Ahmed.; Research Assistant. Dalia, Alansary and Assistant Research.Michael, Kamal. for providing facilities and for their kind help.

My thanks also due to my colleagues members in Pyrogen Departments, National Organization for Drug Control and Research (NODCAR), for their help and support during the preparing of this thesis.

Abstract

Two major objectives have been considered in the present work. The first one was to produce diclofenac hepatotoxicity model in rats. The second one was to investigate the possible protective effect of ginseng against the hepatic toxicity of diclofenac sodium.

The work was carried out using an adult female albino rats (432 rat) and adult male Newzeland rabbits (20 rabbit). Diclofenac sodium was administered orally (p.o) to rats in the different doses (10, 20, 30, 0.625, 1.25, 2.5, 5.00 and 7.5 mg/kg), interperitoneally (i.p) (10, 20, 30 and 40 mg/kg) and intramuscularly (i.m) (20, 40, 80, 120, 140, 2 and 4 mg/kg).In addition, it was administered orally (p.o) to rabbits in the dose (10 mg/kg). This drug was tested in either single or daily repetitive doses (for 3 or 7 days in rats and for 1 and 2 weeks in rabbits).Treatment by ginseng extract (50 mg/kg) to rats and (15 mg/kg) to rabbits was done in three ways, co-administered, pre-administered or post-administered with certain treatments of diclofenac sodium. The examined parameters included estimation of the serum activities of alanine aminotransferase “ALT”, alkaline phosphatase “ALP”, lactate dehydrogenase "LDH", total protein "T.protein", albumin, globulin as well as the serum albumin/globulin ratio (A/G ratio) .Also, triglycerides, hepatic lipid peroxidation (MDA), the liver/body weight

ratio and the hepatic histopathological changes were determined.

The obtained results helped in identifying the most suitable parameters for evaluation of the diclofenac experimental hepatotoxicity model in rats, which include lactate dehydrogenase "LDH", total protein "T.protein", albumin, triglycerides and the level of lipid peroxidation (MDA).The results have also revealent that ginseng extract causes partial protective effect against the hepatotoxicity of diclofenac sodium.

Diclofenac hepatic toxicity may return to the effect of drug on cytochrome p-450.The drug is biotransformed to unstable epoxide metabolites that can destroy the cytochrome p-450.

The hepatoprotective action of ginseng extract is possibly due to its antioxidative property as well as the ability of certain ginsenosides to interact with P-450 enzymes, which may prevent necrosis in the hepatocytes.Therefore, the present study suggests that ginseng extract may be affords an effective role in protection against diclofenac hepatic toxicity .

List of Tables

intramuscular low dose (2 mg/kg) of diclofenac sodium.

Table (III-E): Experiments on rats given daily co-, pre- or post oral ginseng extract for 3 days with intramuscular low dose (4 mg/kg) of diclofenac sodium.

Table (V): Experimental design for rabbits (Oral administration).

Table (1): Effect of single oral administration of different doses of diclofenac sodium (10, 20 and 30 mg/kg) on certain biochemical indicators of hepatic toxicity and the relative liver weight in rats for one week.

Table (2): Effect of different doses of diclofenac sodium (0.625, 1.25, 2.5, 5.0 and 7.5 mg/kg) along one week on certain biochemical indicators of hepatic toxicity and the relative liver weight in rats.

Table (3): Changes in some serum and liver parameters in rats (2hr) orally pre- administered with ginseng extract (50 mg/kg) along with daily administration of diclofenac sodium (2.5 mg/kg) for one week.

Table (4): Effect of single intraperitoneal injection of different doses of diclofenac sodium (10, 20 and 30 mg/kg) on serum biochemical indicators of hepatic toxicity and the relative liver weight in rats.

Table (5): Effect of daily pretreatment with ginseng extract (50 mg/kg) for 3 days and intraperitoneal treated with single doses of diclofenac sodium (10, 20 and 40 mg/kg) on serum proteins and relative liver weight. **Table (6):** Effect of single

List of Figures

Fig.(6): Changes in serum and liver parameters of single intramuscular injection of diclofenac sodium (20, 40, 80, 120 and 140 mg/kg) in rats.

Fig.(7): Changes in serum and liver parameters of rats received pre-or post treatment of ginseng extract 50 mg/kg p.o) for 3 days and injected intramuscularly with a single dose (40 mg/kg) of diclofenac sodium.

Fig.(8): Changes in serum and liver parameters of rats received pre-or post treatment of ginseng extract (50 mg/kg p.o) for 3 days and injected intramuscularly with a single dose (80 mg/kg) of diclofenac sodium.

Fig.(9): Effect of daily intramuscular injection of two doses of diclofenac sodium (2 and 4 mg/kg) for 3 days on certain biochemical indicators of hepatic toxicity and the relative liver weight in rats.

