A CLINICO-PATHOLOGICAL AND BIOCHEMICAL STUDY

OF THE TOXICITY OF

CALLILEPIS LAUREOLA (IMPILA)

BY

KESHAVLAL DAYA NAROTAM BHOOLA

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF MEDICINE IN THE DEPARTMENT OF ANATOMICAL PATHOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF NATAL.

DURBAN 1983

CALLILEPIS LAUREOLA DC. (COMPOSITAE)

The title photograph was reproduced by the kind permission of Janet M. Gibson.

From: *Wild Flowers of Natal (Coastal Region).* Plate III.

Published by: The Trustees of the Natal
Publishing Trust Fund
Durban 1975


5

To My Wife

KUSUM

DECLARATION

I hereby certify that this research, unless specifically indicated to the contrary in the text, is the result of my own investigation and has not already been accepted for any degree and is not being concurrently submitted in candidature for any other degree.

K.D.N. BHOOLA

ACKNOWLEDGEMENTS

I am indebted to the late Professor J. Wainwright for the initiation of this project and his active and enthusiastic encouragement as well as to Professor W.P.P. Leary for his subsequent supervision and helpful criticism. I hereby also express my gratitude to Professor S. Kallichurum for guidance and assistance. It is also a pleasure to record my thanks to members of the Department of Anatomical Pathology who have in one way or another contributed to the successful completion of this study - in particular the technical staff. In addition, I wish to thank the following:

Mrs. K.B. Brookes, Mr. H.A. Candy and Dr. E. Malan for chemical analysis and identification of constituents of *Callilepis laureola*.

Miss A. van Middelkoop and Professor A.I. Dale for evaluation of statistical data.

Members of the Department of Medical Illustration.

ABSTRACT

This study was undertaken as a result of the occurrence of a large number of deaths among the local Black population from the use of herbal medicines prepared from the rootstock of *Callilepis laureola* known to the Zulus as *impila*. The salient clinico-pathological features in these cases were hypoglycaemia, centrilobular zonal liver necrosis and acute renal tubular necrosis. The purpose of this study was to investigate fully the clinical, biochemical and pathological aspects of the toxicity produced by *Callilepis laureola (impila)*.

The first part of the investigation consisted of an assessment of all cases of death due to acute liver necrosis diagnosed by necropsy at King Edward VIII Hospital, Durban. A review of clinical and necropsy records of 21687 consecutive post-mortems performed on Black patients during a 20 year period showed that acute liver necrosis was the major contributing cause of death in 447 patients. In 263 cases the hepatic lesion was centrilobular zonal necrosis with associated acute tubular necrosis (Group A); while in 184 cases the liver necrosis was of the massive or submassive type (Group B). A comparative assessment of these two groups as regards necropsy prevalence, age and sex distribution and the clinical, biochemical and pathological findings was undertaken. This study shows that the combination of hypoglycaemia, centrilobular zonal liver necrosis and acute renal tubular necrosis due to *Callilepis laureola (impila)* poisoning is a distinct clinico-pathological entity and differentiates this group from cases of acute massive and submassive liver necrosis resulting in most cases from fulminant viral hepatitis.

In the search for the toxic components of the root of *Callile*

several compounds were isolated. These were atractyloside, carboxy-atractyloside, two thymol related oils and a carbohydrate. The thymol related oils as well as the carbohydrate were found to be non-toxic in laboratory rats. The crude methanol extract of the root of *Callilepis laureola*, when injected intraperitoneally into laboratory rats, produced centrilobular zonal liver necrosis and acute renal tubular necrosis, the lesions identical to those seen in patients who had died after intake of *impila* prescribed by witchdoctors and other dispensers of herbal medicines.

On the other hand intraperitoneal injections of the purified compound atractyloside caused acute renal tubular necrosis and hypoglycaemia in laboratory rats but failed to produce liver necrosis. Carboxyatractyloside also failed to cause liver necrosis. This indicated that there may be at least two toxins contained in the rootstock of *Callilepis laureola*, one causing the liver lesion and the other (atractyloside) causing nephrotoxicity and hypoglycaemia.

Repeated attempts at isolating the hepatotoxin have failed; the liver toxin or toxins being lost during the process of extraction and purification. Identification of the hepatotoxin awaits further investigation. It is possible that the liver necrosis may be caused by a metabolite or that it may be a synergistic effect of two or more compounds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

SECTION 1

GENERAL INTRODUCTION

Consultation of traditional practitioners such as herbalists and witchdoctors is a widespread practice among the Black people of Natal and the tribal "Medicine Man" is very often the first source of primary health care, the hospital being resorted to only if this contact is unrewarding. Certainly many Black patients have already visited the herbalist before arriving at King Edward VIII Hospital, Durban. The majority of these Black patients belong to the Zulu tribe.

There are three main types of "Medicine Men" among the Zulus (Krige, 1974). The *inyanga* (herbalist) who has knowledge of roots and herbs, simply dispenses his medicines without ceremony and claims no special relation to the spirits. The *isanusi* (diviner) who practices as a mystic and usually combines with his divination a fairly extensive knowledge of herbs and roots by means of which he can treat diseases without having to send his patient to the herbalist. The *umthakathi* (wizard) on the other hand is the enemy of society and uses his power for evil against the welfare of society; he is believed to injure people's health, destroy life, prevent rain and cause all manner of misfortune. He is able to carry out his evil practices by virtue of the herbal medicines he uses. Since the direct cause of illness is very often believed to be due to the black arts of an *umthakathi* it is essential that the traditional Zulu "practitioner" be able to combat these by dispensing his own herbal medicines.

The Zulus traditionally use large numbers of herbs, barks and roots, many of which have real medicinal value. Herbal mixtures and medically prescribed drugs are often used together.

Umuthi omnyama ("Black medicine") is a generic term for all medicines that are administered with the intention of charming away evil. In such preparations leaves and roots or both are powdered and mixed with water; this is then churned until it becomes frothy when it is drunk in a kneeling position outside the kraal (Kirge, 1974).

HISTORICAL BACKGROUND

Hospital pathologists at King Edward VIII Hospital, Durban had for some time been interested in necropsies of children and adults who died due to a combination of hypoglycaemia, centrilobular zonal necrosis of the liver and acute renal tubular necrosis.

In 1964 Neame and Pillay investigated hypoglycaemia in Black patients at King Edward VIII Hospital and noticed that some patients did not respond to the intravenous administration of dextrose solution and died soon after admission. Necropsy findings and histological sections in eleven of these patients showed acute centrilobular zonal necrosis of the liver, in all and in four cases acute renal tubular necrosis was also present. In seven of these patients a history of ingesting a herbal remedy had been obtained.

In 1977 Wainwright and Schonland undertook a sociological investigation of toxic hepatitis. A Zulu social worker, Mrs. S. Ngcobo, was engaged to interview relatives of patients who appeared to have died from toxic liver necrosis. In all, 30 cases were investigated. She encountered considerable difficulty in persuading the relatives to divulge what medicine had been given to the deceased or supply samples of such remedies. Nevertheless, after repeated visits her questioning did reveal that probably in all 30 cases some form of traditional herbal remedy had been administered to the deceased. In some cases the relatives were unaware of the nature of the herbs which had been obtained from the *inyanga*. Several samples of tubers and plants were obtained from the relatives and these were identified botanically at the Natal Herbarium.

The following plants were identified (Zulu names in brackets):

Albizia gummifera		*(Umhlandlothi)*
Trichilia emetica	Vahl	*(Umkhulu)*
Stephani abyssinica	Walp	*(Umtambane)*
Adenia gummifera		*(Imfulwa)*
Acacia caffra	Wield	*(Umtholo)*

All these roots and plants were extracted and administered to laboratory animals but none proved to have any hepatotoxic or nehprotoxic effects.

One case which provided useful information in identifying at least one poisonous plant was reported by Wainwright and Schonland in 1977. A six month old infant was admitted to King Edward VIII Hospital on 29 August 1973 with a history of cough and dyspnoea for 4 days followed by vomiting and abdominal distension for 1 day. On examination, the child had marked tachycardia and acidosis but was not jaundiced. Bronchopneumonia and gastroenteritis were diagnosed clinically and treatment was instituted. However, the child died on the day of admission. Necropsy revealed bilateral bronchopneumonia, a normal heart, distended small intestine and colon filled with watery stool. The liver was pale but showed pronounced centrilobular zonal engorgement. The liver weighed 175 g (expected normal weight 240 g). The cortices of both kidneys appeared pale and swollen. Histological sections of the lungs showed a viral pneumonitis with secondary bacterial pneumonia. The liver showed centrilobular zonal necrosis with collapse of the reticulin, cholestasis and fatty change in the surviving hepatocytes. The kidneys showed degenerating and necrotic tubular epithelium indicative of acute tubular necrosis.

The mother of this child, on questioning by the social worker, admitted to having given the infant an enema which she had prepared from a root. The original extract was not available but a dessicated tuber of the

AIMS OF THE THESIS

Callilepis laureola (impila) has long been known in Natal as a medicinal plant. The root of this plant and other herbs are widely used by many Blacks for self medication or are prescribed by the herbalists.

This study was undertaken at the suggestion of Professor J. Wainwright, Department of Anatomical Pathology, Faculty of Medicine, University of Natal, following upon the occurrence of a large number of deaths among the local Black population (mainly Zulus) from the use of *Callilepis laureola*. The salient clinico-pathological features in these cases were hypoglycaemia, toxic centrilobular liver necrosis and acute renal tubular necrosis.

Under the supervision of Professor Schlemmer, Department of Social Sciences, University of Natal, a survey of a properly randomised sample of residents of the Umlazi African Township was undertaken by a Zulu social worker, Mrs. S. Ngcobo. This survey revealed that 30% of the residents questioned used or had used *"impila"* which was regarded as a valuable medicinal plant.

In view of its widespread usage one would expect fatal toxic liver necrosis to occur even more frequently than encountered. That this is not the case may be explained firstly by the fact that the preparations may have contained low doses of the toxin, secondly that they have an emetic action and when taken by mouth some of the toxin may have been expelled by vomiting and, thirdly, that the toxic content of the root extracts may have varied, e.g. if a dessicated tuber is used the extract might contain more or less toxin than when it is used in the fresh state.

Extracts of *Callilepis laureola* were investigated by Wainwright, Schonland and Candy (1977). Powdered rootstock was extracted with a number of organic solvents and extracts were tested on laboratory rats. The acetone and methanol extracts proved to be both hepatotoxic and nephrotoxic.

The initial part of the study reported in this thesis was designed as a comparative clinical and pathological assessment of (A) toxic centrilobular zonal liver necrosis, and (B) acute massive and submassive liver necrosis. The final objective of such an evaluation was to see if toxic centrilobular zonal necrosis of the liver emerges as an easily recognisable separate entity.

Subsequent investigations involved purification and identification of the toxic principles contained in the root of *Callilepis laureola*. The chemically separated fractions were tested on laboratory rats for hepato-renal toxicity and for their hypoglycaemic effect. The morphological changes produced in rat liver and kidney were examined by light microscopy.

SECTION 2

A COMPARATIVE REVIEW OF TOXIC CENTRILOBULAR ZONAL LIVER NECROSIS AND MASSIVE/SUBMASSIVE LIVER NECROSIS

PURPOSE

The purpose of this study was to investigate clinical, biochemical and pathological aspects of the toxicity produced by *Callilepis laureola (impila)*. In order to achieve this it became necessary to undertake an assessment of all cases of death due to acute liver necrosis diagnosed by necropsy at King Edward VIII Hospital. In addition, the necropsy prevalence, age and sex distribution and the clinical, biochemical and pathological findings in patients with toxic centrilobular zonal liver necrosis were analysed and compared to those with acute massive and submassive liver necrosis. The objective of such a comparison was to establish whether "toxic liver necrosis" seen among the local Black population could be categorised as a recognisable separate clinico-pathological entity.

MATERIAL AND METHODS

This section therefore represents a review of clinical and necropsy records and the material studied consists of 21 687 consecutive post-mortems performed on Black patients at King Edward VIII Hospital during the 20 year period, January 1958 to December 1977. All age groups with the exception of neonatal deaths have been included.

The necropsy material for the survey has been drawn from both King Edward VIII Hospital (2 000 beds) and its subsidiary hospital at Clairwood (1 400 beds). These hospitals are situated in Durban and together serve the majority of the Black population in the city and

its periurban areas. A small proportion of the patients are drawn from other areas of the province, so that the total population served numbers 2 million (Seedat and Reddy, 1976).

During the period under consideration necropsies were performed by various pathologists and every case was routinely studied by standard histological methods. From March 1974, 15% of the post mortems were personally performed.

The Mann-Whitney U-z test was used in evaluating statistical data.

ANALYSIS OF MATERIAL

During the period under review, acute liver necrosis was found to be the major contributing cause of death in 447 patients. In 263 cases the lesion was a centrilobular zonal necrosis (Group A), while in 184 cases the necrosis was of the massive or submassive type (Group B). Cases showing less well defined zonal necrosis associated with congestive cardiac failure, severe shock or severe infection have been excluded from the analysis.

PREVALENCE OF ACUTE LIVER NECROSIS

Figures of annual necropsy prevalence (Table 1 and Figure 1) show that the number of cases of centrilobular zonal liver necrosis has varied from year to year with an annual average of 13 cases. The total number of necropsies carried out on Black patients by the Department of Pathology is given in Table 1. It can be seen from Tables 1 and 2 and Figure 1 that the annual prevalence of massive and submassive liver necrosis varies less than that of centrilobular zonal necrosis. In the period 1959 - 1963 a large number of cases of centrilobular zonal liver necrosis were found at necropsy. During this period the necropsy rate

TABLE 1

ANNUAL NECROPSY PREVALENCE AND SEX DISTRIBUTION OF CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

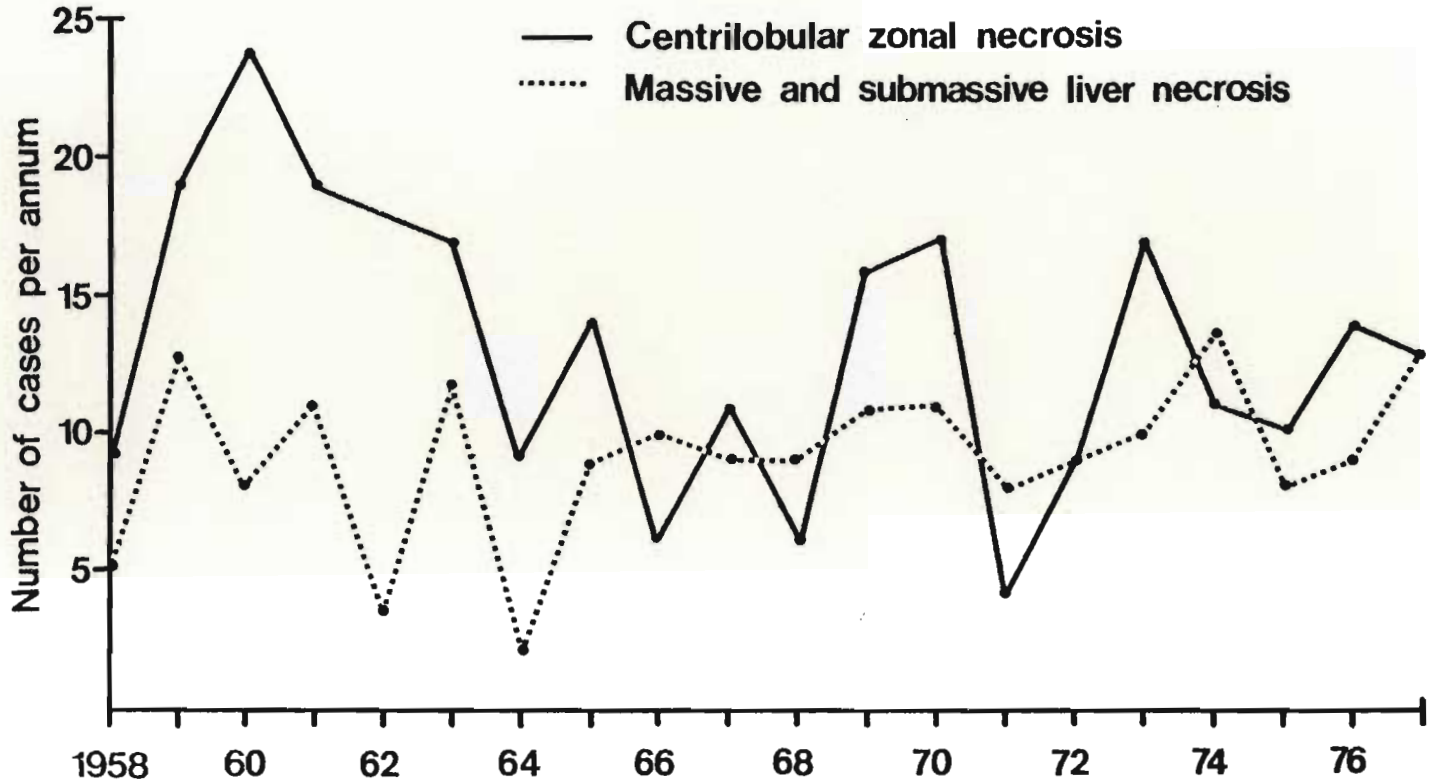
Year	Males	Females	Total	% of Total Necropsies	Total number of Necropsies on Black patients
1958	4	5	9	0,66	1357
1959	8	11	19	0,89	2116
1960	11	13	24	1,23	1938
1961	7	12	19	1,08	1751
1962	7	11	18	0,96	1872
1963	6	11	17	1,13	1501
1964	5	4	9	0,85	1048
1965	3	11	14	1,25	1119
1966	0	6	6	0,61	968
1967	6	5	11	1,62	678
1968	5	1	6	0,72	828
1969	8	8	16	1,88	850
1970	7	10	17	2,41	705
1971	3	1	4	0,55	721
1972	4	5	9	1,44	622
1973	11	6	17	2,44	694
1974	5	6	11	1,46	749
1975	4	6	10	1,40	714
1976	6	8	14	1,92	730
1977	9	4	13	1,79	726
TOTAL	119	144	263	1,21	21687
PERCENT OF TOTAL	45,2	54,8	100		
MEAN PER ANNUM	6	7	13		

Mann-Whitney U-z test: applied in relation to sex difference

$z = 1,1312$

$p = 0,2585$

FIG.1 ANNUAL PREVALENCE OF CENTRILOBULAR ZONAL NECROSIS AND MASSIVE/SUBMASSIVE LIVER NECROSIS



YEAR OF STUDY:

PERIOD OF STUDY: 1958 - 1977

TABLE 2

ANNUAL NECROPSY PREVALENCE AND SEX DISTRIBUTION OF CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

Year	Males	Females	Total	% of Total Necropsies	Total number of Necropsies on Black patients
1958	2	3	5	0,36	1357
1959	6	7	13	0,61	2116
1960	3	5	8	0,41	1938
1961	8	3	11	0,62	1751
1962	1	2	3	0,16	1872
1963	9	3	12	0,79	1501
1964	2	0	2	0,19	1048
1965	4	5	9	0,80	1119
1966	5	5	10	1,03	968
1967	2	7	9	1,32	678
1968	4	5	9	1,09	828
1969	4	7	11	1,29	850
1970	4	7	11	1,56	705
1971	1	7	8	1,10	721
1972	5	4	9	1,44	622
1973	5	5	10	1,44	694
1974	4	10	14	1,86	749
1975	4	4	8	1,12	714
1976	2	7	9	1,23	730
1977	5	8	13	1,79	726
TOTAL	80	104	184	0,85	21687
PERCENT OF TOTAL	43,5	56,5	100		
MEAN PER ANNUM	4	5	9		

Mann-Whitney U-z test: applied in relation to sex difference

$z = 1,781$

$p = 0,0751$

(Table 1) was high, ranging from 1 500 - 2 000 cases per year. Subsequently the annual necropsy number was in the region of 1000 cases or less.

