

**STUDIES ON RAPHANUS SATIVUS AS
HEPATOPROTECTIVE AGENTS**

Thesis Title: Raphanus sativus

A THESIS SUBMITTED
TO
THE UNIVERSITY OF THE PUNJAB
IN
PARTIAL FULFILLMENT OF REQUIREMENTS
FOR THE DEGREE OF

MASTER OF PHILOSOPHY
IN
PHARMACY
(Pharmacology)

Session 1994-96

By

Rukhsana anwar
B.Pharm., R.Ph.

Department of Pharmacy
University of the Punjab,
Lahore-Pakistan.

Roll No.

613



Supervisor/Internal Examiner

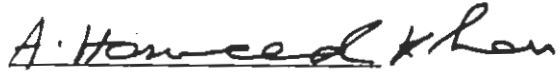
Dr. Mobashir Ahmad
Assistant Professor
Department of Pharmacy,
University of the Punjab,
Lahore.

Chairman

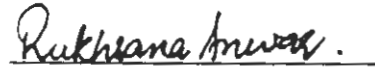


Prof. Dr. Mahbub Alam
B.Sc.(Hons.) M.Sc.(Pb.)
Ph.D.(Wales)

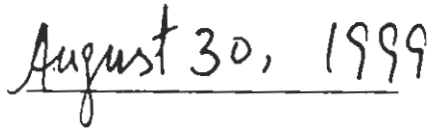
External Examiner



Research Candidate


Rukhsana Anwar

Oral Examination Held On


August 30, 1999

APPROVAL CERTIFICATE

The thesis entitled "**Studies on Raphanus Sativus as Hepatoprotective Agent**", prepared by Rukhsana Anwar under my guidance in partial fulfillment of the requirements for the degree of **Master of Philosophy**, in the subject of **Pharmacology** is hereby approved for submission.



Dr. Mobashir Ahmad
Assistant Professor
Department of Pharmacy,
University of the Punjab,
Lahore.

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

The image features the Basmala (Bismillah) in a highly stylized, bold black calligraphic font. The text is arranged in a compact, horizontal layout. Four prominent vertical lines with arrowheads at the top extend upwards from the baseline of the calligraphy, creating a sense of height and structure. The calligraphy includes various decorative elements such as small squares and dots placed near the letters. A signature or artist's mark is visible at the bottom right of the calligraphic composition.

**Dedicated to My
Parents**

ACKNOWLEDGEMENT

All praise be to Allah Almighty who gave me the required mental and physical capacities to accomplish my research work.

I love to express my debt of gratitude and sincere appreciation to my supervisor, **Dr. Mobashir Ahmad**, B.Pharm., M.Pharm., M.Phil., Ph.D., Assistant Professor in Pharmacology, University of the Punjab, Lahore, for his keen interest, enlightening guidance, friendly and sympathetic vigilance and encouraging attitude germane to the consummation of my research work and writing this thesis. To add his scholarly suggestions, timely availability and perfect monitoring went a long way to engineer me in order to embrace my formidable challenge.

I am very much grateful to **Prof. Dr. M. Afzal Sheikh**, Ex-Chairman, Department of Pharmacy, University of the Punjab, Lahore, for patronising me in my research work and providing me all the requisite paraphernalia to do my research.

I am also very much grateful to **Prof. Dr. Mahbub Alam**, Chairman, Department of Pharmacy, University of the Punjab, Lahore. It was only due to his affection and deep concern that I met no snag in laboratories. Besides his backing me up and motivating me to my ends defies words to express my obligation.

I have deeply felt sense of gratitude to my father for his loving paternal prayers and good wishes. I am very grateful to my elder brother **Munawar Ramzan** for his skill in typing and composing of mine thesis. I am also to remember the eager and loving inspiring words of my husband, brothers and sister to successfully complete my research work.


