

STUDY OF CARBON TETRACHLORIDE HEPATOTOXICITY

THESIS

Submitted to the Faculty of Medicine,
Ain Shams University for the Degree of
Doctor of Philosophy (Biochemistry)

By

EZZAT RUSHDY AWAD

M. B. , B. Ch. M. Sc.

Assistant Lecturer in Biochemistry Department
Faculty of Medicine
Ain Shams University

Supervised by .

Prof. Dr. MOHAMED FARID EL ASMAR
Professor and Head of Biochemistry Department

Prof. Dr. SALAH EL DIN ZAKI EID
Professor in Biochemistry Department

Dr. MONIR BOLOS HANNA
Prof. in Biochemistry Department

(1983)

ACKNOWLEDGEMENT

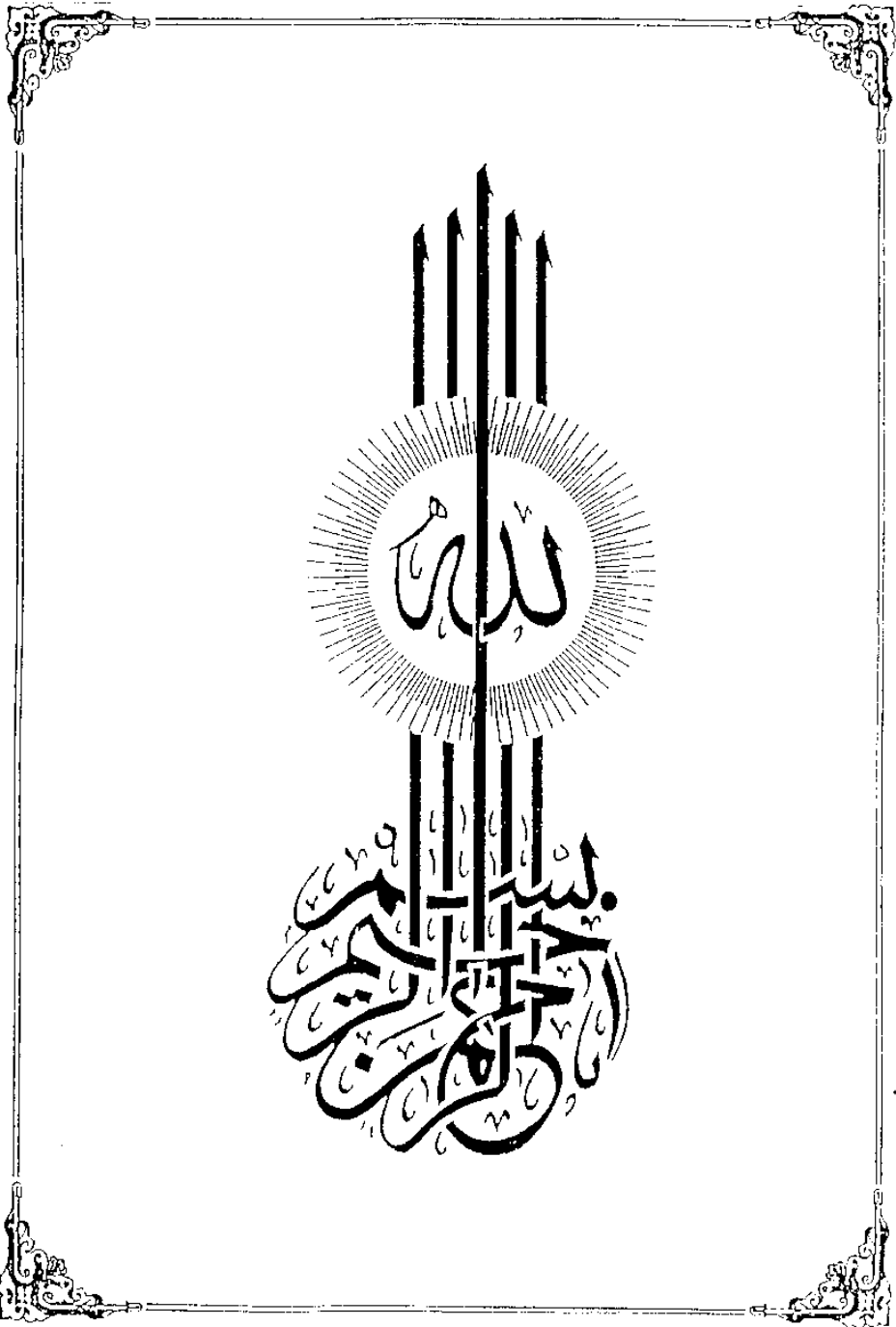
I wish to offer my sincere thanks and gratitude to Prof. Dr. Mohamed Farid El Asmar, Professor and Head of the Biochemistry department for suggesting the research point and his continuous encouragement throughout the entire work.