AGE DISTRIBUTION OF ACUTE LIVER NECROSIS

The age distribution of cases with centrilobular zonal liver necrosis and massive/submassive liver necrosis is shown in Tables 3 and 4 respectively and compared in Figure 2. The age distribution varies amongst the youngest members studied. Centrilobular zonal liver necrosis occurred predominantly in patients under the age of 14 years (about 1/2 of cases), while most cases of massive and submassive liver necrosis occurred in the age group 0 - 24 years. Both types of liver necrosis became less common with age.

SEX DISTRIBUTION OF ACUTE LIVER NECROSIS

The sex distribution of patients with centrilobular zonal liver necrosis and massive/submassive liver necrosis shown in Tables 1 and 2 indicate slight female predominance in both types of liver damage. This difference is not significant (Mann-Whitney U-z test). In both sexes death due to centrilobular zonal liver necrosis was commoner than death due to massive and submassive liver necrosis.

CLINICAL PRESENTATION OF ACUTE LIVER NECROSIS

The clinical presentation of cases of centrilobular zonal liver necrosis and massive/submassive liver necrosis have been analysed separately for convenience.

TABLE 3

AGE DISTRIBUTION IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

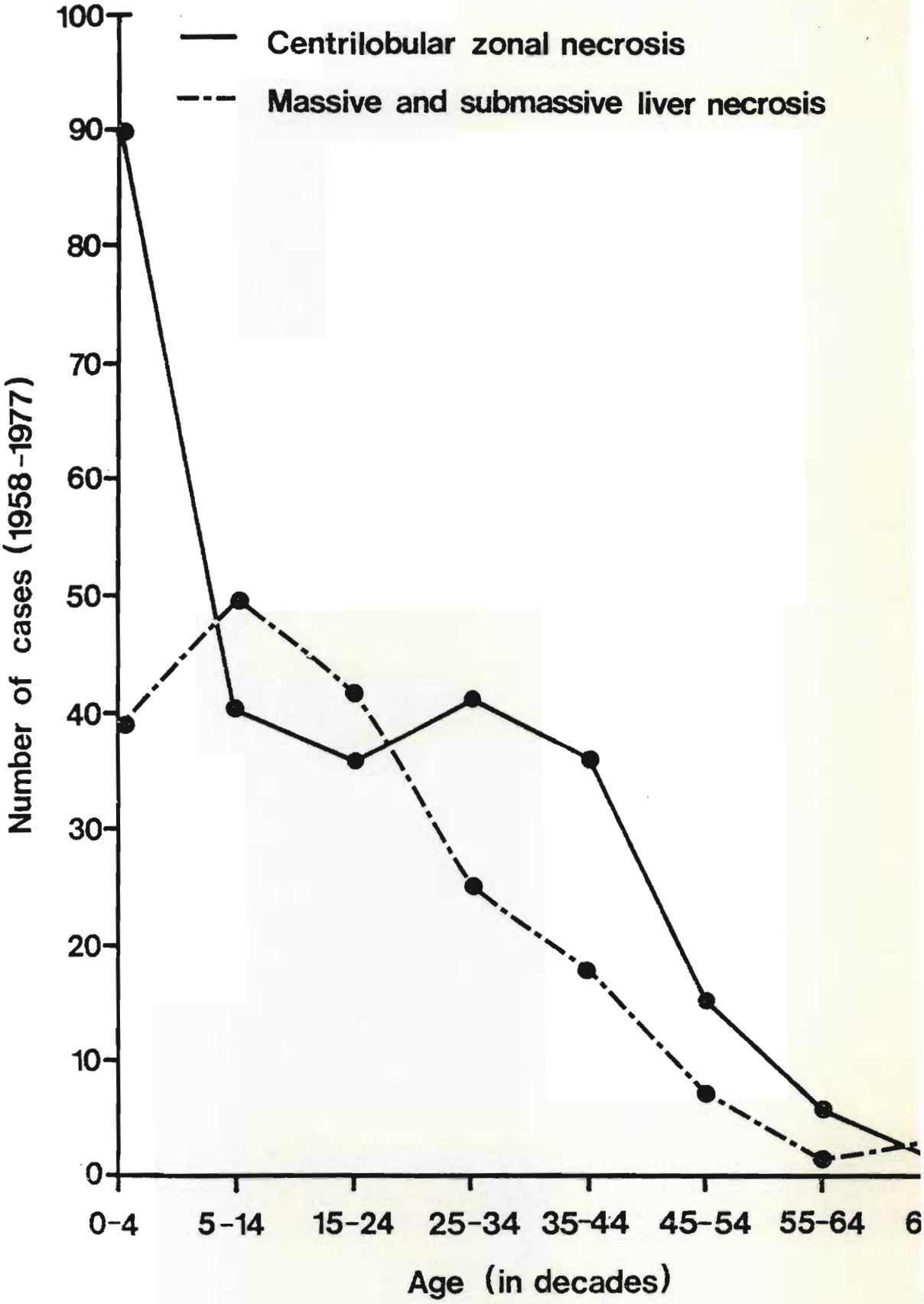
Year	AGE DISTRIBUTION IN YEARS							
	0-4	5-14	15-24	25-34	35-44	45-54	55-64+	Total
1958	1	1	3		1	1	2	9
1959	4	2	7	4		2		19
1960	4	8	1	2	4	2	3	24
1961	7	1	2	4	4	1		19
1962	7	2	2	3	3	1		18
1963	5	1	2	2	4	3		17
1964	3	2	2		2			9
1965	3	2	3	2	4			14
1966	3	1	1			1		6
1967	3	1	3	3	1			11
1968	1	1		1	3			6
1969	10	2		2	1	1		16
1970	8			7		2		17
1971	4							4
1972	2	3	1	2	1			9
1973	6	5	2	1	2		1	17
1974	6	2	2	1				11
1975	2	1	1	4	1	1		10
1976	4	3	2	1	4			14
1977	6	2	2	2	1			13
TOTAL	89	40	36	41	36	15	6	263
% OF TOTAL	33,8	15,2	13,7	15,6	13,7	5,7	2,3	100

TABLE 4

AGE DISTRIBUTION IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

Year	AGE DISTRIBUTION IN YEARS							
	0-4	5-14	15-24	25-34	35-44	45-54	55-64+	Total
1958	2		1	1			1	5
1959	4	3	1	2	1	1	1	13
1960		2	2	3	1			8
1961	1	5	2	1	1	1		11
1962	1	1	1					3
1963	3	4	4			1		12
1964			1		1			2
1965	2	2	1	1	3			9
1966	4	3	1	1	1			10
1967		2	2	1	2	1	1	9
1968	1	2	3	2	1			9
1969	2	6	1		2			11
1970	3	2	3		2	1		11
1971		2	2	3		1		8
1972	5	1	1	2				9
1973	3	4	3					10
1974	1	4	6	3				14
1975	3	3		1	1			8
1976	2	3	3		1			9
1977	2	1	4	4	1	1		13
TOTAL	39	50	42	25	18	7	3	184
% OF TOTAL	21,2	27,2	22,8	13,6	9,8	3,8	1,6	100

FIG. 2 AGE DISTRIBUTION IN CENTRILOBULAR ZONAL NECROSIS AND MASSIVE/SUBMASSIVE LIVER NECROSIS



GROUP A : CENTRILOBULAR ZONAL LIVER NECROSIS

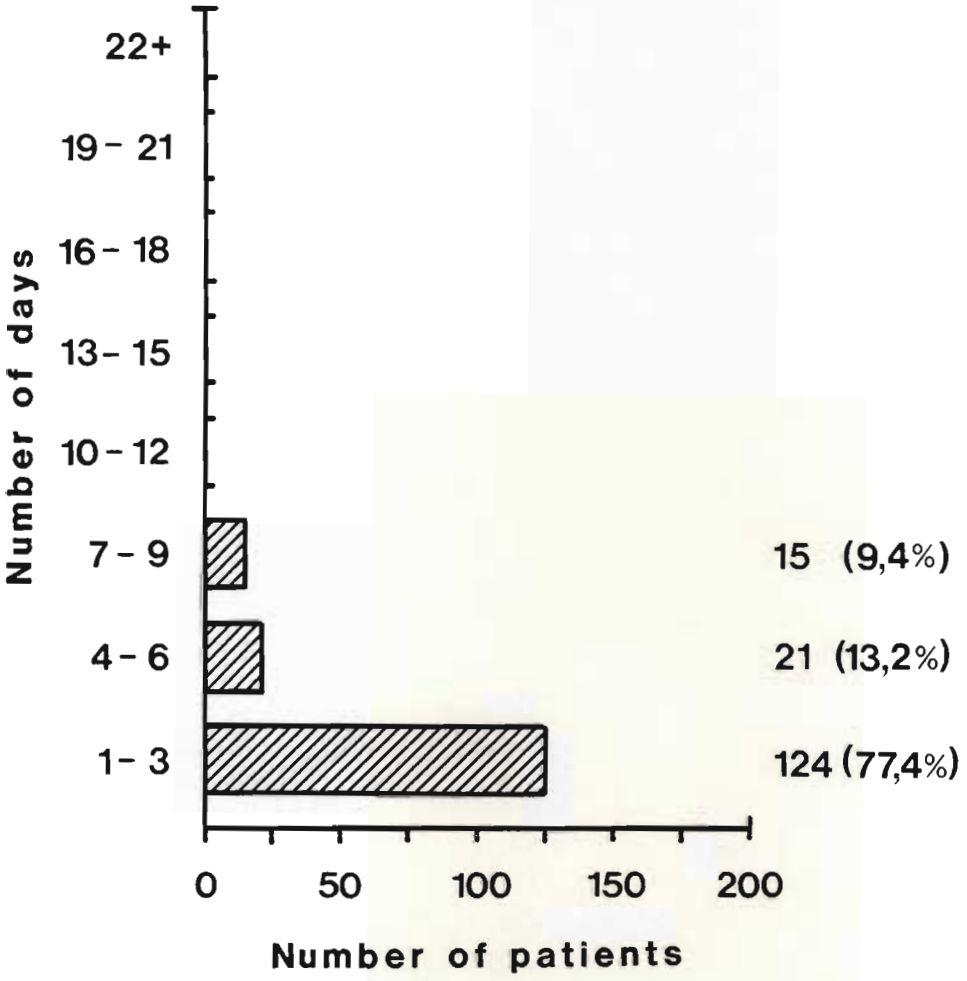
Clinical Features

A positive history of either oral or rectal administration of herbal medicine was obtained in 53 (20%) of the patients. There was no record of the remainder having been questioned specifically regarding herbal medication. The duration of illness before admission to hospital was usually very short. In 35% of cases it was 1 day or less and in 54% 2 - 5 days (see Figure 3). The symptoms recorded on admission and during hospitalisation were:

Disturbed level of consciousness	74%
Vomiting	46%
Abdominal pain	18%
Jaundice	13%
Diarrhoea and vomiting	12%
Headache	11%
Pyrexia	8%

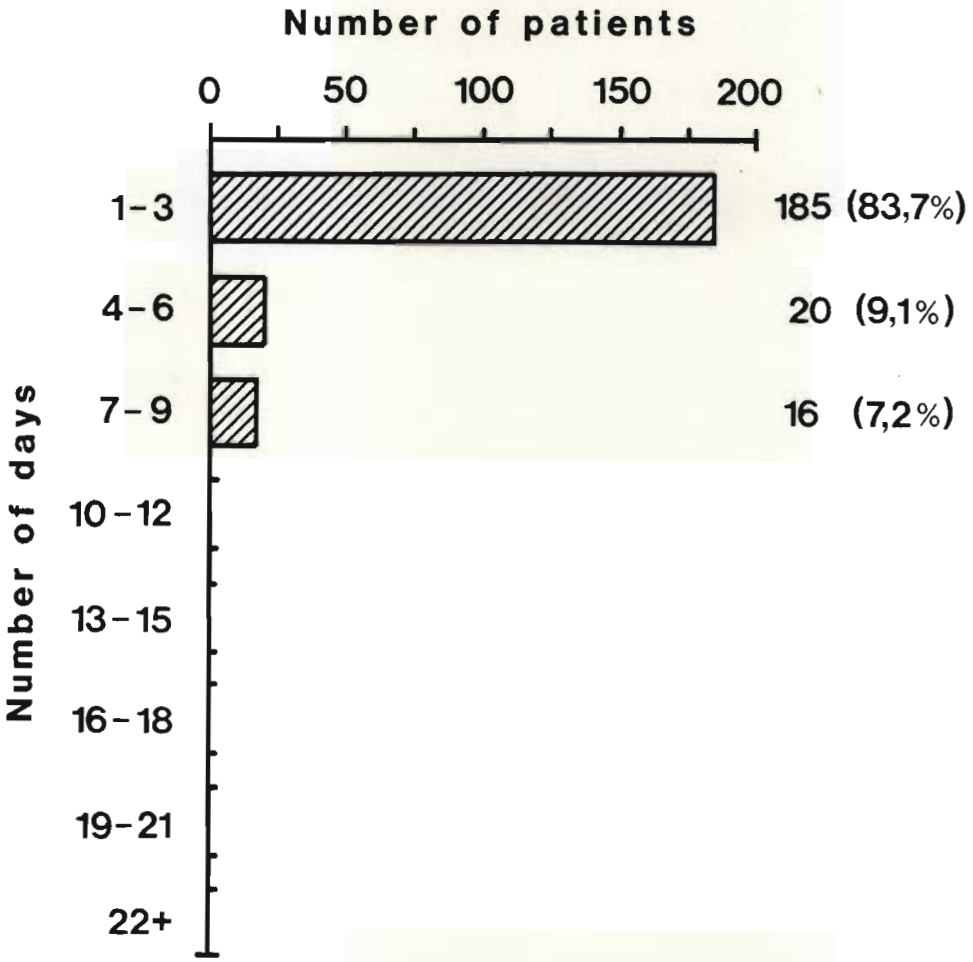
In 11% of the cases, vomiting was accompanied by haemetemesis and in 2% there was blood in the stools. Disturbed level of consciousness was associated with confusion in 10%, convulsions in 23% and 41% became frankly comatose, but focal neurological signs or meningeal irritation was not evident in them. Twelve percent of patients were hypotonic and 6% hyporeflexic. Acidotic breathing was prominent in 77% of patients. The liver was palpable in only 12% of cases and clinical jaundice was noted in 35 patients (13%). Jaundice was recorded as mild in 31, moderate in 2 patients and there were only 2 cases of severe jaundice. The duration of illness in hospital is illustrated in Figure 4.

FIG.3 DURATION OF ILLNESS BEFORE ADMISSION IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS.



This data has been retrieved from history elicited from patients and their relatives. Based on information from 160 cases.

FIG.4. DURATION OF ILLNESS IN HOSPITAL IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS



This data has been elicited from clinical records of patients.
Based on information from 221 cases.

Laboratory Investigations

a) Plasma glucose

Rapidly developing hypoglycaemia was an almost universal finding. Plasma glucose estimations were recorded in 114 patients (Figure 5). Hypoglycaemia was demonstrated in 82 (71%) of these cases with the plasma glucose values falling to very low levels (< 1,0 mmol/litre) in 35 patients (31%). These patients failed to recover despite intravenous dextrose therapy, although there was some initial improvement in their level of consciousness.

In an occasional case where the patient had survived for several days and the plasma glucose had been monitored, the hypoglycaemia persisted despite intravenous glucose therapy. Examples of three such cases are given in Table 5. Patients with plasma glucose values in the normal or above normal range had been given intravenous glucose as an emergency procedure, shortly prior to the taking of blood samples.

b) Plasma urea

Plasma urea estimations were available in 118 patients. Of these 85 (72%) had raised urea values. In 45% of cases the plasma urea values had reached high levels ranging from 16 - 80 mmol/litre (Figure 6). Eight patients had become oliguric or anuric. Twelve patients were dialysed for renal failure. In patients who had survived several days there was progressive rise in plasma urea level as shown in Table 6.

c) Liver function

Disturbed liver function was demonstrable in those patients who lived long enough for tests to be carried out (statistical data are given in

FIG.5 PLASMA GLUCOSE VALUES IN 114 CASES OF CENTRILOBULAR ZONAL NECROSIS

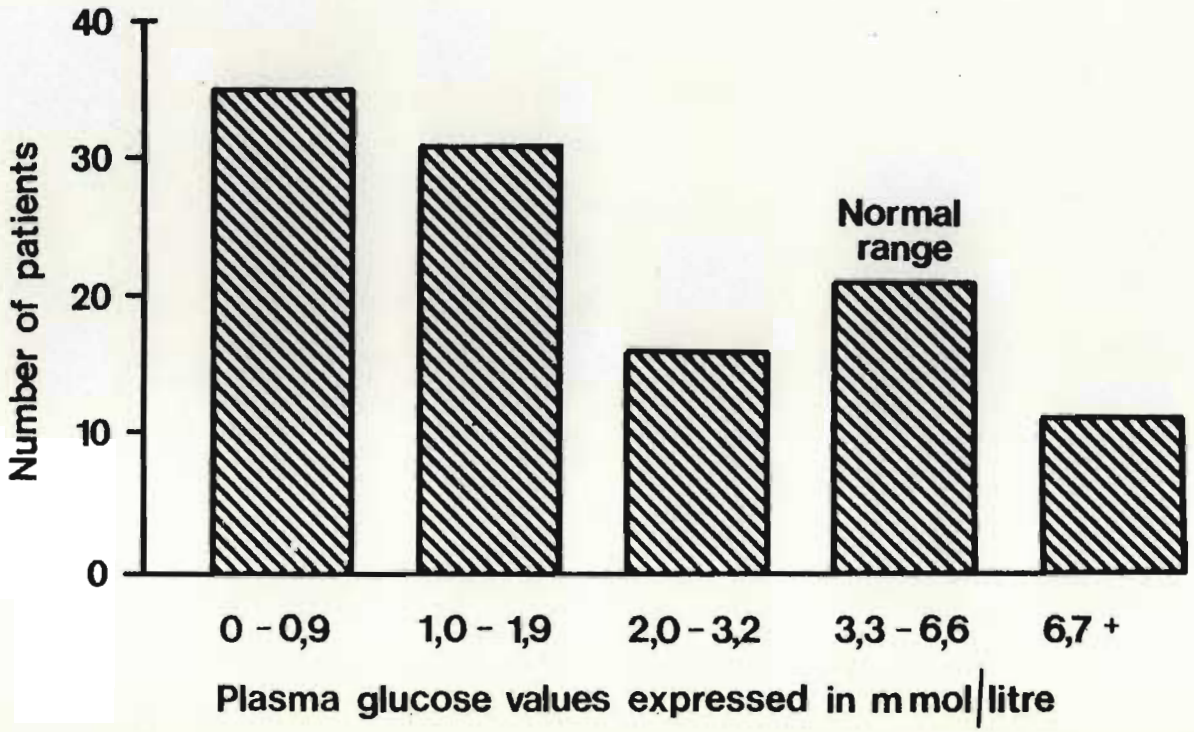


TABLE 5

EXAMPLES OF PERSISTENT HYPOGLYCAEMIA IN 3 CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

	DAYS AFTER ADMISSION			
	1	2	3	4
Case 1	0,8	1,8	2,7	2,5
Case 2	0,6	1,2	2,0	2,3
Case 3	1,8	2,0	2,1	2,2
	Plasma glucose in mmol/litre			