Fig.(10): Changes in serum and liver parameters of rats received co-, pre- or post oral dose of ginseng extract (50 mg/kg) concomitant with intramuscular injection of diclofenac sodium (2 mg/kg) daily for 3 days.

Fig.(11): Changes in serum and liver parameters of rats received co-, pre- or post oral dose of ginseng extract (50 mg/kg) concomitant with intramuscular injection of diclofenac sodium (4 mg/kg) daily for 3 days.

Fig.(12): Changes in serum and liver parameters of rabbits pretreated orally with ginseng extract (15 mg/kg) 2 hr.prior the treatment with diclofenac sodium (10mg/kg) for one and two weeks.

Fig.(13): Light micrograph of liver of control rats, showing normal architecture, where the hepatic lobules are regularly arranged and the hepatocytes are partitioned by hepatic sinusoids with fine distribution of lymphocytes. (H & E stain X 40).

Fig.(14): Light micrograph of liver of rats treated with repetitive doses of GE (50 mg/kg) along 3 days showing normal histological structure (H & E stain, X 40).

Fig.(15): Light micrograph of liver of rats treated with single dose of diclofenac sodium 20 mg/kg. Note the dilatation of portal veins and bile ducts (arrow) associated with oedema (arrowhead) and some inflammatory cells infiltration (small arrow) (H & E stain X40).

Fig.(16): Light micrograph of liver of rats treated with single dose of diclofenac sodium 40 mg/kg, Showing the dilatation of portal veins and bile ducts (arrow) associated with oedema (arrow head) and mild inflammatory cells infiltration (small arrow) (H & E stain X40).

Fig.(17): Light micrograph of liver of rats treated with single dose of diclofenac sodium 80 mg/kg, Showing marked dilatation of portal veins (arrow), degeneration of bile ducts (big arrow) associated with oedema (arrow head) and marked inflammatory cells infiltration (small arrow) (H & E stain X40).

Fig.(18): Light micrograph of liver of rats treated with single dose of diclofenac sodium 120 mg/kg, Showing severe dilatation of central veins (arrow) associated with fatty change (arrow head) and some inflammatory cells infiltration (small arrow) (H & E stain X40).

Fig.(19): Light micrograph of liver of rats treated with single dose of diclofenac sodium 140 mg/kg, Showing the dilatation of portal veins (arrow), oedema (arrow head), and inflammatory cells infiltration (small arrow) (A) and fatty change associated with necrotic area in the hepatocyte (big arrow) (B) (H & E stain X40 & X160).

Fig.(20): Light micrograph of liver of rats treated with repetitive doses of GE (50 mg/kg) along 3 days followed by single dose of DS (40 mg/kg), showing mild fatty change in some of the hepatocytes (arrow) and some picknotic nuclei (small arrow) (H & E stain, X 160).

Fig.(21): Light micrograph of liver of rats treated with single dose of DS (40 mg/kg) followed by repetitive doses of GE (50 mg/kg) along 3 days, showing few inflammatory cells infiltration (arrow) and fatty changes in some hepatocyte (big arrow) (H & E stain, X 160).

Fig.(22): Light micrograph of liver of rats treated with repetitive doses of GE (50 mg/kg) along 3 days followed by single dose of DS (80 mg/kg), showing few inflammation in between the hepatocyte (arrow) and fatty change in some of the hepatocyte (small arrow) (H & E stain, X 160).

Fig.(23): Light micrograph of liver of rats treated with single dose of DS (80 mg/kg) followed by repetitive doses of GE (50 mg/kg) along 3 days, showing granulated and degenerated hepatocytes (arrow), and mild inflammation (small arrow). (H & E stain, X 160).

Fig.(24): Light micrograph of liver of rats treated daily with DS (2 mg/kg) for 3 days, showing dilatation of portal vein (arrow), inflammatory cells infiltration (big arrow) and degenerated hepatocytes (small arrow). (H & E stain, X40).

Fig.(25): Light micrograph of liver of rats treated daily with GE (50 mg/kg) 2 hours before DS (2 mg/kg) for 3 days, showing dilatation of portal and central veins (arrow), inflammatory cells infiltration (big arrow) and some of pyknotic nuclei (small arrow). (H & E stain, X40).

Fig.(26): Light micrograph of liver of rats treated daily with GE (50 mg/kg) for 3 days followed by DS (2 mg/kg) for 3 days more, showing widely dilatation of portal vein (arrow), inflammatory cells infiltration (big arrow) and granular degeneration of hepatocytes (small arrow), (H & E stain, X 40).

Fig.(27): Light micrograph of liver of rats treated daily with DS (2 mg/kg) for 3 days followed by GE (50 mg/kg) for 3 days more, showing massive number of inflammatory cells infiltration (arrow) and some degenerative changes in hepatocytes (big arrow) (H & E stain, X 40).

Fig.(28): Light micrograph of liver of rats treated daily with DS (4 mg/kg) for 3 days, showing dilatation of