His help was far more than I have a right to ask.

I am deeply grateful to Prof. Dr. Salah El Din Zaki Eid for his valuable help and continuous encouragement. I would also like to express my gratitude to Dr. Monir Boles Hanna for his sincere help and true understanding.

Finally, a word of appreciation is due to the faculty, Staff and Colleagues of the Biochemistry department for ever available help and understanding.

We wish also to express our gratitude to Dr. Fawzi Nagib, Assistant Prof. of Pathology for his help in histopathological studies.



C O N T E N T S

	Page
INTRODUCTION	1
- Pharmacological actions of CCl_4	2
- Carbon tetrachloride toxicity in man	2
- The pathological changes in acute carbon tetrachloride toxicity	5
- The clinical manifestation of acute carbon tetrachloride poisoning	9
- Mechanism of action of carbon tetrachloride	12
- Blood lipids.....	31
- Carbon tetrachloride induced fatty liver.	48
- Species difference.....	53
- Glutamic oxaloacetic transaminase (GOT)..	54
AIM OF THE WORK.....	57
MATERIALS AND METHODS.....	59
- Materials.....	59
- Methods.....	62
- Extraction of total lipids.....	62
- Estimation of total lipids.....	63
- Estimation of triglycerides.....	65
- Estimation of total cholesterol.....	67
- Determination of total phospholipids.....	70

C O N T E N T (C O N T .)

	Page
- Thin layer chromatography.....	73
- Determination of non esterified fatty acids.	75
- Determination of esterified fatty acids.....	77
- Estimation of protein content.....	79
- Determination of serum glutamic oxaloacetic transaminase.....	80
- Determination of nucleic acids in liver tissue.....	82
- Serum proteins electrophoresis.....	85
- Determination of clotting factor X.....	91
- Determination of clotting factor V.....	94
- Determination of clotting factorII	96
- Statistical Analysis.....	98
RESULTS AND TABLES.....	99
DISCUSSION.....	184
SUMMARY.....	211
REFERENCES.....	215
ARABIC SUMMARY.	

LIST OF TABLES

	Page
Table 1. Liver total lipids in rats after treatment with CCl ₄	116
Table 2. Serum total lipids in rats after treatment with CCl ₄	117
Table 3. Liver triglycerides in rats after treatment with CCl ₄	118
Table 4. Serum triglycerides in rats after treatment with CCl ₄	119
Table 5. Liver total cholesterol in rats after treatment with CCl ₄	120
Table 6. Serum total cholesterol in rats after treatment with CCl ₄	121
Table 7. Serum total phospholipids in rats after treatment with CCl ₄	122
Table 8. Liver total phospholipids in rats after treatment with CCl ₄	123
Table 9. Serum esterified fatty acids in rats after treatment with CCl ₄	124
Table 10. Serum free fatty acids in rats after treatment with CCl ₄	125
Table 11. Serum total proteins in rats after treatment with CCl ₄	126
Table 12. Serum albumin in rats after treatment with CCl ₄	127

LIST OF TABLES

(Cont.)