Normal range: 3,33 - 6,66 mmol/litre

FIG.6 PLASMA UREA VALUES IN 118 CASES OF CENTRILOBULAR ZONAL NECROSIS

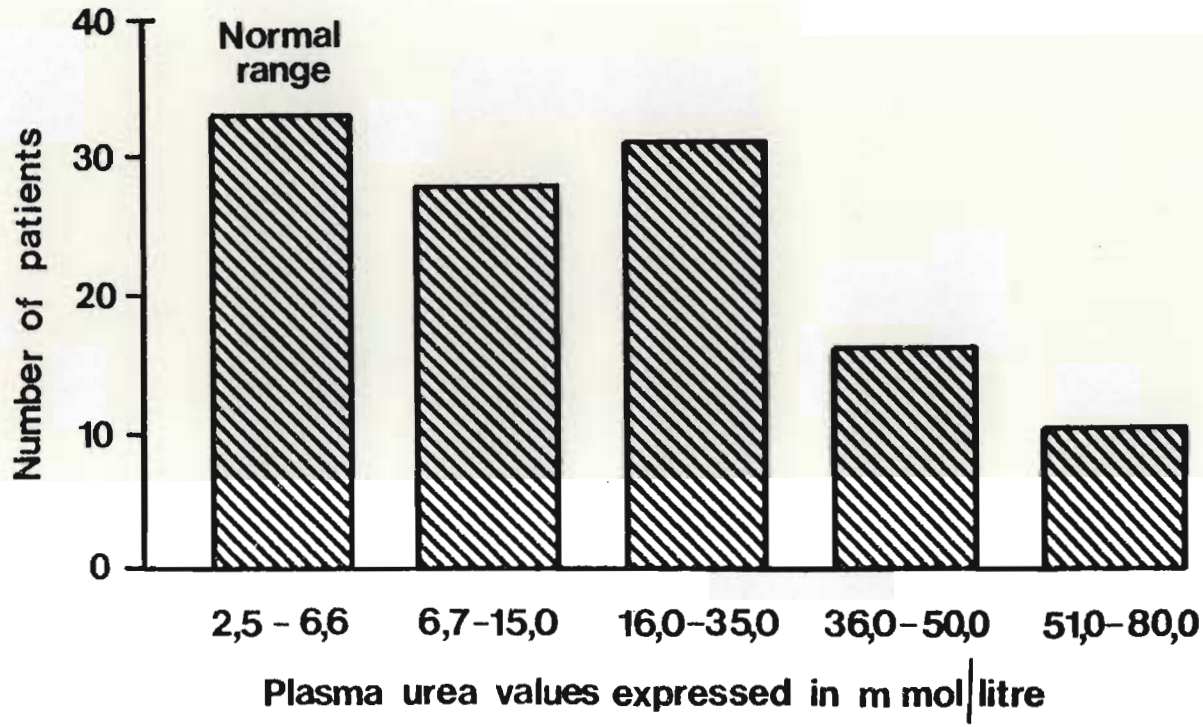


TABLE 6

EXAMPLES OF PROGRESSIVE RISE IN PLASMA UREA VALUES IN 6 CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

	DAYS AFTER ADMISSION				
	1	2	3	4	5
Case 1		17,7	24,3	29,5	41,0
Case 2		19,4	24,3	24,7	
Case 3	22,5	32,3	28,3		29,0
Case 4		39,2	70,8		34,0
Case 5	4,5	13,1	24,0	37,5	39,5
Case 6		29,3		71,3	
	Plasma urea in mmol/litre				

Normal range: 2,49 - 6,64 mmol/litre

Tables 7 A & B). In all cases there was a significant rise in the values of lactate dehydrogenase (LDH) and in 4 cases there was over a four fold increase. Both aspartate transaminase (AST) and alanine transaminase (ALT) were increased in all cases, indicating hepato-cellular damage. The alkaline phophatase (ALP) varied considerably with elevated values in 68% of cases. The values of total serum bilirubin varied from 12 - 291 µmol/litre and were found to be increased in 93% of patients. The prothrombin index was assayed in 19 patients - 10 of these (53%) had values below 50%.

Total serum protein values were found to be within the normal range in the majority of patients. Two patients out of seventeen had values of less than 60 grams/litre. The values of serum albumin however were below and those of serum globulin were higher than the normal accepted range in most patients.

d) Plasma electrolytes

Plasma electrolyte estimations (Table 8) showed hyperkalaemia in 55%, hyponatraemia in 58% and hypochloraemia in 50% of cases. Plasma bicarbonate values were estimated in 44 patients. Thirty-four (77%) of these were significantly acidotic (< 18 mmol/litre) with values ranging between 4,1 to 18 mmol/litre, the average being 11 mmol/litre.

e) Urine analysis

Clinical records showed that the illness followed a rapidly fatal course after admission, about 63% having died within 24 hours and about 28% between 2 - 5 days. The results of urine analysis were available therefore in only 45 patients (Table 9). Albuminuria was found in 80%, glycosuria in 33% and haematuria in 22%. Bilirubin was noted in one patient (2%) and ketone bodies were present in the urine of 3 patients (7%). Only one of these patients was a probable diabetic.

TABLE 7A

SERUM ENZYME VALUES IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

L.D.H. (LACTATE DEHYDROGENASE) E.C. 1.1.1.27

N	10
Median	896 (260 - 4296)
Range	4036
< 120	0
120 - 240	0
> 240	10 (100%)

Normal values: 120 - 240 U/litre

Values expressed in International Units per litre.

A.S.T. (ASPARTATE TRANSAMINASE) E.C. 2.6.1.1.

N	14
Median	143 (27 - 1764)
Range	1737
Up to 12	0
> 12	14 (100%)

Normal values up to 12 U/litre

Values expressed in International Units per litre.

E.C. = Enzyme commission

N = Number of cases

Statistical Analysis: Mann-Whitney Test

TABLE 7A (CONTINUED)

A.L.T. (ALANINE TRANSAMINASE) E.C. 2.6.1.2

N	6
Median	213 (69 - 432)
Range	363
Up to 12	0
> 12	6 (100%)

Normal values: Up to 12 U/litre

Values expressed in International Units per litre.

A.L.P. (ALKALINE PHOSPHATASE) E.C. 3.1.3.1

N	22
Median	145 (21 - 605)
Range	584
Raised values	15 (68%)

NORMAL VALUES

AGE IN YEARS	U/LITRE
2 - 4	68 - 135
5 - 7	52 - 169
8 - 10	78 - 284
11 - 13	32 - 299
14 - 15	32 - 167
16 - 17	32 - 109
Over 18	30 - 85
Borderline	85 - 95

Values expressed in International Units per litre.

E.C. = Enzyme commission

N = Number of cases

Statistical analysis: Mann-Whitney Test

TABLE 7B

LIVER FUNCTION TESTS IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

TOTAL SERUM BILIRUBIN

N	27
Median	65 (12 - 291)
Range	279
3,4 - 17,1	2
> 17,1	25 (93%)

Normal values: 3,4 - 17,1 µmol/litre

Values expressed in µmol/litre

PROTHROMBIN INDEX

N	19
Median	47 (27 - 92)
Range	65
< 50	10 (53%)

Values expressed as %

N = Number of cases

Statistical analysis: Mann-Whitney Test

TABLE 7B (CONTINUED)

SERUM TOTAL PROTEINS

N	17
Median	65 (53 - 84)
Range	31
Mean	67

Normal range: 60 - 82 grams/litre
Values expressed in grams per litre

SERUM ALBUMIN

N	17
Median	29 (16 - 38)
Range	22
Mean	28

Normal range: 35 - 50 grams/litre
Values expressed in grams per litre

SERUM GLOBULIN

N	17
Median	39 (26 - 56)
Range	30
Mean	38

Normal range: 25 - 32 grams/litre
Values expressed in grams per litre

N = Number of cases
Statistical analysis: Mann-Whitney Test

TABLE 8

PLASMA ELECTROLYTE ESTIMATIONS IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

	PLASMA ELECTROLYTE	NUMBER OF PATIENTS	%
Increased Values			
> 148 mmol/litre	Sodium	8	9
> 5,3 mmol/litre	Potassium	48	55
> 106 mmol/litre	Chloride	9	10
Values within Normal Range			
135 - 148 mmol/litre	Sodium	29	33
3,5 - 5,3 mmol/litre	Potassium	36	41
96 - 106 mmol/litre	Chloride	35	40
Decreased Values			
< 135 mmol/litre	Sodium	51	58
< 3,5 mmol/litre	Potassium	4	4
< 96 mmol/litre	Chloride	44	50
Total number of patients		88	
Plasma bicarbonate values: < 18 mmol/litre in 34 out of 44 cases -			77

Normal Values:

Sodium	135 - 148 mmol/litre
Potassium	3,5 - 5,3 mmol/litre
Chloride	96 - 106 mmol/litre
Plasma bicarbonate	24 - 32 mmol/litre

TABLE 9

RESULTS OF URINE ANALYSIS IN 45 CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

TEST	RESULT	NO. OF PATIENTS
Albumin	+	11
	++	16
	+++	8
	++++	1
TOTAL		36 (80%)
Glucose	+	10
	++	2
	++++	3
TOTAL		15 (33%)
Blood	+	3
	++	6
	+++	1
TOTAL		10 (22%)
Bilirubin	+	1
TOTAL		1 (2%)
Ketone bodies	++	3
TOTAL		3 (7%)

KEY:

+ - trace
++ - mild
+++ - moderate
++++ - severe

SUMMARY

Retrospective study of necropsy records of post mortems performed at King Edward VIII Hospital, Durban, during the period 1958 - 1977 showed that acute liver necrosis was the major cause of death in 447 (2,06%) patients. In 263 (59%) of these patients, the hepatic lesion was CENTRILOBULAR ZONAL NECROSIS and considered to be due to *Callilepis laureola (impila)* poisoning. The significant clinical and biochemical findings in cases of centrilobular zonal necrosis were:

1. About one third of the patients were under the age of 5 years.
2. Short duration of illness before admission to hospital.
3. In 20% of patients a definite history of oral or rectal administration of a herbal remedy was obtained. The rest either did not volunteer the information or were not questioned by the clinicians.
4. The main presenting symptoms were disturbed level of consciousness (74%), vomiting (46%), abdominal pain (18%), jaundice (13%), diarrhoea and vomiting (12%) and headache (11%). The gastrointestinal symptoms may be due to the irritant effect of the herbal remedy or to pre-existing gastroenteritis. The disturbed level of consciousness with associated confusion (10%), convulsions (23%) and coma (41%) was explained by hypoglycaemia. A specific hypoglycaemic agent has been isolated from the root of *Callilepis laureola* (see Section 3).
5. Laboratory investigations showed:
   a) Hypoglycaemia in 71% with plasma glucose values falling below 1,0 mm/litre in 31% of cases.
   b) Uraemia in 72% was due to acute renal tubular necrosis. A specific nephrotoxic agent has been isolated from the root of *Callilepis laureola* (see Section 3).

ADDENDUM

CENTRILOBULAR ZONAL LIVER NECROSIS

Although a definite history of administration of herbal medicine was obtained in only 20% of cases, patients in this group were selected on the basis of the histological finding of centrilobular zonal liver necrosis which was a constant feature in all 263 cases. When these 20% of cases were compared with those in whom no history of administration of a herbal remedy had been obtained, no gross anatomical and histopathological differences were noted. The lack of a positive history in 80% of cases was attributed to the fact that patients or their relatives were reluctant to divulge what medicines had been administered prior to admission. In many cases clinicians had omitted to enquire specifically about herbal remedies.

In 80% of patients in this group acute tubular necrosis was also present. It was found that cases which did not show tubular necrosis had died within a day or two of admission, the time span being insufficient to produce the renal lesion. Hypoglycaemia was noted in 71% of cases in group. Patients with normal plasma glucose values had been given intravenous dextrose prior to the taking of blood samples.

Methanol extracts of the root of *Callilepis laureola (impila)* when administered to laboratory rats (Section 3), have produced a similar triad of centrilobular zonal liver necrosis, acute renal tubular necrosis and hypoglycaemia.

c) Disturbed liver function resulting from liver cell necrosis attributed to hepatotoxin(s) contained in the root of *Callilepis laureola*. Raised values of serum aspartate transaminase (AST) and alanine transaminase (ALT) were indicative of hepatocellular damage. The significant rise in serum lactate dehydrogenase (LDH) was consistent with liver cell necrosis together with renal tubular necrosis. Raised total serum bilirubin was found in 93% of patients and the prothrombin index was below 50% in 53% of cases.

d) Electrolyte imbalance (hyperkalaemia in 55%, hyponatraemia in 58% and hypochloraemia in 50%) as well as decreased values of plasma bicarbonate leading to acidosis (77%).

e) Albuminuria (80%), glycosuria (33%) and haematuria in 22% were due to acute renal tubular necrosis.

6. Clinical records showed that this was an illness of short duration and followed a rapidly fatal course; 91% of the patients died within 5 days of admission.

GROUP B : MASSIVE AND SUBMASSIVE LIVER NECROSIS

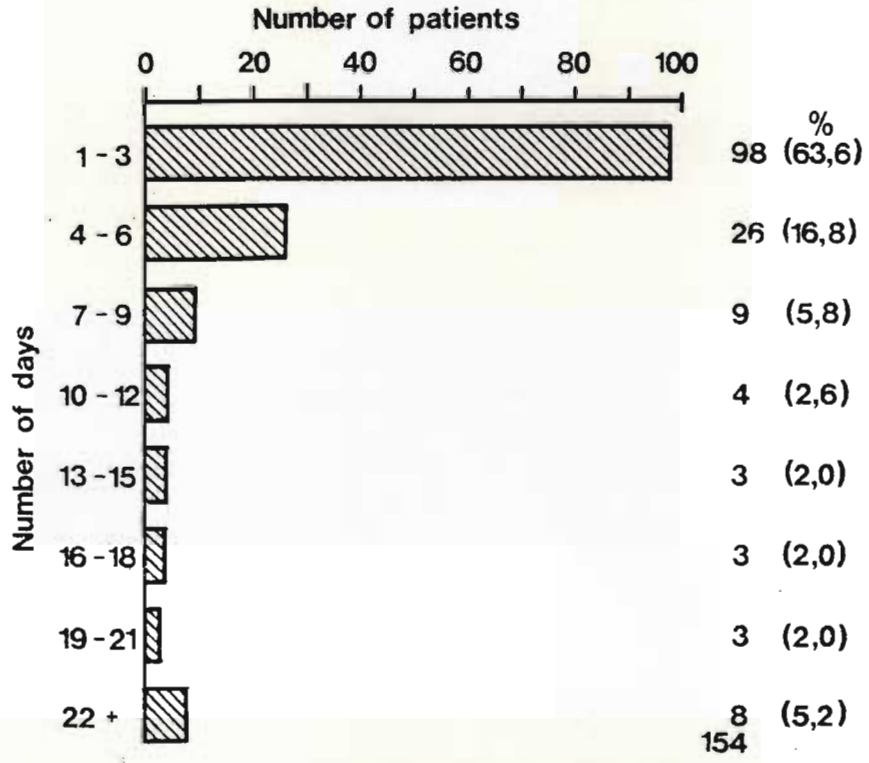
Clinical Features

There was usually no history of ingestion of a herbal remedy. In this series of 184 cases only 3 patients admitted to having taken herbal extracts. The duration of illness before admission to hospital ranged from 1 - 22 days (Figure 7).

The initial symptoms encountered were:

Nausea and vomiting	34%
Pyrexia	23%
"Dark urine"	22%
"Yellow eyes"	20%
[illegible]	20%

FIG.8 DURATION OF ILLNESS IN HOSPITAL IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS



This data has been elicited from clinical records of patients–based on information from 154 cases.

FIG.9 PLASMA GLUCOSE VALUES IN 69 CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

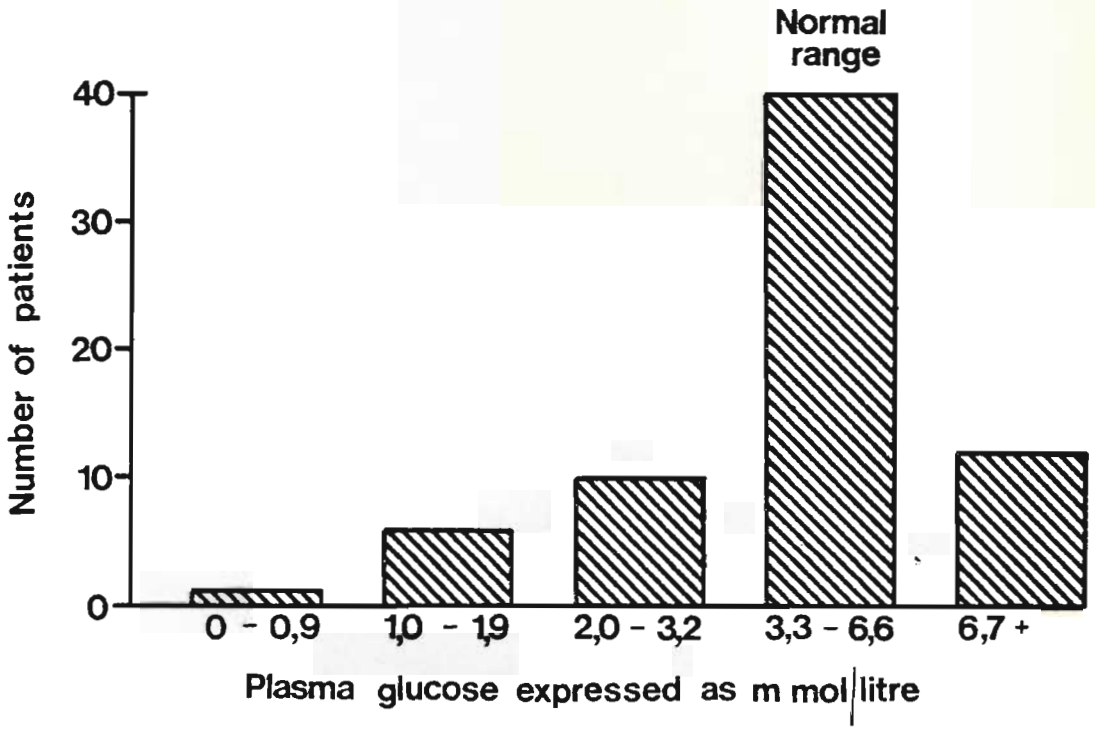


FIG. 10. PLASMA UREA VALUES IN 71 CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