RUKHSANA ANWAR

CONTENTS

Discription	Page
List of Table	
List of Figures	
Abstract	
Section - 1	
<u>Introduction</u>	
Raphanus sativus	1
Composition	1
Chemical Nature	2
Pharmacological Action	5
Hepato Protective Agents	7
Section - 2	
Jaundice	12
Bilirubin, Its Metabolism	13
Classification	15
Test for Disclosing Hepatic Dysfunction	19
Section - 3	
Paracetamol	24
Pharmacokinetics	24
Dose and Administration	25
Pharmacodynamics	26
Hepatotoxicity	29

Section - 4

Material and Method

2.1	Apparatus	33
2.2	Chemicals	34
2.3	Procurement and Maintenance of Animals	34
2.4	Preparation of Crude Drug from R. sativus Leaves	35
2.5	Collection of Blood	36
2.6	Experimental Scheme	36
2.7	Biochemical Investigation	38
2.7.1	Determination of Serum Glutamate Oxalacetate Transaminase "SGOT" Activity.	38
2.7.2	Determination of Serum Glutamate Pyruvate Transaminase "SGPT" Activity.	44
2.7.3	Determination of Serum Lactate Dehydrogenase "LDH" Activity.	50
2.7.4	Determination of Serum Alkaline Phosphatase "AP" Activity.	52
2.7.5	Determination of Serum Total Bilirubin	54
2.7.6	Determination of Serum Total Protein	55
2.8	Determination of Acute toxic Dose of Paracetamol	58

Section - 5

Results	66
---------	----

Section - 6

Discussion	147
------------	-----

Section - 7

Appendix	168
----------	-----

Section - 8

Bibliography	172
--------------	-----

LIST OF TABLES

<u>Table No.</u>	<u>Title</u>	<u>Page</u>
2.8.1	The Effect of Different Dose of Paracetamol on SGOT u/l Activity at Different time Intervals	60
2.8.2	The Effect of Different Dose of Paracetamol on SGPT u/l Activity at Different time Intervals	61
2.8.3	The Effect of Different Dose of Paracetamol on LDH u/l Activity at Different time Intervals	62
2.8.4	The Effect of Different Dose of Paracetamol on Serum AP u/l Activity at Different time Intervals	63
2.8.5	The Effect of Different Dose of Paracetamol on Serum Total Bilirubin mg/dl at Different time Intervals.	64
2.8.6	The Effect of Different Dose of Paracetamol on Serum Total Protein g/dl at Different time Intervals.	65
3.1.1	Effect of Crude Raphanus sativus Leaves Powder on SGOT u/l Activity in the Paracetamol Induced Rabbits.	69
3.1.2	Comparison on the Activity of SGOT u/l Between Paracetamol Induced and Paracetamol + Raphanus Crude Powder.	70
3.1.3	Effect of Water Extract of Raphanus sativus Crude Powder on SGOT u/l Activity in the Paracetamol Induced Rabbits.	72
3.1.4	Comparison on the Activity of SGOT u/l Between Paracetamol Induced and Paracetamol + Water Extract of R. sativus Crude Powder.	73
3.1.5	Effect of Ethanol Extract of Raphanus sativus Crude Powder on SGOT u/l Activity in the Paracetamol Induced Rabbits.	76

- 3.1.6 Comparison on the Activity of SGOT u/l Between 77
Paracetamol Induced and Paracetamol + R. sativus
Crude Powder.
- 3.2.1 Effect of Crude Raphanus sativus Leaves Powder 81
on SGPT u/l Activity in the Paracetamol
Induced Rabbits.
- 3.2.2 Comparison on the Activity of SGPT u/l Between 82
Paracetamol Induced and Paracetamol + Raphanus
sativus Crude Powder.
- 3.2.3 Effect of Water Extract of Raphanus sativus 84
Leaves Powder on SGPT u/l Activity in the
Paracetamol Induced Rabbits.
- 3.2.4 Comparison on the Activity of SGPT u/l Between 85
Paracetamol Induced and Paracetamol + Water
Extract of R. sativus Crude Powder.
- 3.2.5 Effect of Ethanol Extract of Raphanus sativus 88
Leaves Powder on SGPT u/l Activity in the
Paracetamol Induced Rabbits.
- 3.2.6 Comparison on the Activity of SGPT u/l Between 89
Paracetamol Induced and Paracetamol + Ethanol
Extract of R. sativus Crude Powder.
- 3.3.1 Effect of Crude Raphanus sativus Leaves Powder 93
on Serum LDH u/l Activity in the Paracetamol
Induced Rabbits.
- 3.3.2 Comparison on the Activity of Serum LDH u/l 94
Between Paracetamol Induced and Paracetamol +
R. sativus Crude Powder.
- 3.3.3 Effect of Water Extract of Raphanus sativus 96
Leaves Powder on Serum LDH u/l Activity in the
Paracetamol Induced Rabbits.
- 3.3.4 Comparison on the Activity of Serum LDH u/l 97
Between Paracetamol Induced and Paracetamol +
Water Extract of R. sativus Crude Powder.
- 3.3.5 Effect of Ethanol Extract of Raphanus sativus 100
Leaves Powder on Serum LDH u/l Activity in the
Paracetamol Induced Rabbits.