	Page
Table 13. Serum alpha ₁ globulins in rats after treatment with CCl ₄	128
Table 14. Serum alpha ₂ globulins in rats after treatment with CCl ₄	129
Table 15. Serum beta-globulins in rats after treatment with CCl ₄	130
Table 16. Serum gamma globulins in rats after treatment with CCl ₄	131
Table 17. Total liver proteins in rats after treatment with CCl ₄	132
Table 18(A). Liver "RNA" in rats after treatment with CCl ₄	133
Table 19(A). Liver "DNA" in rats after treatment with CCl ₄	134
Table 18(B). Liver "RNA" in rats after treatment with CCl ₄ (mg/mg tissue proteins).....	135
Table 19(B). Liver "DNA" in rats after treatment with CCl ₄ (mg/mg tissue proteins).....	136
Table 20(I). Serum phospholipid fractions by thin layer chromatography.....	137
Table 20(II). Effect of CCl ₄ poisoning on clotting factors X,V,II.....	138
Table 20(III). Serum total lipids in rats after treatment with CCl ₄ and EDTA.....	139

LIST OF TABLES

(Cont.)

	<u>Page</u>
Table 21. Liver total lipids in rats after treatment with CCl_4 and EDTA.	140
Table 22. Liver triglycerides in rats after treatment with CCl_4 and EDTA.....	141
Table 23. Serum triglycerides after treatment with CCl_4 and EDTA.	142
Table 24. Liver total cholesterol in rats after treatment with CCl_4 and EDTA.....	143
Table 25. Serum total cholesterol in rats after treatment with CCl_4 and EDTA.....	144
Table 26. Serum glutamic oxalo-acetic transaminase in rats after treatment with CCl_4 and EDTA.	145
Table 27. Serum total lipids in rats after treatment with EDTA and CCl_4	146
Table 28. Serum triglycerides in rats after treatment with EDTA and CCl_4	147
Table 29. Serum total cholesterol in rats after treatment with EDTA and CCl_4	148
Table 30. Serum glutamic oxalo-acetic transaminase in rats after treatment with EDTA and CCl_4	149
Table 31. Serum total lipids in rats after treatment with CCl_4 and EDTA(at end of one month).....	150

LIST OF TABLES

(Contd.)

	Page
Table 32. Liver total lipids in rats after treatment with CCl ₄ and EDTA (at end of one month).....	151
Table 33. Serum triglycerides in rats after treatment with CCl ₄ and EDTA(at end of one month).....	152
Table 34. Liver triglycerides in rats after treatment with CCl ₄ and EDTA(at end of one month).....	153
Table 35. Serum total cholesterol in rats after treatment with CCl ₄ and EDTA (at end of one month).....	154
Table 36. Liver cholesterol in rats after treatment with CCl ₄ and EDTA(at end of one month)..	155
Table 37. Serum glutamic oxalo-acetic transaminase in rats after treatment with CCl ₄ and EDTA (at end of one month).....	156
Table 38. Serum total lipids in rats after treatment with CCl ₄ and verapamil	157
Table 39. Liver total lipids in rats after treatment with CCl ₄ and verapamil	158
Table 40. Serum triglycerides in rats after treatment with CCl ₄ and verapamil.....	159

LIST OF TABLES

(Cont.)

	Page
Table 41. Liver triglycerides in rats after treatment with CCl ₄ and verapamil.....	160
Table 42. Serum total cholesterol in rats after treatment with CCl ₄ and verapamil	161
Table 43. Liver total cholesterol in rats after treatment with CCl ₄ and verapamil.....	162
Table 44. Serum glutamic oxalo-acetic transaminase in rats after treatment with CCl ₄ and verapamil	163
Table 45. Serum total lipids in rats after treatment with CCl ₄ and verapamil (at end of one month)	164
Table 46. Liver total lipids in rats after treatment with CCl ₄ and verapamil (at end of one month)	165
Table 47. Serum triglycerides in rats after treatment with CCl ₄ and verapamil (at end of one month)	166
Table 48. Liver triglycerides in rats after treatment with CCl ₄ and verapamil (at end of one month)	167
Table 49. Serum total cholesterol in rats after treatment with CCl ₄ and verapamil (at end of one month)	168

LIST OF TABLES

(Cont.)