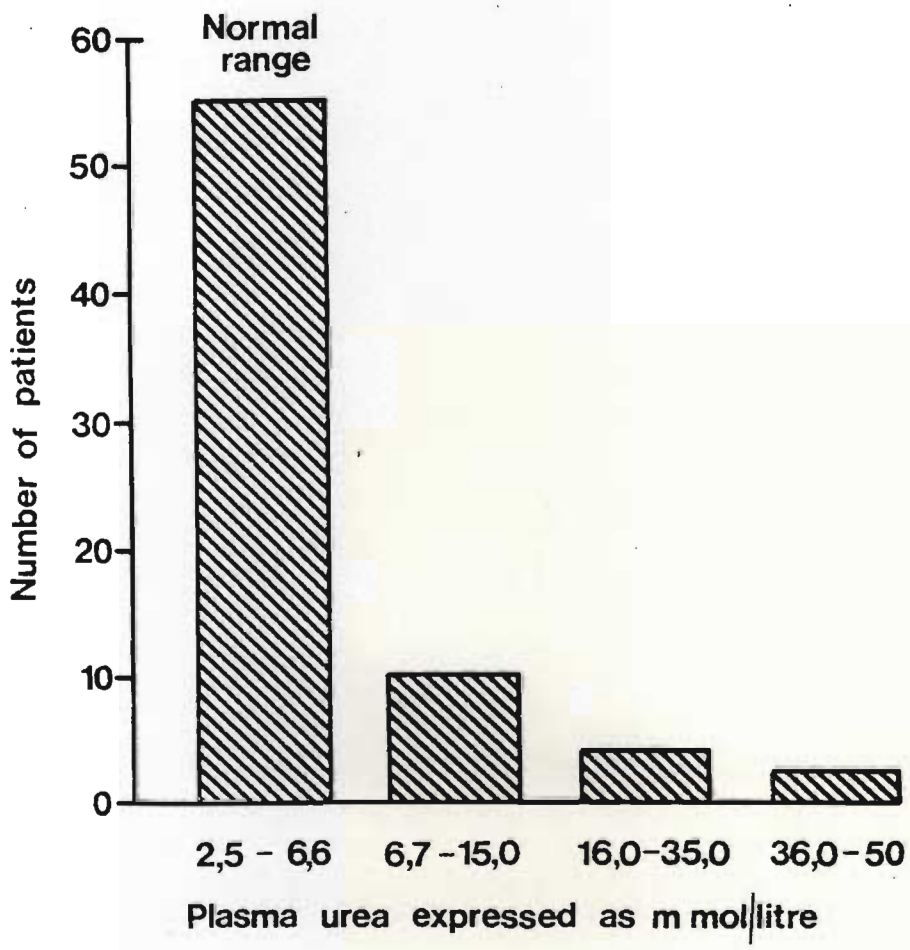


TABLE 10A

SERUM ENZYME VALUES IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

L.D.H. (LACTATE DEHYDROGENASE) E.C. 1.1.1.27

N	25
Median	478 (36 - 1660)
Range	1624
< 120	2
120 - 240	4
> 240	19 (76%)

Normal values: 120 - 240 U/litre

Values expressed in International Units per litre

A.S.T. (ASPARTATE TRANSAMINASE) E.C. 2.6.1.1

N	43
Median	254 (62 - 1175)
Range	1113
Up to 12	0
> 12	43 (100%)

Normal values up to 12 U/litre

Values expressed in International Units per litre

E.C. = Enzyme commission

N = Number of cases

Statistical Analysis: Mann-Whitney Test

TABLE 10A (CONTINUED)

A.L.T. (ALANINE TRANSAMINASE) E.C. 2.6.1.2

N	22
Median	210 (10 - >300)
Range	300
Up to 12	1
> 12	21 (95%)

Normal values: up to 12 U/litre

Values expressed in International Units per litre

A.L.P. (ALKALINE PHOSPHATASE) E.C. 3.1.3.1

N	65
Median	156 (28 - 765)
Range	737
Raised Values	40 (62%)

NORMAL VALUES

AGE IN YEARS	U/LITRE
2 - 4	68 - 135
5 - 7	52 - 169
8 - 10	78 - 284
11 - 13	32 - 299
14 - 15	32 - 167
16 - 17	32 - 109
Over 18	30 - 85
Borderline	85 - 95

Values expressed in International Units per litre

E.C. = Enzyme commission

N = Number of cases

Statistical analysis: Mann-Whitney test

TABLE 10B

LIVER FUNCTION TESTS IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

TOTAL SERUM BILIRUBIN

N	74
Median	250 (12 - 684)
Range	672
3,4 - 17,1	2
> 17,1	72 (97%)

Normal values: 3,4 - 17,1 µmol/litre
Values expressed in µmol/litre

PROTHROMBIN INDEX

N	50
Median	42 (20 - 80)
Range	60
< 50	36 (72%)

Values expressed as %

N = Number of cases
Statistical analysis: Mann-Whitney test

TABLE 10B (CONTINUED)

SERUM TOTAL PROTEINS

N	52
Median	69 (37 - 92)
Range	55
Mean	68

Normal range: 60 - 82 grams/litre
Values expressed in grams per litre

SERUM ALBUMIN

N	52
Median	30 (10 - 42)
Range	32
Mean	29

Normal range: 35 - 50 grams/litre
Values expressed in grams per litre

SERUM GLOBULIN

N	52
Median	39 (18 - 64)
Range	46
Mean	39

Normal range: 25 - 32 grams/litre
Values expressed in grams per litre

N = Number of cases
Statistical analysis: Mann-Whitney test

(LDH) was >240 U/litre in 76% of cases. Aspartate transaminase (AST) and alanine transaminase (ALT) were significantly raised in all but one patient.

The serum alkaline phosphatase values were raised in 62% of patients and total serum bilirubin was markedly raised in 97%. The prothrombin index was estimated in 50 patients; 36 of these (72%) had values below 50%. The serum protein pattern was found to be similar to that found in cases of centrilobular zonal liver necrosis (see Table 7B).

d) Plasma electrolytes

Plasma electrolyte estimations were available in 43 patients (Table 11) and were within the normal range in most cases. Hyperkalaemia was noted in 7%, hypokalaemia in 9% and hyponatraemia in 33%. Chloride values were raised in 12% and decreased in 23%. The plasma bicarbonate values were estimated in 32 patients. Thirteen (40%) of these were within the acidotic range with values of 6,6 to 18 mmol/litre, the average value being 14 mmol/litre.

e) Urine analysis

The main significant finding was the presence of bilirubin in the urine of 89% of patients. A few cases showed albuminuria, glycosuria and haematuria (Table 12).

With progression of the disease patients showed mental changes including irritability (4%), restlessness (9%), confusion (20%), convulsions (10%) and rapidly advancing stupor (15%) or coma (42%). Death occurred from 1 - 22+ days after admission (see Figure 8).

TABLE 11

PLASMA ELECTROLYTE ESTIMATIONS IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

	PLASMA ELECTROLYTE	NUMBER OF PATIENTS	%
Increased Values			
> 148 mmol/litre	Sodium	0	-
> 5,3 mmol/litre	Potassium	3	7
> 106 mmol/litre	Chloride	5	12
Values within normal range			
135 - 148 mmol/litre	Sodium	29	67
3,5 - 5,3 mmol/litre	Potassium	36	84
96 - 106 mmol/litre	Chloride	28	65
Decreased Values			
<135 mmol/litre	Sodium	14	33
<3,5 mmol/litre	Potassium	4	9
< 96 mmol/litre	Chloride	10	23
Total number of patients		43	
Plasma bicarbonate values: <18 mmol/litre in 13 out of 32 cases			40

NORMAL VALUES

Sodium	135 - 148 mmol/litre
Potassium	3,5 - 5,3 mmol/litre
Chloride	96 - 106 mmol/litre
Plasma bicarbonate	24 - 32 mmol/litre

TABLE 12

RESULTS OF URINE ANALYSIS IN 18 CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

TEST	RESULT	NUMBER OF PATIENTS
Albumin	+	2
Total		2 (11%)
Glucose	+	1
	++	1
Total		2 (11%)
Blood	++	1
Total		1 (5%)
Bilirubin	+	3
	++	7
	+++	6
Total		16 (89%)
Ketone bodies		0

Key:

+	-	trace
++	-	mild
+++	-	moderate
++++	-	severe

SUMMARY

The significant clinical and biochemical findings in cases of MASSIVE AND SUBMASSIVE LIVER NECROSIS (184 patients) were:

1. About half the cases occurred in the age group 0 - 14 years.
2. Duration of illness before admission to hospital ranged from 1 - 22 days. Except in 3 cases there was no history of ingestion of a herbal remedy.
3. The main presenting symptoms were nausea and vomiting (34%), pyrexia (23%), "dark urine" (22%), "yellow eyes" (20%), confusion (20%), abdominal pain and discomfort (17%), anorexia (14%), headache (12%) and irritability and drowsiness (4%). There was rapidly increasing jaundice after admission in 85%, with foetor hepaticus in 18% and asterixis in 5%. Gastrointestinal haemorrhage was manifested by "coffee ground" vomitus in 9% and malaena in 5%.
4. Laboratory investigations showed:
   a) Hypoglycaemia in 25% of patients but only in 1,45% of the cases the plasma glucose values were below 1,0 mmol/litre.
   b) In 77% of patients the blood urea values were within normal limits. Mild to moderate increases in plasma urea levels were noted in 23%.
   c) Serum enzyme estimations showed marked progressive disturbance of liver function indicating widespread liver cell necrosis. Values of LDH, AST and ALT were significantly raised in the majority of patients. Serum alkaline phosphatase was raised in 62% and values of total serum bilirubin were markedly raised in nearly all the cases. The prothrombin index was below 50% in 72% of patients.
   d) Plasma electrolyte values were within normal range in most patients.

e) Urine analysis showed presence of bilirubin in 89% of cases. A few patients had albuminuria, glycosuria and haematuria.

5. Patients in this group showed mental changes manifested by irritability, restlessness, confusion, convulsions, stupor and coma. Death occurred from 1 - 22+ days after admission.

MORBID ANATOMY AND HISTOPATHOLOGY

Analysis of 263 postmortems on cases of centrilobular zonal liver necrosis and 184 postmortems on cases of massive and submassive liver necrosis permitted a comparison between the morbid anatomical and histopathological findings in the two conditions.

GROUP A : CENTRILOBULAR ZONAL LIVER NECROSIS

Morbid Anatomy

On external examination the nutritional state of the patients was estimated to be good in 59%, satisfactory in 28% and poor in 13%. Mild to moderate jaundice was noted in 13%.

The liver appeared pale and yellow. The capsular and cut surface showed sharply demarcated, centrilobular punctate congested areas, which gave a speckled appearance to the liver (Plates 1, 2 and 3). The weight of the liver was reduced by an average of 22% in 66% of cases. In 12% of cases it was slightly increased - the average increase in weight being 9%. The liver weight was within normal limits in 22%.

The renal weight was increased in 53% of cases - the average increase being 30% of the normal weight for age. In 33% of cases the weight of the kidneys was reduced by an average of 22%. The renal weight was normal in 14%. The cut surface of the renal cortex appeared pale and swollen and the medulla was congested in many cases (Plates 4 and 5).

Haemorrhages were often present in other organs, particularly in the lungs and less often in the brain and intestines (Table 13).

ADDENDUM

MORBID ANATOMY AND HISTOPATHOLOGY

CENTRILOBULAR ZONAL LIVER NECROSIS

Autopsies were performed 1 - 7 days after death (mean 2,9 days : median 2,8 days). The macroscopic appearance of the liver was distinct, showing sharply demarcated centrilobular areas of congestion and was seen in all cases in this group of patients. Allowing for autolytic changes which were present to varying degrees, the histological pattern of centrilobular zonal liver necrosis was easily distinguishable in all cases. Patients with hepatic changes associated with shock, cardiac failure, severe trauma, burns and severe infection were excluded from this study.

Histological sections of the kidneys, although autolytic changes were present, showed evidence of acute tubular necrosis, which could be recognised with certainty in 80% of cases. The remainder (20%) showed the hepatic changes of centrilobular zonal necrosis but renal tubular necrosis was not evident. This group of patients had died within a day or two of admission, there being insufficient time for the renal lesion to be recognised under light microscopy.

The main criterion which was applied to the assignation of cases in this group was the presence of centrilobular zonal liver necrosis.

PLATES 1 - 3

A macroscopic view of the liver in a case of *impila* poisoning

PLATE 1 (upper plate)

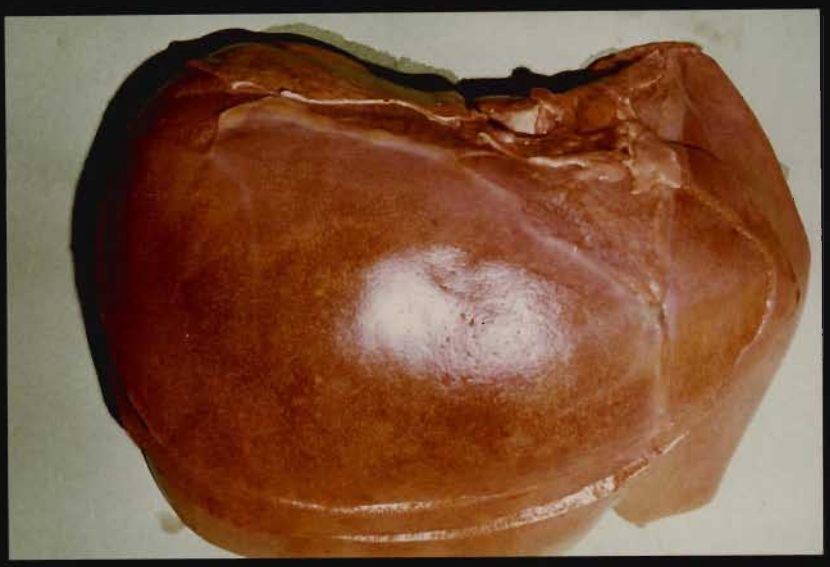
This plate shows the capsular surface of the liver with a pattern simulating acute centrilobular congestion. The liver is pale and yellow.
(Reduced by a factor of 2,5)

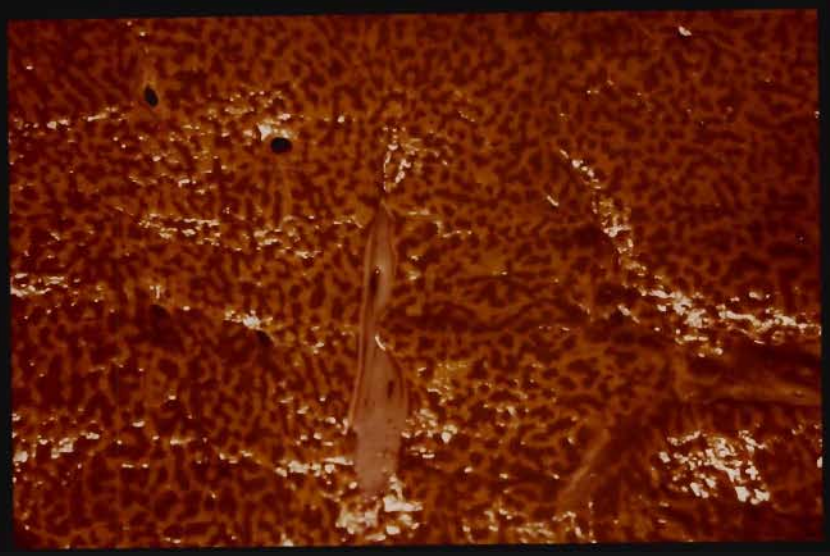
PLATE 2 (middle plate)

The cut surface of the liver with the characteristic speckled appearance seen in *impila* poisoning is shown in this plate.
(Reduced by a factor of 2,7)

PLATE 3 (lower plate)

A high power view of the above liver is shown in this plate. Sharply demarcated centrilobular punctate congested areas are seen.
(Magnified by a factor of 0,76)





PLATES 4 AND 5

A macroscopic view of the kidneys in a case of *impila* poisoning

PLATE 4 (upper plate)

The cut surfaces of both kidneys are shown in this plate. The cortices are pale and swollen and the medullae are congested. (Reduced by a factor of 1,5)

PLATE 5 (lower plate)

This plate shows a closer view of one kidney with pale and swollen cortex and congested medulla characteristic of acute tubular necrosis. (Actual size)

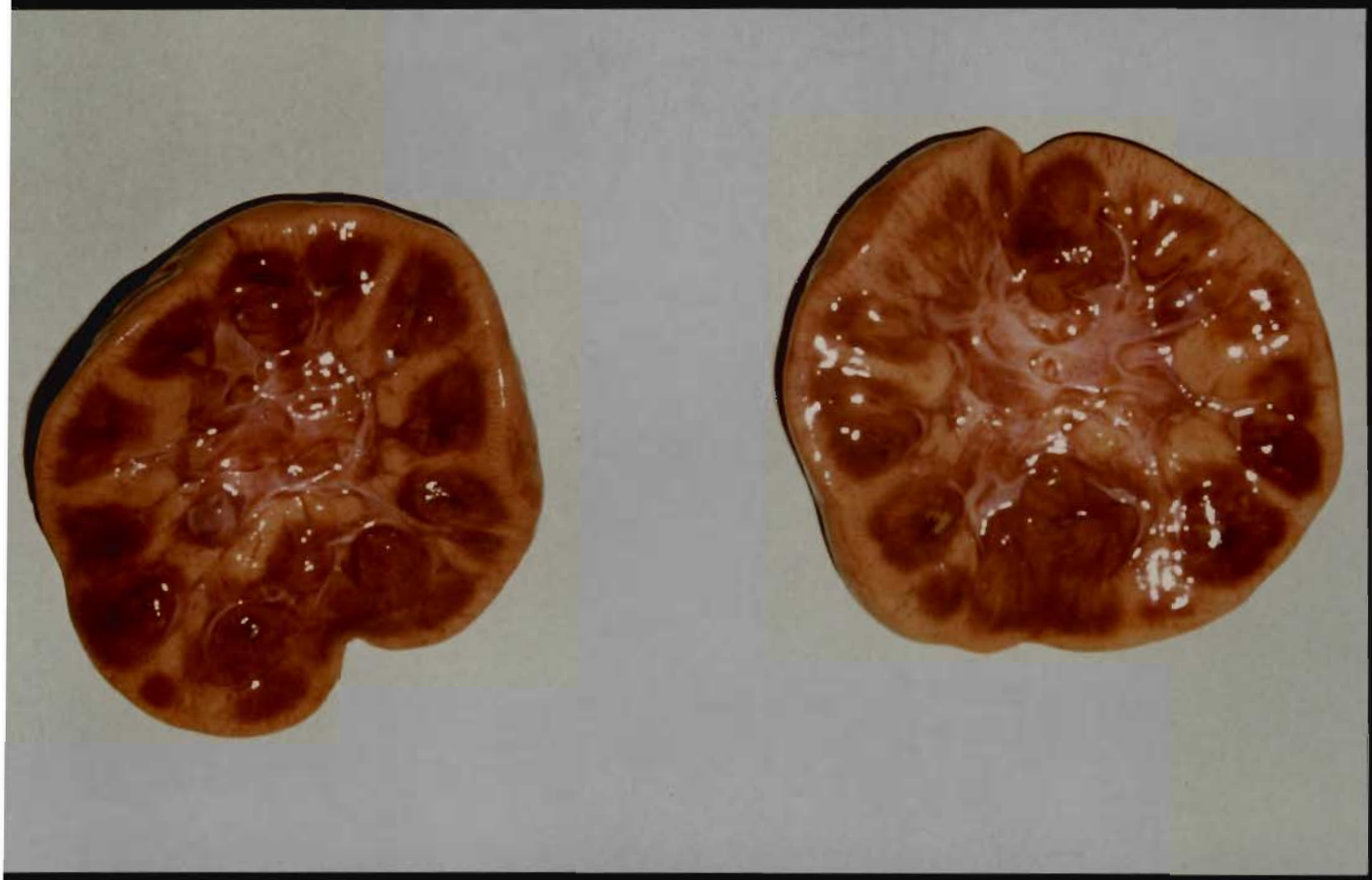


TABLE 13

SITES OF HAEMORRHAGES AND EFFUSIONS IN CASES OF CENTRILOBULAR ZONAL LIVER NECROSIS

	NUMBER OF CASES	%
HAEMORRHAGES		
1. Intrapulmonary	56	21,3
2. Gastrointestinal tract	59	22,4
3. Subendocardial and myocardial	29	11,0
4. Brain and meninges	16	6,0
5. Serosal surfaces (Petechial)	54	21,0
(a) Pericardial	30	11,0
(b) Pleural	13	5,0
(c) Peritoneal	11	4,2
EFFUSIONS (including blood stained effusions)		
(a) Pericardial	2	0,8
(b) Pleural	20	7,6
(c) Ascites	12	4,6

Histopathology

Examination of histological sections of the liver by light microscopy showed sharply demarcated centrilobular areas of necrosis (Plate 6).

In patients who had died early from hypoglycaemia, the centrilobular hepatocytes were swollen and showed karyolysis and contained fat vacuoles. In cases surviving longer and dying from liver or renal failure, many of the necrosed hepatocytes had been reabsorbed and the central sinusoids had become distended with blood (Plate 8) giving the characteristic macroscopic appearance of centrilobular zonal congestion.