3.3.6	Comparison on the Activity of Serum LDH u/l Between Paracetamol Induced and Paracetamol + Ethanol Extract of <i>R. sativus</i> Crude Powder.	101
3.4.1	Effect of Crude <i>Raphanus sativus</i> Leaves Powder on Serum AP u/l Activity in the Paracetamol Induced Rabbits.	105
3.4.2	Comparison on the Activity of Serum AP u/l Between Paracetamol Induced and Paracetamol + <i>R. sativus</i> Crude Powder.	106
3.4.3	Effect of Water Extract of <i>Raphanus sativus</i> Leaves Powder on Serum AP u/l Activity in the Paracetamol Induced Rabbits.	108
3.4.4	Comparison on the Activity of Serum AP u/l Between Paracetamol Induced and Paracetamol + Water Extract of <i>Raphanus sativus</i> Crude Powder.	109
3.4.5	Effect of Ethanol Extract of <i>Raphanus sativus</i> Leaves Powder on Serum AP u/l Activity in the Paracetamol Induced Rabbits.	112
3.4.6	Comparison on the Activity of Serum AP u/l Between Paracetamol Induced and Paracetamol + Ethanol Extract of <i>R. sativus</i> Crude Powder.	113
3.5.1	Effect of Crude <i>Raphanus sativus</i> Leaves Powder on Serum Total Bilirubin mg/dl in the Paracetamol Induced Rabbits.	117
3.5.2	Comparison on the Serum Total Bilirubin mg/dl Between Paracetamol Induced and Paracetamol + <i>R. sativus</i> Crude Powder.	118
3.5.3	Effect of Water Extract of <i>Raphanus sativus</i> Leaves Powder on Serum Total Bilirubin mg/dl in the Paracetamol Induced Rabbits.	120
3.5.4	Comparison on the Serum Total Bilirubin mg/dl Between Paracetamol Induced and Paracetamol + Water Extract of <i>R. sativus</i> Crude Powder.	121

3.5.5	Effect of Ethanol Extract of <i>Raphanus sativus</i> Leaves Powder on Serum Total Bilirubin mg/dl in the Paracetamol Induced Rabbits.	124
3.5.6	Comparison on the Serum Total Bilirubin mg/dl Between Paracetamol Induced and Paracetamol + Ethanol Extract of <i>R. sativus</i> Crude Powder.	125
3.6.1	Effect of Crude <i>Raphanus sativus</i> Leaves Powder on Serum Total Protein g/dl in the Paracetamol Induced Rabbits.	129
3.6.2	Comparison on the Serum Total Protein g/dl Between Paracetamol Induced and Paracetamol + <i>R. sativus</i> Crude Powder.	130
3.6.3	Effect of Water Extract of <i>Raphanus sativus</i> Leaves Powder on Serum Total Protein g/dl in the Paracetamol Induced Rabbits.	132
3.6.4	Comparison on the Serum Total Protein g/dl Between Paracetamol Induced and Paracetamol + Water Extract of <i>Raphanus sativus</i> Crude Powder.	133
3.6.5	Effect of Ethanol Extract of <i>Raphanus sativus</i> Leaves Powder on Serum Total Protein g/dl in the Paracetamol Induced Rabbits.	136
3.6.6	Comparison on the Serum Total Protein g/dl Between Paracetamol Induced and Paracetamol + Ethanol Extract of <i>R. sativus</i> Crude Powder.	137
3.7.1	Effect of Chronic Administration of Paracetamol on SGOT, SGPT, Serum LDH, Serum AP, Serum Total Bilirubin and Serum Total Protein and its Blocking with <i>R. sativus</i> Leaves Powder.	140
4.1	Comparison of Effects of Paracetamol Crude Drug, Its Water and Ethanol Extracts on SGOT u/l Activity in Paracetamol Induced Rabbits.	148
4.2	Comparison of Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on SGPT u/l Activity in Paracetamol Induced Rabbits.	151