	Page
Table 50. Liver total cholesterol in rats after treatment with CCl_4 and verapamil (at end of one month).....	169
Table 51. Serum glutamic oxalo-acetic transaminase in rats after treatment with CCl_4 and verapamil (at end of one month)	170

LIST OF FIGURES

	Page
Fig. 1 Twenty four hours after I.P.CCl ₄ in paraffin oil and show central zonal necrosis with evidence of fatty degeneration	171
Fig. 2 Twenty four hours after I.P. paraffin oil showing normal liver cells.....	171
Fig. 3 Twenty four hours after I.P. CCl ₄ in paraffin oil and received five I.P. doses of EDTA before CCl ₄ administration and show no necrosis of liver cells but there is vacuolar degeneration	172
Fig. 4 Twenty four hours after I.P. paraffin oil and received five I.P. doses of EDTA before paraffin oil administration and show normal liver cells with minimal dilatation of blood vessels...	172
Fig. 5 Six days after I.P.CCl ₄ in paraffin oil and show marked necrosis surrounded by liver cells showing fatty degeneration with few scattered normal liver cells.....	173
Fig. 6 Six days after I.P. paraffin oil and show normal liver cells.....	173
Fig. 7 Six days after I.P. CCl ₄ in paraffin oil followed by five I.P. doses of EDTA after CCl ₄ administration and show normal liver cells with slight blood vessel dilatation.....	174
Fig. 8 Six days after I.P. paraffin oil followed by five I.P. doses of EDTA after paraffin oil administration and show normal liver cells with minimal dilatation of blood vessels..	174
Fig. 9 One month after I.P. CCl ₄ in paraffin oil followed by five doses of EDTA and show normal liver cells	175
Fig. 10 One month after I.P. paraffin oil followed by five I.P. doses of EDTA after paraffin oil administration and show normal liver cells..	175

LIST OF FIGURES

(Cont.)

	Page
Fig.11 Six days after I.P. CCl ₄ in paraffin oil followed by I.P. verapamil for five successive days after CCl ₄ administration and show normal liver cells	176
Fig. 12 Six days after I.P. paraffin oil followed by I.P. verapamil for five successive days after paraffin oil administration and show normal liver cells.....	176
Fig. 13 Six days after I.P. paraffin oil and show normal liver cells.....	177
Fig. 14 One month after I.P. paraffin oil and show normal liver cells.....	178
Fig. 15 One month after I.P. CCl ₄ in paraffin oil followed by I.P. verapamil for five successive days and show normal liver cells...	178
Fig.16 One month after I.P. paraffin oil followed by I.P. verapamil for five successive days and show normal liver cells.....	179
Fig. 17 Fractionation of serum phospholipids by thin layer chromatography	179
Fig. 18 Serum electrophoresis on cellulose acetate membrane in normal rats.....	180
Fig. 19 Serum electrophoresis on cellulose acetate membrane in rats after CCl ₄ treatment	180

INTRODUCTION

INTRODUCTION

Carbon tetrachloride (CCl_4) is a chlorinated hydrocarbon chemically related to chloroform. The compound, first prepared in 1849, was used initially as an anaesthetic by Simpson, the discoverer of chloroform anaesthesia, but soon discarded because of its toxicity. It was later introduced for the treatment of hookworm infestations (Tomb and Helmy, 1933) and was used for this purpose for many years; but the compound has now been almost abandoned in favor of less toxic anthelmintics. Nevertheless, CCl_4 remains of interest to physicians because its industrial and household uses make it a source of poisoning. Carbon tetrachloride is widely employed as a reactant and a solvent of the chemical ingredient in fire extinguisher fuel and insecticide sprays. It has been used in the manufacture of rubber, and as a nonflammable solvent in factories, garages, floorwaxes, cleaners and as a disinfectant.