The hepatocytes peripheral to the necrosed cells in the centrilobular area contained abundant small fat vacuoles (Plate 9). Inflammatory cell infiltration was either absent or minimal. When present the inflammatory cells were mainly mononuclear and lymphocytic in type. In cases of late death the liver showed centrilobular and midzonal necrosis with collapse of reticulin framework (Plate 7); regenerative activity by the peripheral hepatocytes and mitotic activity was sometimes seen. There was occasional evidence of bile ductular proliferation if the necrosis had been severe and had encroached on a portal tract. In some cases cholestasis was seen as thrombi in bile canaliculi and bile pigments in hepatocytes.

There was evidence of renal tubular necrosis in 80% of cases. This was more marked in longer surviving patients. The necrosis involved the proximal convoluted tubules and hyaline, granular and red cell casts were seen in the lumina of the tubules (Plates 10, 11 and 12). Generally there was interstitial oedema and in some cases tubular rupture with escape of filtrate into the interstitial tissue evoking an inflammatory response. In longer surviving cases tubular regenera-

PLATES 6 - 9

Histological sections of the liver in a case of *impila* poisoning

PLATE 6 (upper plate)

This plate shows sharply demarcated centrilobular and midzonal necrosis with sparing of periportal hepatocytes.
(Mayers haematoxylin and eosin x 50)

PLATE 7 (upper middle plate)

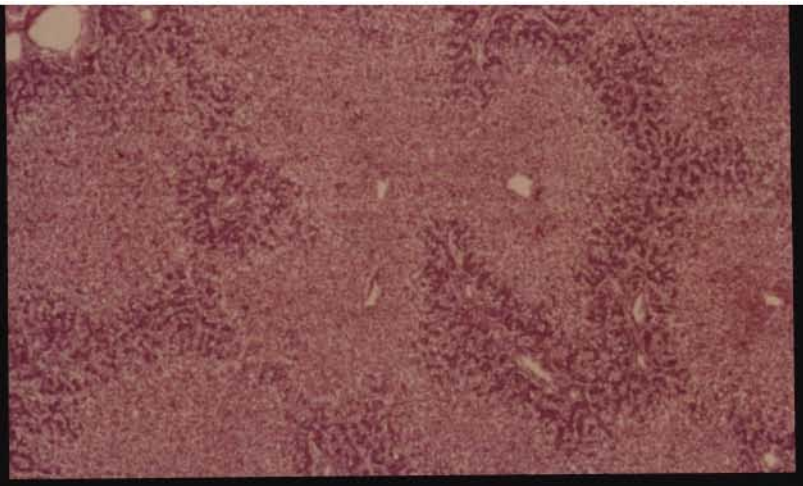
Centrilobular collapse of reticulin framework corresponding to areas of necrosis is shown in this plate.
(Gordon and Sweet silver reticulin impregnation x 50)

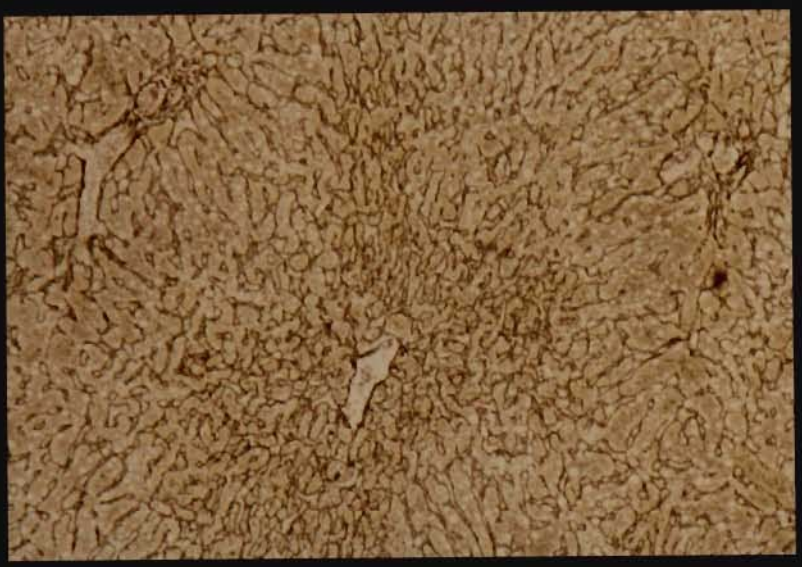
PLATE 8 (lower middle plate)

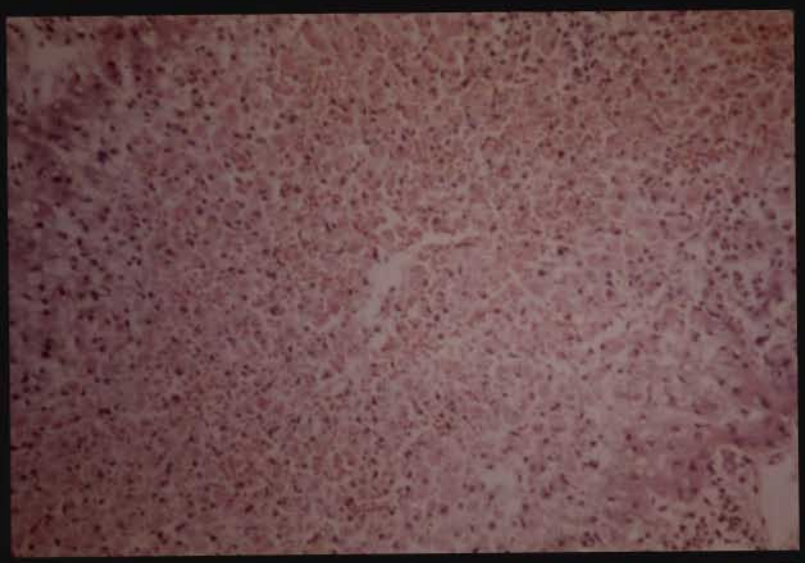
A high power magnification showing centrilobular and midzonal necrosis; the central sinusoids are distended with blood.
(Mayers haematoxylin and eosin x 150)

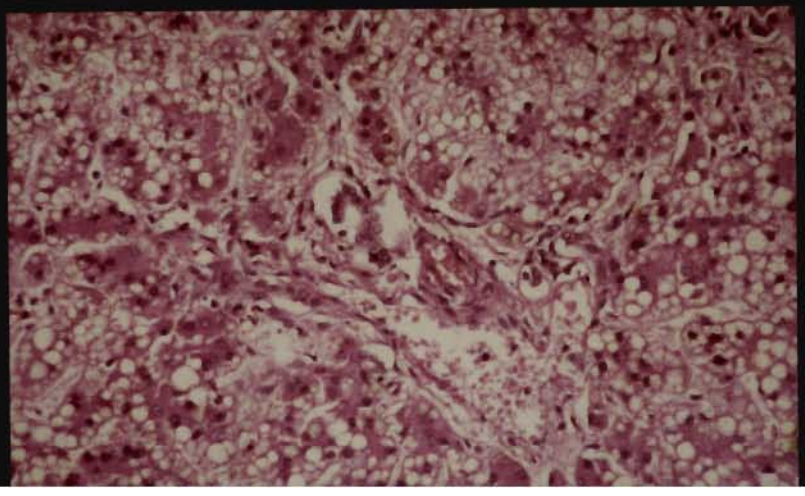
PLATE 9 (lower plate)

A high power magnification showing periportal surviving hepatocytes containing abundant fat vacuoles.
(Mayers haematoxylin and eosin x 400)









PLATES 10 - 12

Histological sections of the kidney in a case of *impila* poisoning

PLATE 10 (upper plate)

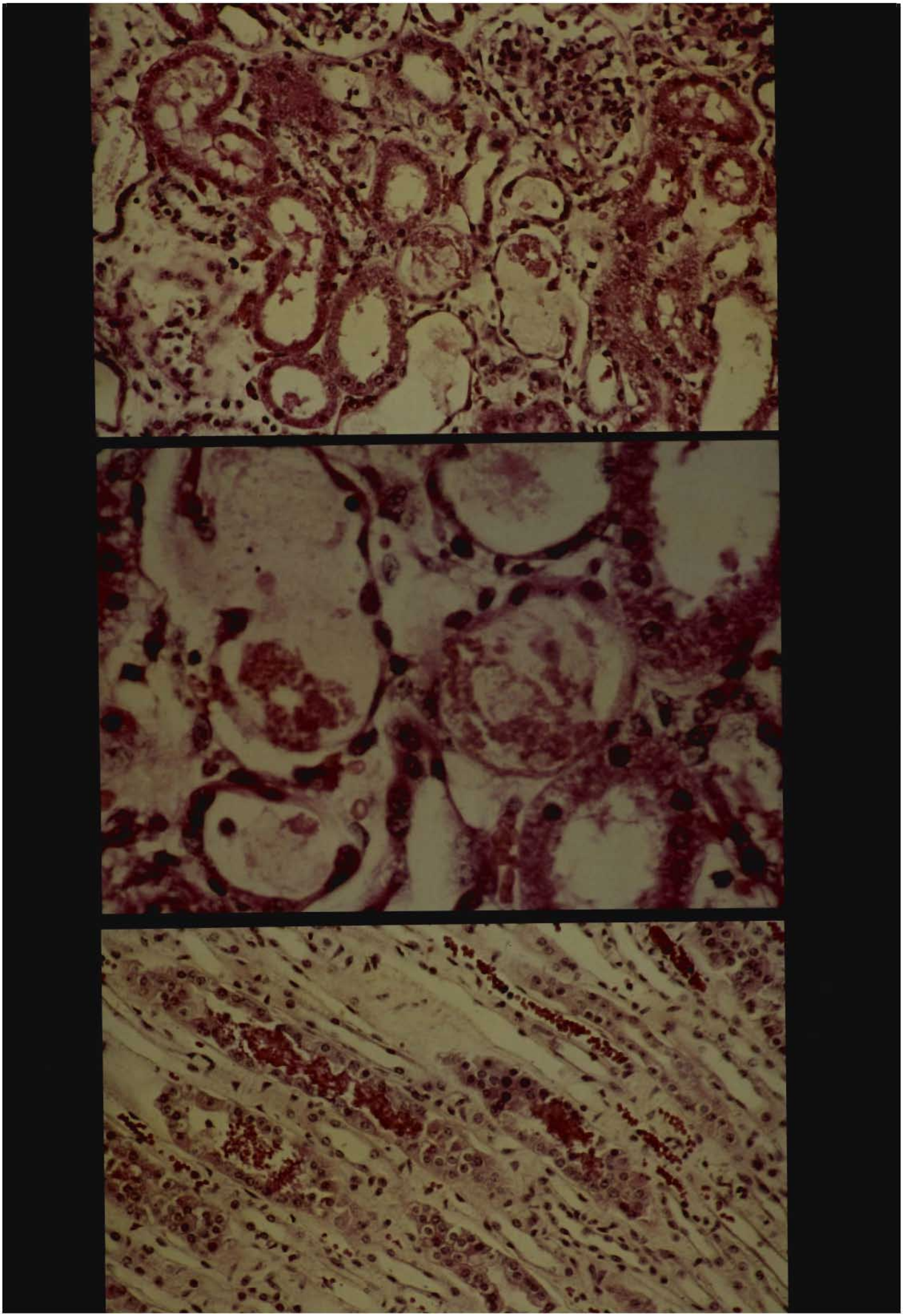
This plate shows acute renal tubular necrosis; the cells lining the proximal convoluted tubules appear thin and eosinophilic with pyknotic nuclei. Some tubules are denuded of cells and lined by basement membrane only. Red cell casts are seen in the lumina of some tubules. Interstitial oedema is present.
(Mayers haematoxylin and eosin x 200)

PLATE 11 (middle plate)

A high power magnification of the section pictured in Plate 10 showing red cell casts and desquamated epithelial cells in the tubular lumina. The intact basement membrane is visible. Regeneration in the form of large flat cells with granular cytoplasm and prominent vescicular nuclei is evident.
(Mayers haematoxylin and eosin x 400)

PLATE 12 (lower plate)

This plate shows a section of the renal medulla. The collecting tubules contain numerous red cell casts.
(Mayers haematoxylin and eosin x 200)



tion was usually evident with proliferating epithelial cells relining the necrosed tubules.

Intrapulmonary haemorrhage was noted in 56 patients and petechial haemorrhages were present in the heart (29 cases) and in the brain (16 cases). No other structural abnormalities were found in these organs.

SUMMARY

Retrospective study of necropsy findings in 263 cases of centrilobular zonal liver necrosis showed:

1. Mild to moderate jaundice in 13% of patients.
2. The liver weight was decreased in 66%. The capsular and cut surface showed sharply demarcated centrilobular punctate congested areas giving the liver a speckled appearance.
3. The renal weight was increased in 53% of cases. Cut surface of the kidneys showed pale and swollen cortices and congested medullae.
4. Gastrointestinal haemorrhage, intrapulmonary haemorrhage and petechial haemorrhages in heart, brain and serosal surfaces were recorded.
5. Histological sections of the liver showed sharply demarcated centrilobular areas of necrosis.
6. Histological sections of the kidneys showed acute tubular necrosis involving the proximal convoluted tubules. Hyaline, granular and red cell casts were present in the lumina of the tubules.
7. Apart from intrapulmonary haemorrhage and petechial haemorrhages in the heart and brain, no structural abnormalities were noted in these organs.

GROUP B : MASSIVE AND SUBMASSIVE LIVER NECROSIS

Morbid Anatomy

The nutritional state was noted to be good in 38% of patients, satisfactory in 46% and poor in 16%. Moderate to severe jaundice was recorded in 92% of cases.

The morphological changes seen in cases of massive and submassive liver necrosis depended upon the extent of necrosis, amount of regeneration and duration of survival of the patient. In rapidly fatal cases with acute massive necrosis the liver was slightly increased in weight, the capsule was smooth and the cut surface showed congestion with a dusky red colour. Patients with massive and submassive necrosis (Plates 13, 14 and 15) who had survived more than one week, the liver was reduced in weight, was soft and flabby and deeply icteric and the cut surface was either yellow or had a mottled appearance of red and yellow. Some patients who had survived a longer period of time, yellow regenerative nodules were seen against a red background of collapsed liver framework. The weight of the liver was reduced in 92% of cases.

The average reduction in weight was 41% of the normal weight for age. In 5% of cases the liver weight was increased by an average of 16% above the maximum of the normal range. The weight of the liver was within the normal range in 3%.

The kidneys were slightly swollen and had increased in weight in 36% of cases; the average increase being 28% of the normal value (Plate 16). In 43% the renal weight was reduced by an average of 19% of normal and in 21% of cases the kidneys were of normal weight. On cut surface some kidneys were dark from congestion, while others appeared pale with or without bile staining (Plate 17).

ADDENDUM

MORBID ANATOMY AND HISTOPATHOLOGY

MASSIVE AND SUBMASSIVE LIVER NECROSIS

Autopsies were performed 1 - 9 days after death (mean 3,1 days : median 2,9 days). The cases in this group were selected on the histological appearance of the liver which showed extensive necrosis, confluent in many areas and involving several adjacent acini. No distinctive pattern of zonal necrosis was evident. Autolytic changes were noted but the presence of confluent areas of necrosis together with an inflammatory response and regenerative changes validated the diagnosis of massive liver necrosis for inclusion in this group.

Three patients in this group had given a history of having ingested herbal medicines. However, the clinical presentation, autopsy and histological findings in these cases closely approximated those of massive liver necrosis and for this reason included in this group. It was assumed that the herbal medicine was either innocuous or did not originate from *Callilepis laureola*. In the rest of the patients in this group there was no mention, in the clinical records, of administration of herbal remedies.

PLATES 13 - 15

PLATE 13 (upper plate)

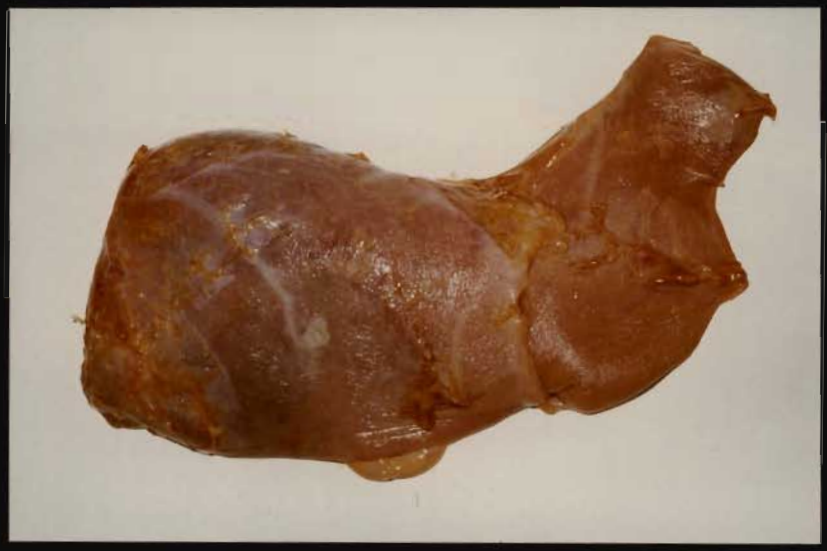
A macroscopic view of the liver in a case of massive liver necrosis. The liver is shrunken with a wrinkled capsule.
(Reduced by a factor of 2,5)

PLATE 14 (middle plate)

The cut surface of the liver pictured in Plate 13 is shown in this plate.
The liver is bile stained with a mottled appearance of red and yellow areas.
(Reduced by a factor of 1,25)

PLATE 15 (lower plate)

This plate shows the macroscopic appearance of the liver in a case of submassive liver necrosis. Yellow regenerative nodules are present on the capsular and cut surfaces. The haemorrhagic areas seen represent recent necrosis.
(Reduced by a factor of 2,4)



PLATES 16 AND 17

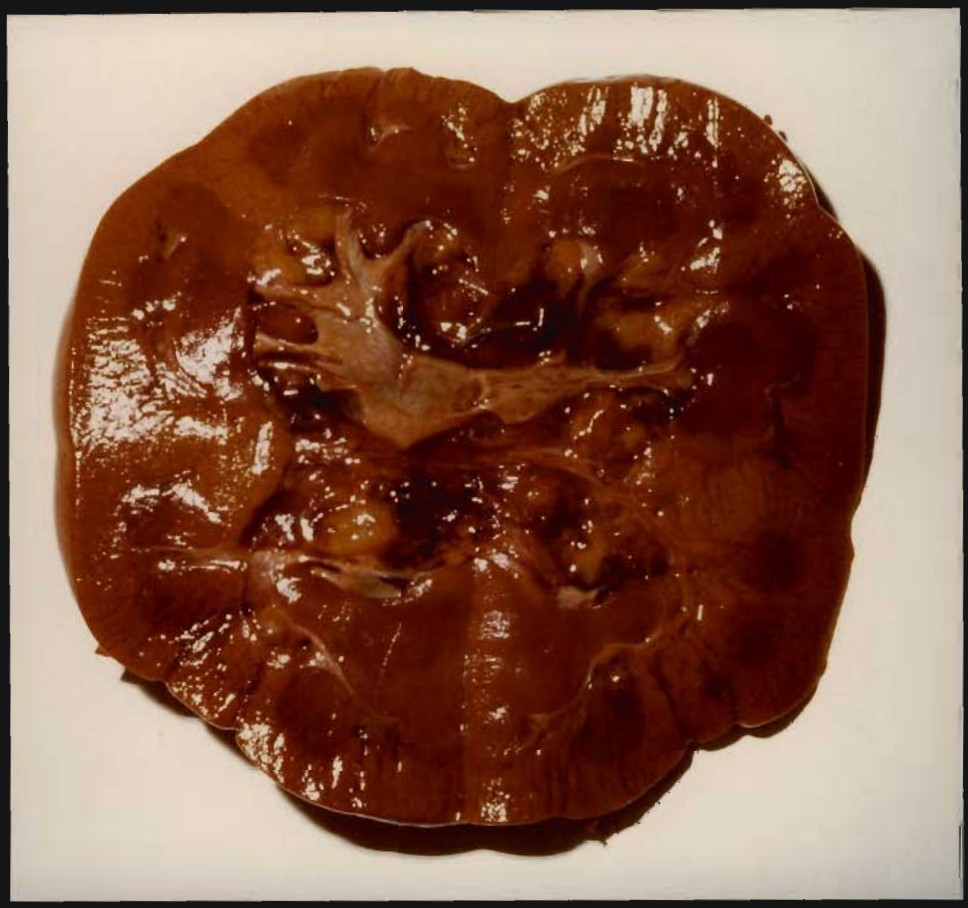
A macroscopic view of the kidney in a case of massive liver necrosis

PLATE 16 (upper plate)

This plate shows the surface of the kidney which appears swollen and bile stained.
(Actual size)

PLATE 17 (lower plate)

The cut surface of the kidney shown in Plate 16. The cortex is bile stained and the medulla is mildly congested.
(Actual size)



Extra hepatic changes included minimal ascites, pleural effusions (Table 14) and peripheral oedema. Haemorrhagic phenomena were seen in various tissues (see Table 14) because of deficiency of coagulation factors resulting from hepatic destruction. Gastrointestinal haemorrhage was found in 28% but in 10% it was severe enough to have contributed to death.