4.3	Comparison of Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on Serum LDH u/l Activity in Paracetamol Induced Rabbits.	154
4.4	Comparison of Effect of Paracetamol Crude Drugs, Its Water and Ethanol Extracts on Serum AP u/l Activity in Paracetamol Induced Rabbits.	156
4.5	Comparison of Effect of Paracetamol Crude Drugs, Its Water and Ethanol Extracts on Serum Total Bilirubin mg/dl in Paracetamol Induced Rabbits.	158
4.6	Comparison of Effect of Paracetamol Crude Drugs, Its Water and Ethanol Extracts on Serum Total Protein g/dl in Paracetamol Induced Rabbits.	160

LIST OF FIGURES

<u>Fig.No.</u>	<u>Title</u>	<u>Page</u>
3.1.1	Graph Showing the Effect of Paracetamol on SGOT Activity.	67
3.1.2	Graph Showing the Effect of <i>R. sativus</i> Crude Powder on SGOT Activity in Paracetamol Induced Rabbits.	71
3.1.3	Graph Showing the Effect of Water Extract of <i>R. sativus</i> Crude Powder on SGOT Activity in Paracetamol Induced Rabbits.	74
3.1.4	Graph Showing the Effect of Ethanol Extract of <i>R. sativus</i> Crude Powder on SGOT Activity in Paracetamol Induced Rabbits.	75
3.2.1	Graph Showing the Effect of Paracetamol on SGPT Activity.	79
3.2.2	Graph Showing the Effect of <i>R. sativus</i> Crude Powder on SGPT Activity in Paracetamol Induced Rabbits.	83
3.2.3	Graph Showing the Effect of Water Extract of <i>R. sativus</i> Crude Powder on SGPT Activity in Paracetamol Induced Rabbits.	86
3.2.4	Graph Showing the Effect of Ethanol Extract of <i>R. sativus</i> Crude Powder on SGPT Activity in Paracetamol Induced Rabbits.	90
3.3.1	Graph Showing the Effect of Paracetamol on Serum LDH Activity.	91
3.3.2	Graph Showing the Effect of <i>R. sativus</i> Crude Powder on Serum LDH Activity in Paracetamol Induced Rabbits.	95
3.3.3	Graph Showing the Effect of Water Extract of <i>R. sativus</i> Crude Powder on Serum LDH Activity in Paracetamol Induced Rabbits.	98

3.3.4	Graph Showing the Effect of Ethanol Extract of R. sativus Crude Powder on Serum LDH Activity in Paracetamol Induced Rabbits.	102
3.4.1	Graph Showing the Effect of Paracetamol on Serum AP Activity.	103
3.4.2	Graph Showing the Effect of R. sativus Crude Powder on Serum AP Activity in Paracetamol Induced Rabbits.	107
3.4.3	Graph Showing the Effect of Water Extract of R. sativus Crude Powder on Serum AP Activity in Paracetamol Induced Rabbits.	110
3.4.4	Graph Showing the Effect of Ethanol Extract of R. sativus Crude Powder on Serum AP Activity in Paracetamol Induced Rabbits.	114
3.5.1	Graph Showing the Effect of Paracetamol on Serum Total Bilirubin Level.	115
3.5.2	Graph Showing the Effect of R. sativus Crude Powder on Serum Total Bilirubin Level in Paracetamol Induced Rabbits.	119
3.5.3	Graph Showing the Effect of Water Extract of R. sativus Crude Powder on Serum Total Bilirubin Level in Paracetamol Induced Rabbits	122
3.5.4	Graph Showing the Effect of Ethanol Extract of R. sativus Crude Powder on Serum Total Bilirubin Level in Paracetamol Induced Rabbits.	126
3.6.1	Graph Showing the Effect of Paracetamol on Serum Total Protein Level.	127
3.6.2	Graph Showing the Effect of R. sativus Crude Powder on Serum Total Protein Level in Paracetamol Induced Rabbits.	131
3.6.3	Graph Showing the Effect of Water Extract of R. sativus Crude Powder on Serum Total Protein Level in Paracetamol Induced Rabbits.	134
3.6.4	Graph Showing the Effect of Ethanol Extract of R. sativus Crude Powder on Serum Total Protein Level in Paracetamol Induced Rabbits.	138