Histopathology

Cases of early death from acute massive necrosis (47% of cases) showed extensive destruction of liver cells involving all parts of the liver uniformly with little inflammatory reaction (Plate 18). The liver reticulin was intact and little collapse was noted. The sinusoids were usually widely engorged with blood. In longer surviving cases of massive necrosis (46%) parenchymal cells had disappeared in large areas with regenerative hyperplasia of surviving liver tissue. Stromal collapse was noted in areas of previous necrosis and there was a variable degree of bile duct proliferation.

In cases of submassive necrosis (5%), islands of surviving hepatocytes were noted (Plate 19). In 3 cases (2%) sections showed post necrotic scarring with signs of further acute necrosis. Inflammatory cellular reaction was variable and was more marked in the submassive form (Plate 20). The inflammatory infiltrate was chiefly composed of lymphocytes and plasma cells but small numbers of neutrophils were also present in some cases.

Histological sections of the kidneys showed tubular degenerative changes of varying severity in 48%, frank tubular necrosis in 11% and bile casts were present in 47% of cases (Plate 21). The kidneys of the remaining 41% were either histologically normal or showed congestion only.

TABLE 14

SITES OF HAEMORRHAGES AND EFFUSIONS IN CASES OF MASSIVE AND SUBMASSIVE LIVER NECROSIS

	NUMBER OF CASES	%
HAEMORRHAGES		
1. Intrapulmonary	46	25,0
2. Gastrointestinal tract	52	28,3
3. Subendocardial and myocardial	31	16,8
4. Brain and meninges	5	2,7
5. Serosal surfaces (Petechial)	23	12,5
(a) Pericardial	11	6,0
(b) Pleural	7	3,8
(c) Peritoneal	5	2,7
EFFUSIONS (including blood stained effusions)		
(a) Pericardial	0	-
(b) Pleural	4	2,2
(c) Ascites	6	3,3

PLATE 18 (upper plate)

A histological section of the liver showing extensive confluent necrosis with near total loss of hepatocytes. Autolytic changes are present. Nevertheless the features are those of acute massive liver necrosis.
(Mayers haematoxylin and eosin x 50)

PLATE 19 (upper middle plate)

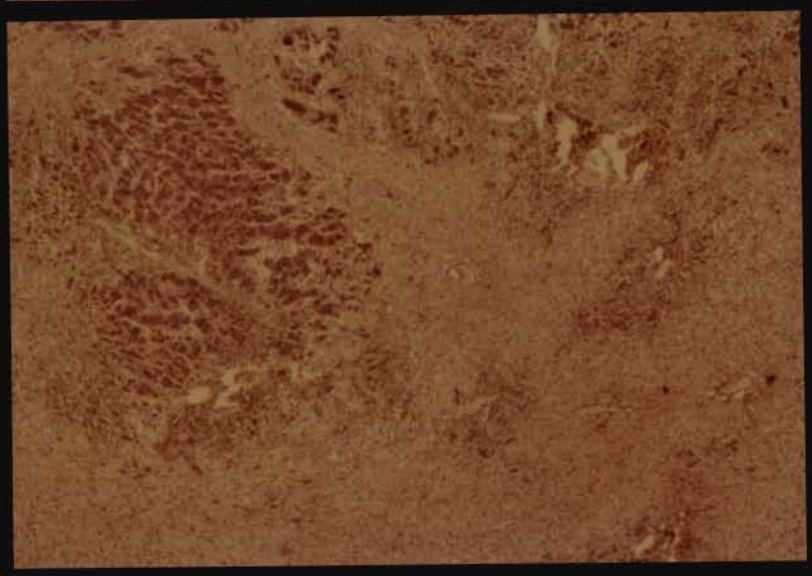
A histological section of the liver in a case of submassive liver necrosis showing large irregular areas of necrosis involving adjacent acini. Areas of surviving hepatocytes are seen. Some autolysis is present.
(Mayers haematoxylin and eosin x 50)

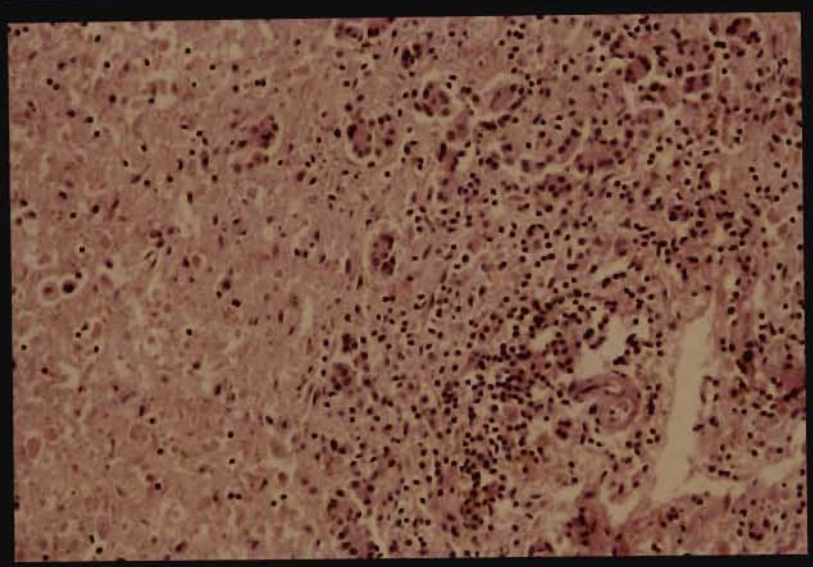
PLATE 20 (lower middle plate)

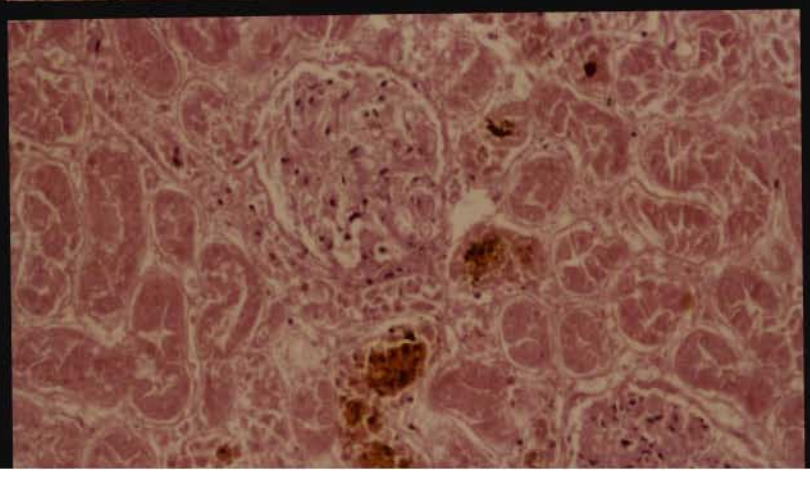
Inflammatory cellular infiltrate and bile duct proliferation is shown in this histological section of the liver, in a case of submassive liver necrosis.
(Mayers haematoxylin and eosin x 150)

PLATE 21 (lower plate)

This plate shows a histological section of the kidney in a case of massive liver necrosis in which the renal tubules are seen to contain bile casts.
(Mayers haematoxylin and eosin x 150)







SUMMARY

The significant necropsy findings in 184 cases of massive and submassive liver necrosis were:

1. Moderate to severe jaundice in 92% of cases.
2. In rapidly fatal cases of acute massive necrosis the liver weight was slightly increased and the cut surface showed a dusky red colour. In patients with massive and submassive necrosis who had survived more than 1 week, the liver was markedly reduced in weight ($\frac{1}{2}$ - 1/3 normal weight), was flabby and deeply icteric. The cut surface of the liver was either yellow or had a mottled appearance of red and yellow areas. In longer surviving cases yellow regenerative nodules were present.
3. The kidneys were swollen and increased in weight in 36%, of normal weight in 21% and slightly decreased in weight in 43%. The cut surface of the kidneys showed congestion in some cases while in others they appeared pale with or without bile staining.
4. Other necropsy findings included minimal ascites, pleural effusions, peripheral oedema, and haemorrhagic phenomena in various tissues. In 10% of cases gastrointestinal haemorrhage was severe enough to contribute to death.
5. Histological sections of the liver in rapidly fatal cases of acute massive necrosis showed extensive destruction of liver cells involving all parts of the liver uniformly. In longer surviving patients parenchymal cells had disappeared in large areas with regenerative hyperplasia of surviving liver tissue. Submassive necrosis with bile duct proliferation and evidence of regeneration was noted in 5% of cases. A few cases (2%) showed post necrotic scarring with signs of further acute necrosis. Inflammatory cellular reaction consisting of lymphocytes and plasma cells

was variable but more marked in the submassive form.

6. Histological sections of the kidneys showed tubular degenerative changes of varying severity in 48% of patients. Bile casts were present in 47% of cases. Frank tubular necrosis was noted in 11%.

A comparison of the variables among patients with centrilobular zonal liver necrosis and massive/submassive liver necrosis as regards clinical features, laboratory investigations and morbid anatomical and histopathological findings is shown in Table 15.

TABLE 15

COMPARATIVE SUMMARY OF THE SIGNIFICANT DIFFERENCES BETWEEN CENTRILOBULAR ZONAL LIVER NECROSIS AND MASSIVE/SUBMASSIVE LIVER NECROSIS

	CENTRILOBULAR ZONAL LIVER NECROSIS GROUP A	MASSIVE AND SUBMASSIVE LIVER NECROSIS GROUP B
NECROPSY PREVALENCE 20 year period: 1958 - 1977	Mean per annum 13 % of total Black necropsies 1,21	Mean per annum 9 % of total Black necropsies 0,85
AGE INCIDENCE	Peak incidence: < 5 years 34%	Peak incidence: 5 - 14 years 27%
CLINICAL PRESENTATION	Illness of rapid onset and short duration characterised by: History of herbal remedy 20% Disturbed level of consciousness 74% Vomiting 46% Abdominal pain 18% Jaundice (mild to moderate) 13% Diarrhoea and vomiting 12% Headache 11% Pyrexia 8% Confusion 10% Convulsions 23% Coma (rapid onset) 41%	Illness of longer duration characterised by: Seldom history of herbal remedy < 2% Nausea and vomiting 34% Pyrexia 23% "Dark urine" 22% "Yellow eyes" 20% Abdominal pain and discomfort 17% Anorexia 14% Headache 12% Irritability and drowsiness 4% Rapidly increasing jaundice 85% Foetor hepaticus 18% Asterixis 5% Confusion 20% Convulsions 10% Coma (late manifestation) 42%

TABLE 15 (CONTD)

	CENTRILOBULAR ZONAL LIVER NECROSIS GROUP A	MASSIVE AND SUBMASSIVE LIVER NECROSIS GROUP B
LABORATORY INVESTIGATIONS		
a) Plasma glucose	Rapidly developing hypoglycaemia 71% Plasma glucose values often < 1 mmol/litre 31%	Hypoglycaemia uncommon 25% Plasma glucose values < 1 mm/litre in only 1,45%
b) Plasma urea	Plasma urea raised in 72% High values (16-80 mmol/litre) 45%	Plasma urea raised in 23% Values of 16-50 mmol/litre in 10%
c) Liver function	Disturbed liver function in patients surviving long enough for tests to be carried out	Marked disturbance of liver function Evidence of progressive liver failure
d) Plasma electrolytes	Electrolyte imbalance: Hyponatraemia 58% Hyperkalaemia 55% Hypochloraemia 50%	Mostly normal: Hyponatraemia 33% Hyperkalaemia 7% Hypochloraemia 23%
acidosis	Plasma bicarbonate values: < 18 mmol/litre in 77%	Plasma bicarbonate values: < 18 mmol/litre in 40%
e) Urine analysis	Albuminuria 80% Glycosuria 33% Haematuria 22% Bilirubinuria 2%	Albuminuria 11% Glycosuria 11% Haematuria 5% Bilirubinuria 89%
SURVIVAL IN HOSPITAL	Fatal outcome; death after admission 1 - 6 days 92,8% 7 - 12 days 7,2% 13 - 18 days - 19 - 22+ days -	Fatal outcome; death after admission 1 - 6 days 80,4% 7 - 12 days 8,4% 13 - 18 days 4,0% 19 - 22+ days 7,2%

TABLE 15 (CONTD)

NECROPSY FINDINGS	CENTRILOBULAR ZONAL LIVER NECROSIS GROUP A	MASSIVE AND SUBMASSIVE LIVER NECROSIS GROUP B
MORBID ANATOMY	External examination: Nutritional state: good 59% satisfactory 28% poor 13% Jaundice (mild to moderate) 13%	External examination: Nutritional state: good 38% satisfactory 46% poor 16% Jaundice (moderate to severe) 92%
	Organ changes Liver: Pale and yellow. Capsular and cut surfaces showed speckled appearance with sharply demarcated centrilobular punctate congested areas. Weight reduced by average of 22% in 66% of cases. Kidney: Cut surface showed pale swollen cortex and congested medulla. Weight increased by average of 30% in 53% of cases.	Organ changes Liver: Appearance variable. In most cases liver was soft, flabby and deeply icteric. Cut surface yellow or mottled areas of red and yellow. Weight reduced by average of 41% in 92% of cases. Kidney: Cut surface often bile stained. Weight increased by average of 28% in 36% of cases.
HISTOPATHOLOGY	Liver: Histological sections showed sharply demarcated centrilobular zonal necrosis; all lobules being affected. Kidney: Acute tubular necrosis in 80% of cases.	Liver: Histological sections showed extensive destruction of liver cells in acute massive necrosis. In cases of submassive necrosis islands of surviving hepatocytes were noted. Kidney: Acute tubular necrosis in 11% of cases; bile casts in 47%.

DISCUSSION OF THE COMPARATIVE RETROSPECTIVE STUDY

In the past two decades clinicians and pathologists at King Edward VIII Hospital, Durban, have encountered patients with an illness of short duration characterised by sudden onset, with a short history of diminishing level of consciousness, convulsions, gastrointestinal symptoms, acidosis, hypoglycaemic coma, disturbance of liver function, uraemia and death within a few days. Analysis of necropsy records of 21 687 consecutive post mortems in this study showed that in 447 cases death was directly attributed to acute liver necrosis. Further analysis of cases of death due to acute liver necrosis, at this hospital, showed these to fall into two major groups:

A. Cases of centrilobular zonal liver necrosis with associated acute renal tubular necrosis and hypoglycaemia, where the aetiological factor was considered to be toxin(s) contained in the root of *Callilepis laureola (impila)*, a plant often used by herbalists in the preparation of traditional medicines. All other causes of centrilobular zonal necrosis were eliminated from this study.

B. Cases of acute massive and submassive liver necrosis where the aetiology was believed to be probably fulminant viral hepatitis in the majority of patients. However, no attempt was made to exclude other causes of the massive or submassive necrosis.

Analysis of data shows that necropsy incidence of cases with centrilobular zonal necrosis was higher (1,21%) when compared with those of massive and submassive necrosis (0,85%). Since many cases of toxic liver necrosis are unsuspected clinically and the true nature of the illness is established only at necropsy, there is little doubt that many more deaths could be due to this cause. The number of cases in this study therefore probably represents a proportion of the total

prevalence of *impila* poisoning during the past 20 years. Also, because this is an acute disease of short duration many patients do not reach hospital prior to death.

The high prevalence of toxic centrilobular zonal liver necrosis in children (49% under 15 years of age) is no doubt due to the fact that many Black parents employ herbal medications for minor illnesses or to ward off "evil spirits" in their children.

The clinical features, laboratory investigations and necropsy and histopathological findings showed essential differences between the two groups. In cases of toxic centrilobular zonal liver necrosis definite history of herbal administration was obtained in 20% of patients. Clinicians have at times encountered considerable difficulty in persuading relatives to divulge what medicines have been given to the patients prior to admission. Relatives are particularly reluctant to admit to the administration of herbal mixtures. This, together with the fact that in many cases the medical officer had specifically omitted to enquire about administration of herbal remedies no doubt account for the low percentage of patients in whom a positive history was obtained.

This retrospective study has shown that the syndrome of *impila (Callilepis laureola)* poisoning is characterised by an illness of short duration of sudden onset, accompanied by vomiting (46%), haemetemesis (11%), abdominal pain (18%), headache (11%), confusion (10%), convulsions (23%) and coma (41%). Mild to moderate jaundice was observed in 13% of patients and severe jaundice in two patients.

Watson, Coovadia and Bhoola (1979) analysed the clinical records of 50 Black children (0 - 12 years), admitted to King Edward VIII Hospital between 1969 and 1977, whose post mortem findings were compatible with

impila poisoning. Their findings of disturbed level of consciousness in 80% of patients with coma in 73% and convulsions in 52% suggests that symptoms related to the central nervous system are more often encountered in children.

Blood glucose estimations showed rapidly developing hypoglycaemia (71%) with levels falling below 1,0 mmol/litre in 31% of cases. Uraemia was a common finding. Blood urea values were available in 118 patients. Of these 72% had a raised blood urea and in 45% the values were very high (16 - 80 mmol/litre). A progressive rise in blood urea was seen in patients surviving several days. Twelve patients were dialysed for renal failure. Disturbed liver function was demonstrated by significant rise in serum enzyme values, raised total serum bilirubin and low prothrombin indices. Plasma electrolyte estimations showed hyponatraemia in 58% (serum sodium <135 mmol/litre), decreased chloride values in 50% (serum chloride < 96 mmol/litre) and hyperkalaemia in 55% of patients (serum potassium values > 5,3 mmol/litre). The electrolyte imbalance was no doubt due to a combination of vomiting and the loss of renal tubular regulation. Plasma bicarbonate values were < 18 mmol/litre (Table 8) in 34 out of 44 patients (77%) indicating that the majority of patients became acidotic before death. Albuminuria in 80% and haematuria in 22% of patients is explained by renal tubular damage and the glycosuria (33%) by failure to reabsorb glucose in the proximal convoluted tubules due to toxic necrosis of the tubular epithelial cells, together with intravenous glucose therapy raising the blood glucose levels.

The illness follows a rapidly fatal course, despite supportive therapy with intravenous glucose and dialysis in those patients who became anuric or oliguric. The majority of these patients (91%) die within 5 days of admission and in this series only 9% survived for more than 5 days with eventual fatal outcome.

Liver biopsies had not been undertaken when the diagnosis of *impila* poisoning was suspected clinically and therefore no reliable records of surviving patients are available. Seedat and Hitchcock (1971) reported a case of survival after *impila* medication. The patient presented with epigastric pain and diarrhoea with blood and pus and was tentatively diagnosed as a case of amoebic dysentery. Four days after admission he became uraemic and anuric. He was treated with 20 ml of 10% calcium gluconate, 40 ml of 4% sodium bicarbonate and 20 units of soluble insulin with 50 grams dextrose intravenously, followed by peritoneal dialysis over 48 hours. On recovery the patient gave a history of visiting a witchdoctor for impotence; he was given *impila* which he took orally. This patient presented with acute renal failure but showed no evidence of toxic hepatitis and had no disturbance of liver function. This certainly appears to be an unusual response to *impila* and may possibly be an example of the nephrotoxicity of *Callilepis laureola* appearing without its associated hepatotoxic component. This patient may have been given a mixture prepared from the flowers of *Callilepis laureola*. Experimental evidence (see Section 3 - toxic effects in laboratory rats) shows that extracts of flowers of *Callilepis laureola* cause severe renal tubular necrosis but no liver cell necrosis.

In patients with hepatic failure due to massive and submassive liver necrosis the onset of the illness was more gradual and the duration of the illness before admission ranged from 1 - 22 days. The clinical presentation includes pyrexia, malaise, anorexia, nausea and vomiting. Most patients had tenderness over the liver and rapidly increasing jaundice was a constant feature. With progress of the disease grave neurological manifestations were seen in 40% of the patients, starting with irritability, drowsiness, confusion and coma. In destructive disease of the liver the blood glucose would be expected to fall to

low levels. In this series 25% had hypoglycaemia and only in 10% the values were below 2,0 mmol/litre. However therapeutic administration of glucose probably obscured the hypoglycaemia in some of these patients. By comparison, in cases of *impila* poisoning the blood glucose values fell to very low values and the hypoglycaemia persisted in many cases despite intravenous glucose therapy.

A specific hypoglycaemic agent has been isolated from the root of *Callilepis laureola* (see Section 3) which accounts for the profound hypoglycaemia seen in patients with *impila* intoxication.

In cases of massive and submassive liver necrosis the blood urea was raised in only 22% of cases. On the other hand, patients with *impila* poisoning showed progressive rise in blood urea and in some patients who had survived for several days very high values were recorded. Chemical analysis of extracts of the root of *Callilepis laureola* has revealed a specific renal tubular toxin (see Section 3).

Increasing jaundice was found to be a constant feature in massive and submassive liver necrosis, with progressive disturbance in liver function. The serum lactate dehydrogenase (LDH) activity was found to be greater in toxic liver necrosis than in massive and submassive liver necrosis. Whereas it is accepted that the extent and severity of cell necrosis is greater in massive liver necrosis the results can nevertheless be explained on the basis that lactate dehydrogenase is also found to a great extent in kidney tissue (Wroblewski, 1963) and in *impila* poisoning liver cell necrosis is accompanied by renal tubular necrosis. It is possible to determine the site of origin of lactate dehydrogenase activity by isoenzyme separation (liver composed predominantly of LD_5 while kidney has relatively greater LD_1 and LD_2) but isoenzyme separations are not routinely requested in this hospital.

The increase in activity of aspartate transaminase (AST) in the serum is related to the severity and extent of cell damage. When comparing massive and submassive liver necrosis with toxic liver necrosis the cell damage occurring in the former is much greater and this is reflected in the mean AST activity in the former being much higher (254) than in the latter (143). In liver disease an increase in alanine transaminase (ALT) parallels that of aspartate transaminase (AST). However, the low levels of these enzymes in cases of massive liver necrosis could be a reflection of the liver being in "near exhaustion" state.

Increase in alkaline phosphatase (ALP) activity in relation to liver pathology is due to increased production of this enzyme by the cells lining the biliary system and increased activity is noted in the serum when there is bile duct obstruction. Mild to moderate increase in alkaline phosphatase activity noted in cases of both toxic liver necrosis and massive liver necrosis appears to be a secondary phenomenon, due to liver cell damage rather than to a primary obstructive lesion.

The much greater increase in total serum bilirubin in patients with massive and submassive liver necrosis compared with cases of toxic liver necrosis is again related to the type of lesions produced in the two conditions. In massive and submassive necrosis most of the cells of the liver lobule are damaged and therefore bilirubin cannot be excreted. In toxic liver necrosis due to *impila* there is sparing of the peripheral cells which are able to excrete some of the bilirubin.

Prothrombin index, which is representative of the synthetic function of the liver, was found to be low in both groups of patients indicating poor prognosis. The serum protein pattern found in both toxic liver necrosis and massive and submassive liver necrosis is generally found in the Black population (Powell, 1958). The lower mean values of total serum protein and serum albumin, when compared with mean values for

Europeans (total protein 70 grams/litre, albumin 39 grams/litre - Powell, 1958) is in keeping with the malnourished state of the Black periurban and rural population.

Plasma electrolyte imbalance was less commonly encountered in cases of massive and submassive liver necrosis. Plasma electrolyte estimations showed diminished values of sodium in 33%, potassium in 9% and chloride in 23% of patients. In some cases potassium (7%) and chloride (12%) values were raised. This is accounted for by the fact that although 24% of patients presented with vomiting, this was neither severe nor intractable and also to the fact that only 11% had renal tubular damage. Urine analysis showed bilirubinuria in 89% of patients but a few cases had albuminuria and haematuria.

Morbid anatomical findings and histopathological features in centrilobular zonal liver necrosis due to *impila* poisoning and massive or submassive liver necrosis, have been compared in this study. Necropsy records showed that 13% of patients in the toxic liver necrosis group and 16% in the massive and submassive necrosis group were malnourished. In a study of 50 cases of *impila* poisoning in children under the age of 12 years, Watson et al (1979) found that almost a third were malnourished with weights below the 3rd centile for age.

Mild to moderate jaundice was recorded in 13% of cases of toxic liver necrosis. In massive and submassive necrosis 92% showed moderate to severe jaundice. The prevalence of jaundice in *impila* poisoning was low because this is an illness of rapid onset and short duration; 91% of patients had died within 5 days. Those patients surviving a longer period of time when liver cell necrosis was more extensive, developed clinical jaundice. At necropsy the liver in toxic liver necrosis generally appeared pale and yellow. The capsular surface as well as the cut surface showed a uniformly speckled appearance with sharply

demarcated centrilobular punctate areas resembling congestion. The liver weight was reduced in 66% of cases. The speckled appearance of the liver, due to centrilobular necrosis with surrounding zone of congestion is often confusingly reminiscent of the pattern seen in acute passive venous congestion but in such cases the liver weight is usually increased.

On the other hand in 92% of cases of massive and submassive liver necrosis, the liver was significantly reduced in weight, the average reduction in weight being 41% of normal weight. The liver was flabby with a shrunken capsule and deeply icteric. The cut surface had a mottled appearance of red and yellow areas and in some patients who had survived a longer period of time, yellow regenerative nodules were seen.

The kidneys in cases of *impila* poisoning were increased in weight in 53%, the average increase being 30% of normal weight. On cut surface the renal cortex appeared pale and swollen. On macroscopic examination the kidneys of patients with massive and submassive liver necrosis appeared either normal or congested or bile stained with a pale cortex. The renal weight was increased in 36% of cases; the average increase in weight being 28% of the normal renal weight.

The haemorrhagic phenomena seen in both groups of patients were no doubt due to defects in clotting mechanism resulting from hepatic dysfunction and gastrointestinal haemorrhage may have contributed to death in some cases in both groups.

The striking histological feature in cases of toxic liver necrosis due to *impila* poisoning was the sharply demarcated centrilobular zonal necrosis with the central sinusoids distended with blood giving the characteristic zonal congestion seen macroscopically.

Hepatocytes peripheral to the necrosed cells showed abundant fat vacuoles. In cases of late death there was centrilobular (sometimes midzonal) collapse of reticulin framework and the hepatocytes peripheral to the central area of necrosis showed evidence of regeneration. The liver morphology in cases of massive and submassive necrosis was determined by the extent of necrosis, amount of regeneration and duration of survival of the patients. In cases of early death from acute massive necrosis there was extensive destruction of liver cells with little inflammatory reaction. In longer surviving patients and in cases of submassive liver necrosis evidence of regeneration, varying degree of bile duct proliferation and variable infiltration of inflammatory cells were noted.

Histological sections of the kidneys in cases of *impila* poisoning showed acute tubular necrosis involving the proximal convoluted tubules in 80% of cases. In those patients who had succumbed rapidly to the toxicity of *impila* (profound hypoglycaemia and hepatic necrosis) tubular necrosis was not evident, the time course being insufficient to induce the renal lesion. Histological sections of the kidneys in cases of massive and submassive liver necrosis showed bile casts (47%) and varying degrees of tubular degenerative changes in 48% of cases and frank tubular necrosis in 11%.

GENERAL DISCUSSION

FACTORS INVOLVED IN THE CAUSATION OF ACUTE LIVER NECROSIS

The various aetiological factors causing acute liver necrosis are discussed and compared with *impila* induced centrilobular zonal liver necrosis.

Reye's syndrome

Encephalopathy with fatty degeneration of viscera (Reye et al, 1963) has many clinical similarities to *impila* poisoning with an acute onset of vomiting, hypoglycaemia, disturbance of consciousness with convulsions and coma. The striking difference is in the pathological findings in the liver. In Reye's syndrome the capsular and cut surface of the liver is bright yellow in colour (Reye et al, 1963; Becroft, 1966; Huttenlocher et al, 1969). Microscopically, there is uniform fatty change; the fat being present as small vacuoles within the cell cytoplasm with no distortion of the nucleus and the cell not greatly enlarged. Despite the extensive fatty change, Reye et al (1963) and Becroft (1966) found no evidence of hepatocellular necrosis either of zonal distribution or of individual cells. However, Huttenlocher et al (1969) and Bourgeois et al (1971) have reported focal areas of liver cell necrosis located in the periphery of the lobules in some cases of Reye's syndrome.

The renal cortex in Reye's syndrome appears swollen, yellow and somewhat greasy. Microscopically there is fatty degeneration in the proximal convoluted tubules and in the loops of Henle but no necrotic or nuclear changes are present (Bourgeois et al, 1971).

Recent ultrastructural studies by Tang et al (1976), Mendoza et al

(1976) and Hernandez et al (1976) suggests a viral aetiology in Reye's syndrome.

Whilst Reye's syndrome may occur in any population, the "syndrome" of *impila* poisoning, characterised by acute centrilobular zonal necrosis affecting all the lobules of the liver with associated profound hypoglycaemia and acute renal tubular necrosis has been encountered only in the Black population of South Africa because of the traditional use of herbal remedies by this group. Even though one patient in this series showed histological features of Reye's syndrome as well as toxic centrilobular zonal necrosis and renal tubular necrosis, there was a definite history of herbal enema administration shortly prior to admission to hospital.

Jamaican "vomiting sickness"

The initial presentation of *impila* poisoning has many features in common with "vomiting sickness of Jamaica" (Hill, 1952), which is characterised by a sudden onset of severe vomiting, epigastric pain, hypoglycaemia, followed soon thereafter by convulsions and coma. Death occurs within 2 or 3 days in 80% of such cases. Autopsy findings include fatty infiltration of the liver and kidneys, petechial haemorrhages in the liver, spleen, heart, lungs and brain and degenerative changes in the renal tubules. The causative agent is considered to be a hypoglycin from the unripe ackee fruit (Tanaka et al, 1972).

Mushroom poisoning

Amanita phalloides (mushroom) poisoning presents with nausea, vomiting, abdominal cramps, dehydration, vasomotor collapse and in some cases jaundice and hypoglycaemia. The principal pathological changes in cases of mushroom poisoning are found in the liver, kidneys and central

nervous system. The liver is small, pale yellow in colour and very friable (Dubash and Teare, 1946). Histological sections show massive hepatocellular necrosis which may be centrilobular in distribution (Davidson, 1963) with extensive fatty change (Cook and Haggerty, 1960). The kidneys show varying degrees of swelling. There is degeneration and fatty infiltration of the tubular epithelium. Dubash and Teare (1946) reported abundant yellow pigment in the renal tubular epithelium. The findings in the central nervous system are widespread notably swelling of cerebral ganglion cells, scattered petechial haemorrhages and perivascular lymphocytic exudates. Not infrequently the myocardium also shows fatty infiltration and degeneration (Klatsin, 1975).

The hepatic changes in mushroom poisoning have been described as indistinguishable from acute yellow atrophy (Dubash and Teare, 1946; Cook and Haggerty, 1960) and do not resemble the well defined centrilobular zonal necrosis seen in *impila* poisoning.

Senecio poisoning

The "necrodegenerative hepatitis" associated with herbs, described by Mokhobo (1976) seems most likely to be due to pyrrolizidine alkaloids contained in the senecio type plants. A similar form of toxic hepatitis affects natives of Central Asia who eat cereal grains contaminated with seeds of Heliotropium lasiocarpine (Bull and Dick, 1959). Veno-occlusive disease of the liver in Jamaican children (Stuart and Bras, 1957) was found to be due to the use of "bush tea", containing extracts of senecio and crotalaria retusia, used as a medicinal decoction.

It is well known that ingestion of senecio plants can induce centrilobular zonal necrosis but this is associated with enlargement of the liver and widespread fibrous occlusion of the central and sublobular

hepatic veins. Organised thrombi may be demonstrable in the larger hepatic veins and in chronic cases there is in addition fibrosis and round cell inflammatory infiltration of the portal tracts, giving rise to a picture of cirrhosis (Selzer and Parker, 1951; Wilmot and Robertson, 1920). These changes were however not seen in the liver in our cases of poisoning with *Callilepis laureola (impila)*.

Aflatoxins

The lesions produced by hepatotoxic fungus, aspergillus flavus are characterised by focal liver cell necrosis, fatty infiltration, bile duct proliferation and centrilobular obliterating endophlebitis. Prolonged administration of aflotoxins may give rise to cirrhosis or hepatoma (Davidson, 1963). The necrosis may be peripheral or centrizonal in distribution (Zimmerman and Ishak, 1979). These features differ from centrilobular zonal necrosis of the liver, acute renal tubular necrosis and hypoglycaemia seen in *impila* poisoning.

Industrial poisons

Carbon tetrachloride poisoning is known to cause centrilobular zonal liver necrosis and renal tubular necrosis (Moon, 1950; Smetana, 1939). Poisoning usually follows inhalation of the vapour but may also occur after ingestion of the compound.

Dimethylnitrosamine poisoning is characterised by centrilobular and midzonal liver necrosis with fatty change in the peripheral hepatocytes (Barnes and Magee, 1954) similar to *impila* poisoning. However, renal tubular necrosis does not occur and the toxic actions are limited to the liver exclusively (Magee, 1956). Small doses of dimethyl nitrosamine given over a long period produce cirrhosis in the rat and dog (Madden et al, 1970) and may give rise to tumours in the liver, kidney

and lung (Magee and Barnes, 1962).

Because of the lack of easy access to industrial compounds or a history of exposure to them and the fact that 50% of the cases of centrilobular zonal necrosis were under the age of 15 years, industrial poisoning was confidently excluded as a cause of liver damage in the cases analysed.

Drug induced hepatitis

In drug induced hepatitis, two types of hepatic lesions are encountered. The cholestatic form is characterised by bile stasis, a portal inflammatory reaction with a variable number of infiltrating monocytes, histiocytes, neutrophils and eosinophils. Foci of hepatocellular necrosis are seen in some cases (Zelman, 1959). In the hepatitic form of drug induced hepatitis the lesions are similar to those of acute viral hepatitis producing a picture of subacute or massive necrosis.

Histological changes after overdose of paracetamol (acetominophen) range from liver cell swelling, vacuolation and nuclear changes to frank confluent necrosis (Portmann et al, 1975), which is usually localised to the centrilobular areas. Occasionally bridges of necrosis link adjacent vascular structures and in severe cases the greater part of the liver cell mass is destroyed.

Drug induced hepatic injury has been excluded from cases of toxic centrilobular zonal necrosis in this study on the basis of history, clinical presentation and the histological lesions observed.

Anaesthetic drugs

Chloroform, trichlorethylene, vinyl ether and tribromoethylalcohol are known to cause centrizonal liver necrosis. None of these are used

as anaesthetics today and their toxicity is largely of historical and toxicological interest (Zimmerman and Ishak, 1979).

Fatal cases of halothane induced hepatic injury are characterised by progressively deepening jaundice, haemorrhagic phenomena, ascites and coma (Moult and Sherlock, 1975). There are differences in the literature regarding the type of necrosis. Massive necrosis, zonal necrosis or diffuse hepatitis-like degeneration and necrosis have all been reported. Morgenstern et al (1965) and Peters et al (1969) maintain that centrizonal necrosis is the most characteristic lesion in halothane toxicity. Methoxyflurane may also produce hepatic necrosis resembling that following exposure to halothane (Joshi and Conn, 1974).

As none of the cases of centrilobular zonal necrosis in this study had undergone any surgical procedures prior to the onset of illness, hepatotoxicity due to anaesthetic agents may be confidently excluded.

Cardiac failure

Patients with hepatic changes associated with shock, acute cardiac failure and other conditions including severe trauma, burns and severe infection have been excluded from this study. The histological changes in these cases (Bywaters, 1948; Ellenberg and Osserman, 1951) are centrilobular zonal necrosis with hydropic changes in the hepatocytes and pyknosis and disintegration of the nuclei. Fatty change is absent. The reticulin structure of the liver is preserved within the necrotic zone (Clarke, 1950).

In congestive cardiac failure the macroscopic appearance of the liver at necropsy simulates to some extent the speckled appearance found in toxic centrilobular zonal necrosis. However, in these cases the liver is increased in weight and purplish in colour with rounded edges. The

cut surface shows prominence of the hepatic veins and alternating yellow and red areas ("nutmeg pattern"); the red areas represent congested centres of the lobules and the yellow, the fatty periphery. Microscopically centrilobular necrosis with severe congestion is less well defined and not as sharply demarcated as in toxic centrilobular zonal necrosis. Electron microscopy shows that in cases of congestive cardiac failure the centrizonal cells disappear because of atrophy rather than necrosis (Safran and Schaffner, 1967). Patients with clinical and post mortem evidence of congestive cardiac failure were excluded from this study.

Acute massive and submassive liver necrosis

The entity of acute massive and submassive liver necrosis may be caused by many agents. The majority are due to fulminant viral hepatitis. Massive and submassive liver necrosis can follow accidental industrial poisoning. Examples of these are chlorinated naphthalenes and diphenyls (Flinn and Jarvik, 1938), tetrachlorethane (Spilsbury, 1917) and trinitrotoluene (Stewart, 1920). Drugs known to cause massive liver necrosis are cinchopen (Beaver and Robertson, 1931); zoxazolamine (Carr and Knauer, 1961); pyrazinamide (McDermott et al, 1954) isoniazid (Cohen et al, 1961); iproniazid (Popper, 1958); indomethacin (Fenech et al, 1967 and Kelsey and Scharyj, 1967) and imipramine (Powell et al, 1968).

For the purpose of comparison with *impila* induced centrilobular zonal liver necrosis, no distinction was made between the various aetiological factors causing acute massive and submassive liver necrosis.

FACTORS INVOLVED IN THE CAUSATION OF ACUTE RENAL TUBULAR NECROSIS

Acute renal tubular necrosis may be associated with a variety of conditions but may be roughly divided into two main groups:

(1) Those cases in which there is a direct poisoning of tubules by various drugs, chemicals or their metabolites; and

(2) Those cases which have the common bond of a preceding hypotensive episode and severe renal ischaemia with different precipitating causes such as severe crushing injuries, septicaemia and burns.