3.7.1	Graph Showing the Effect of Chronic Administration of Paracetamol on SGOT Activity and Blocking with <i>R. sativus</i> Crude Powder.	141
3.7.2	Graph Showing the Effect of Chronic Administration of Paracetamol on SGPT Activity and Blocking with <i>R. sativus</i> Crude Powder.	142
3.7.3	Graph Showing the Effect of Chronic Administration of Paracetamol on Serum LDH Activity and Blocking with <i>R. sativus</i> Crude Powder.	143
3.7.4	Graph Showing the Effect of Chronic Administration of Paracetamol on Serum AP Activity and Blocking with <i>R. sativus</i> Crude Powder.	144
3.7.5	Graph Showing the Effect of Chronic Administration of Paracetamol on Serum Total Bilirubin Level and Blocking with <i>R. sativus</i> Crude Powder.	145
3.7.6	Graph Showing the Effect of Chronic Administration of Paracetamol on Serum Total Protein Level and Blocking with <i>R. sativus</i> Crude Powder	146
4.1	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on SGOT Activity in Paracetamol Induced Rabbits	149
4.2	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on SGPT Activity in Paracetamol Induced Rabbits	152
4.3	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on Serum LDH Activity in Paracetamol Induced Rabbits	155
4.4	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on Serum AP Activity in Paracetamol Induced Rabbits	157
4.5	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on Serum Total Bilirubin Level in Paracetamol Induced Rabbits	159
4.6	Graph Showing the Effect of Paracetamol Crude Drug, Its Water and Ethanol Extracts on Serum Total Protein Level in Paracetamol Induced Rabbits	161

ABSTRACT

The protective effect of *Raphanus sativus* leaves powder, its water and ethanol extracts against paracetamol hepatotoxicity was investigated. Paracetamol increased the activity of hepatic enzymes (SGOT, SGPT, Serum LDH, Serum AP). The crude *Raphanus sativus* leaves powder in paracetamol induced rabbits, decrease SGOT, SGPT, SERUM LDH, SERUM AP, SERUM TOTAL BILIRUBIN AND SERUM TOTAL PROTEIN LEVELS.

Hepatoprotective activity of water and ethanolic extracts of *Raphanus sativus* leaves powder was also investigated. the results indicates that the *Raphanus sativus* ethanolic extract contains hepatoprotective constituents and suggest further work on the isolation and characterization of these constituents which may potentially be used as hepatoprotective agents.

OBJECTS AND SCOPE

Despite the great adventures and inventions made by the synthetic chemists in providing effective tools to help the health of poor humanity, the ideal drugs could not be synthesized for the treatment of the conditions such as hypertension, asthma, cancer, diabetes and liver disorder etc.

Research on indigenous medicinal plants has resulted in many therapeutic agents becoming available and many more remains to be discovered. Thus in search of the best, the scientific research was started on the pharmacological activity of *Raphanus sativus*. In traditional medicine *Raphanus sativus* was used to cure different disease i.e. Jaundice, Syphilis, Piles (Kirtikar & Basu, 1987, Qadoos, 1989). The leaves, root and fruit of the plant proved to be useful. But all this owed to the human observations and no research was done in this connection on the scientific bases. For this reason present scientific work was planned to study the effects of crude *Raphanus sativus* leaf powder its water and ethanol extracts on the activity of the enzymes i.e. SGOT, SGPT, SERUM LDH, SERUM AP, SERUM TOTAL BILIRUBIN and SERUM TOTAL PROTEIN LEVELS. Paracetamol produced liver damage as manifested by significant rise in liver weight and AST activity & high bilirubin concentration (Rasheed et al., 1995). Toxic doses of paracetamol destroy the cellular defence system in hepatic tissue.

The experimental work was planned not only to see the effects of *Raphanus sativus* leaves on normal but also on paracetamol induced rabbits. As such the information may be helpful for the treatment of patient with hepatic toxicity.