Although carbon tetrachloride (Hans Smetana, 1939) and amanita phalloides (vander Veer and Farley, 1935) cause hepatic centrilobular zonal necrosis as well as renal toxicity, hypoglycaemia is not a feature of such cases. Furthermore, none of the nephrotoxic agents listed in Table 16 have been reported to cause consistently the triad of centrilobular zonal liver necrosis, acute renal tubular necrosis and hypoglycaemia as seen in cases of *impila* poisoning.

FACTORS INVOLVED IN THE CAUSATION OF HYPOGLYCAEMIA

Hypoglycaemia may be produced by a variety of factors and the common causes are grouped together in Table 17. At post mortem, no neoplastic or adrenal, pituitary, pancreatic, thyroid or central nervous system disease which could have given rise to hypoglycaemia was detected in the group of patients studied. Amongst functional hypoglycaemias, the female patients were in non pregnant and non lactational state and no evidence of hypoglycaemia arising from episodes of alcoholism was noted clinically.

TABLE 16

CHEMICAL AGENTS KNOWN TO CAUSE ACUTE TUBULAR NECROSIS

METALS	Compounds of mercury, bismuth, cadmium, lead, gold and arsenic (Schreiner and Maher, 1965)
ORGANIC SOLVENTS	Carbon tetrachloride (Guild et al, 1958), tetrachlorethylene (Schreiner and Maher, 1965)
GLYCOLS	Diethylene glycol (Geiling and Cannon, 1938), ethylene glycol dinitrite and prophylene glycol (Schreiner and Maher, 1965)
THERAPEUTIC AGENTS	Sulphonamides, salicylates, phenylbutazone, kanamycin, polymyxin (Schreiner and Maher, 1965), gentamycin and cephalothin (Bobrow et al, 1972)
ANAESTHETIC AGENTS	Methoxyflurane (Frascino et al, 1970; Hollenberg et al, 1972; Hook, 1971)

TABLE 17

FACTORS WHICH MAY PRODUCE HYPOGLYCAEMIA

HARVEY AND BORDLEY, 1970

FUNCTIONAL HYPOGLYCAEMIA

1. Increased islet cell function
2. Anterior pituitary or adreno-cortical hypofunction
3. During pregnancy and lactation
4. Idiopathic
5. Alcoholic hypoglycaemia

SECONDARY TO HEPATIC DISEASE

1. Carcinoma a) secondary b) primary
2. Congestive heart failure
3. Acute hepatitis
4. Glycogen disease
5. Diffuse cholangitis
6. Cirrhosis a) Laennec's b) Biliary
7. Toxic hepatitis due to chemical agents
8. Fatty infiltration

SECONDARY TO HYPOPHYSEAL DEFICIENCY (ANTERIOR LOBE)

1. Due to chromophobe adenoma
2. Compression or invasion by tumours, cysts, inflammatory lesions, granulomatous disease
3. Infection : abscess formation
4. Atrophy
5. Infarction and necrosis

SECONDARY TO ADRENOCORTICAL INSUFFICIENCY

1. Tuberculosis
2. Atrophy of undetermined cause
3. Bilateral haemorrhage in diseases associated with haemorrhagic phenomena

HYPOTHYROIDISM

SECONDARY TO DISEASE OF THE NERVOUS SYSTEM

Hypothalamic invasion or destruction

NEOPLASTIC DISEASE

1. Islet cell tumour
2. Other tumours which produce a substance with insulin-like activity

The aetiology of hypoglycaemia in cases of *impila* poisoning is related primarily to a specific hypoglycaemic agent (atractyloside) and secondarily to the hepatic necrosis caused by toxins contained in the root of *Callilepis laureola (impila)* (see Section 3).

SUMMARY AND CONCLUSIONS

A review of 21 687 consecutive post mortems performed over a period of 20 years (1958 - 1977) has shown that in 447 cases the major contributing cause of death was acute liver necrosis. Since 1974, 15% of the post mortems have been personally performed.

These cases were subdivided into two groups. In Group A (263 cases) the hepatic lesion was acute centrilobular zonal necrosis. The patients presented with an illness of rapid onset and short duration, usually 1 - 4 days before admission. Careful history-taking in some of these patients revealed that a herbal remedy had been administered, usually as an enema but sometimes as a draught, shortly prior to the onset of symptoms. The illness was characterised by diminishing level of consciousness, acidosis, gastrointestinal symptoms and convulsions. There was no evidence of focal neurological signs or hepatic foetor and jaundice when present was usually mild. Hypoglycaemia was almost invariable and very frequently accompanied by signs of severe renal and metabolic impairment with uraemia, albuminuria, haematuria, hypochlorae hyperkalaemia, hyponatraemia and acidosis. Disturbed liver function was manifested by elevated serum enzyme values, raised total serum bilirubin and low prothrombin indices. The illness followed a rapidly fatal course - 63% of patients had died within a day of admission and 91% had succumbed within 5 days; 41% becoming comatose before death.

At necropsy the liver weight was usually reduced and showed a characteristic speckled appearance with sharply demarcated pale centrilobular areas surrounded by a zone of congestion. The kidneys were often increased in weight and the cortex appeared pale and swollen.

Histological sections of the liver showed sharply demarcated centrilobular zonal necrosis, all the liver lobules being uniformly affected. In longer surviving patients the necrosis had extended to the midzonal areas. The kidneys showed acute tubular necrosis in the majority of cases.

Although a definite history of administration of a herbal remedy was obtained in only 20% of patients, the similarity of presentation and of clinico-pathological and biochemical findings, the aetiological factor in all the patients falling under this group may be presumed to be toxins contained in the root of *Callilepis laureola (impila)*. Extracts of the root of *Callilepis laureola* when administered to laboratory rats have produced hypoglycaemia and identical hepatic lesions of centrilobular zonal necrosis and acute renal tubular necrosis (see Section 3).

The patients in Group B (184 cases) where the lesion was either acute massive or submassive liver necrosis, presented with an illness of more gradual onset with pyrexia, malaise, anorexia and sometimes vomiting. Increasing jaundice was a constant feature with mental changes of hepatic encephalopathy, followed by stupor and finally coma. Hypoglycaemia and severe renal or metabolic impairment were seldom encountered. This group showed severe disturbances of hepatic function with marked elevation of serum enzyme values and serum total bilirubin and low prothrombin indices. Death was common in the first 5 days after admission but some patients survived up till 2 - 3 weeks after onset of illness.

At necropsy the liver was often reduced in weight with a wrinkled capsule and the cut surface was either red or yellow or had a mottled appearance of red and yellow areas. The kidneys were either normal or slightly swollen and often bile stained. Histological sections

of the liver showed either acute massive or submassive necrosis. Sections of the kidneys showed tubular degenerative changes in 48%, frank tubular necrosis in 11% and bile casts in 47%.

This retrospective study clearly establishes the syndrome of *Callilepis laureola (impila)* poisoning as a distinct, separate clinico-pathological entity and differentiates this group from cases of acute massive and submassive liver necrosis resulting in most cases from fulminant viral hepatitis.

SECTION 3

CALLILEPIS LAUREOLA

Callilepis laureola DC. family compositae, (Plates 1 - 4) is an attractive perennial herb, with tuberous or subwoody root stock producing annual stems up to 60 cms tall (Dyer, 1975). Flowering from September to November, it is widespread in the grasslands of the South Eastern Transvaal, Swaziland, Natal and Transkei about as far south as the Umzimvubu river (Hilliard, 1977). The combination of colours in the flower heads, cream or white ray-florets surrounding reddish-black disc florets, is an unusual feature (Letty, 1962). The heads are terminal, solitary or occasionally in clusters in very open corymbs. The involucre is hemispherical subequal and glabrous. Achenes are 5 cms long and dimorphic; those of the ray flowers are angled. There is considerable variation in leaf shape with a wide range from relatively short, broad leaves to longer narrower ones (Hilliard, 1977).

The plant is commonly known as "ox-eye daisy" (English), "Wildemagriet" (Afrikaans), *"intsika yo-mhlaba"* (Xhosa) and *"impila"* (Zulu). *Impila* is classed by the local Zulu population as an *"ikhubala"*, a medicinal plant which is "dug up" and the root described as a sweet potato but with an unpleasant smell. The root is gathered in winter and extracts are prepared by chopping a piece of the root and boiling the pieces. The liquid extracted is used as a medicant for a wide variety of ailments and is given as an enema or taken by mouth. In the latter case it may have an emetic effect.

The extract is reputed to be effective against "evil spirits" and its usage is closely interwoven with superstition, traditional Black beliefs and customs. The extracts are often used as a vermifuge,

PLATES 1 - 4

CALLILEPIS LAUREOLA DC. (COMPOSITAE)
(IMPILA)

PLATE 1 (upper left)

This plate shows *Callilepis laureola* in its natural habitat.

PLATE 2 (upper right)

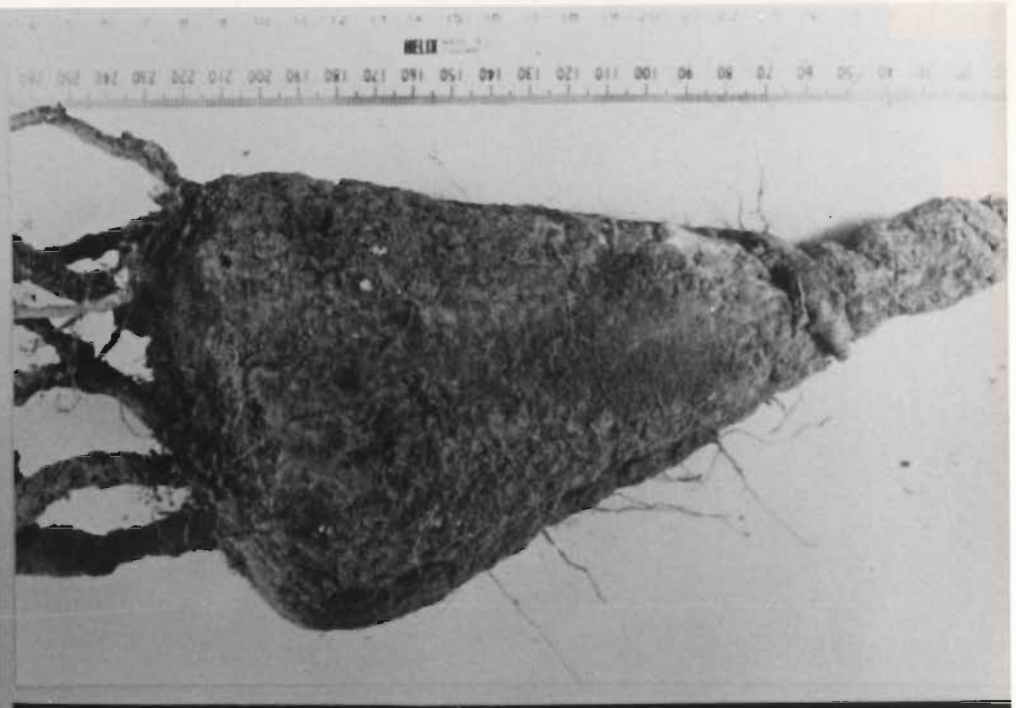
The entire "dug up" plant of *Callilepis laureola* is shown in this plate.

PLATE 3 (lower left)

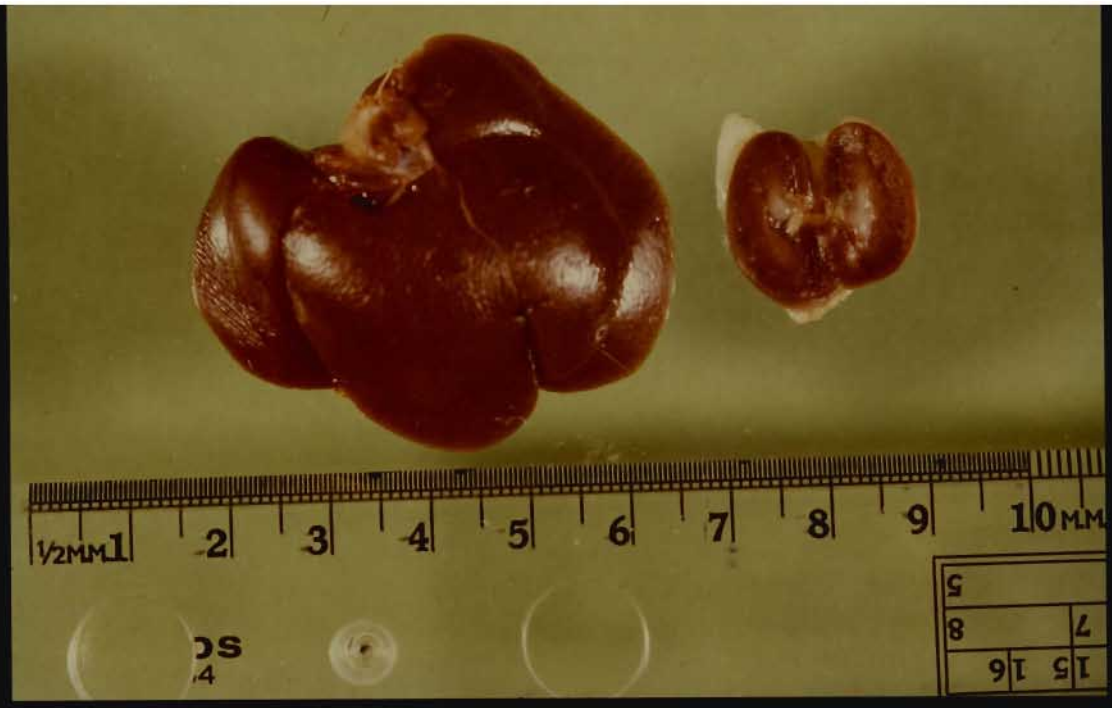
A closer view of the flower of *Callilepis laureola* is shown in this plate.

PLATE 4 (lower right)

This plate shows a closer view of the tuberous rootstock of *Callilepis laureola*.



purgative, cough remedy, for gastroenteritis and for infertility. The Zulus apply a paste of the root to kill maggots in cattle (Wood - Natal Plants 1898 - 1912). In 1905, Maberley recorded that the root of *Callilepis laureola* was used as a cough remedy and that he, himself, had found it useful in the treatment of whooping cough. Bryant (1909) reported the strongly toxic nature of *Callilepis laureola*, which had caused several deaths among the Zulus. "The Zulus take a decoction of the root as a vermifuge and an infusion as a purgative." Githens (1949) stated that the root was used for tapeworm infestation. Among the Swati, the macerated leaf is used as an external disinfectant (Watt and Breyer-Brandwijk, 1962).


½MM1
2
3
4
5
6
7
8
9
10MM

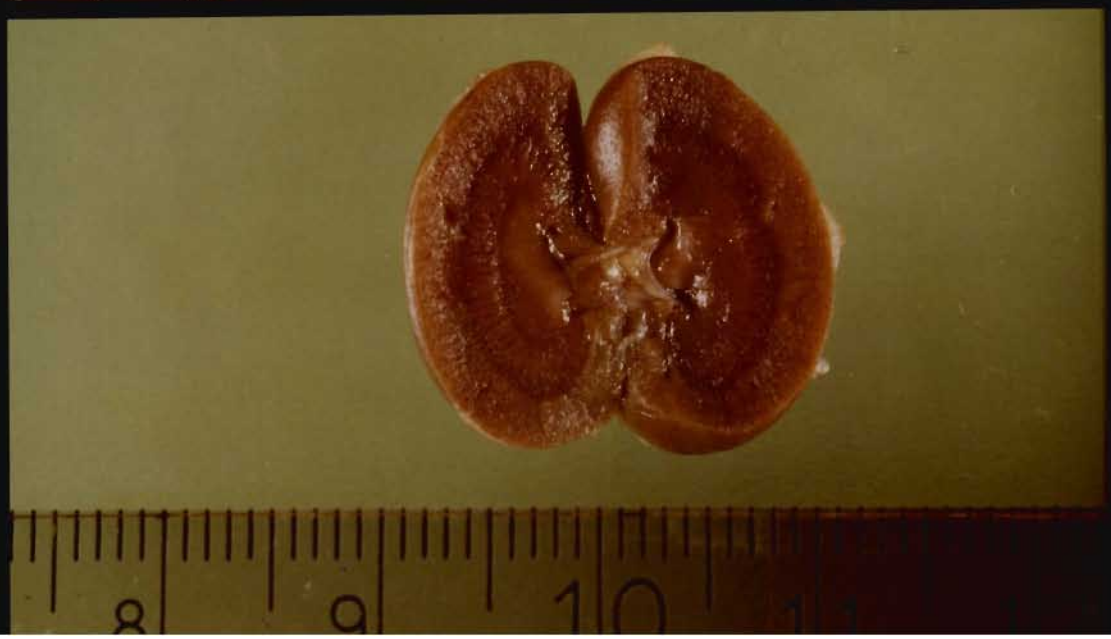


PLATE 8 (upper plate)

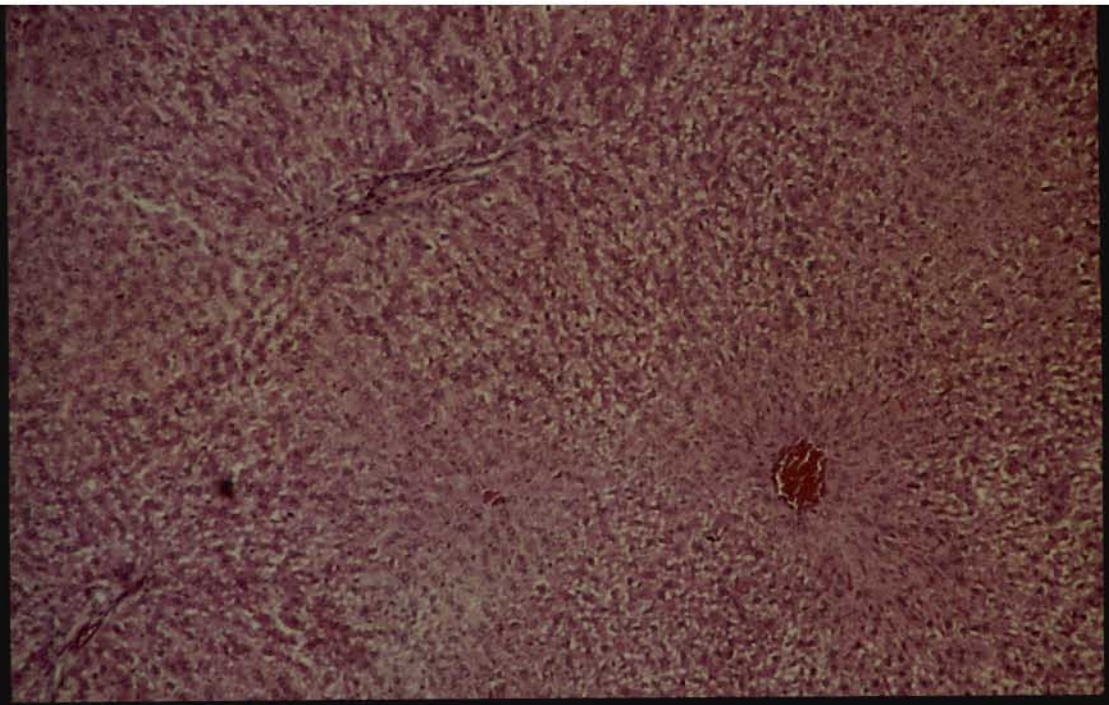
A histological section of a normal rat liver is shown in this plate.
(Mayers haematoxylin and eosin x 50)

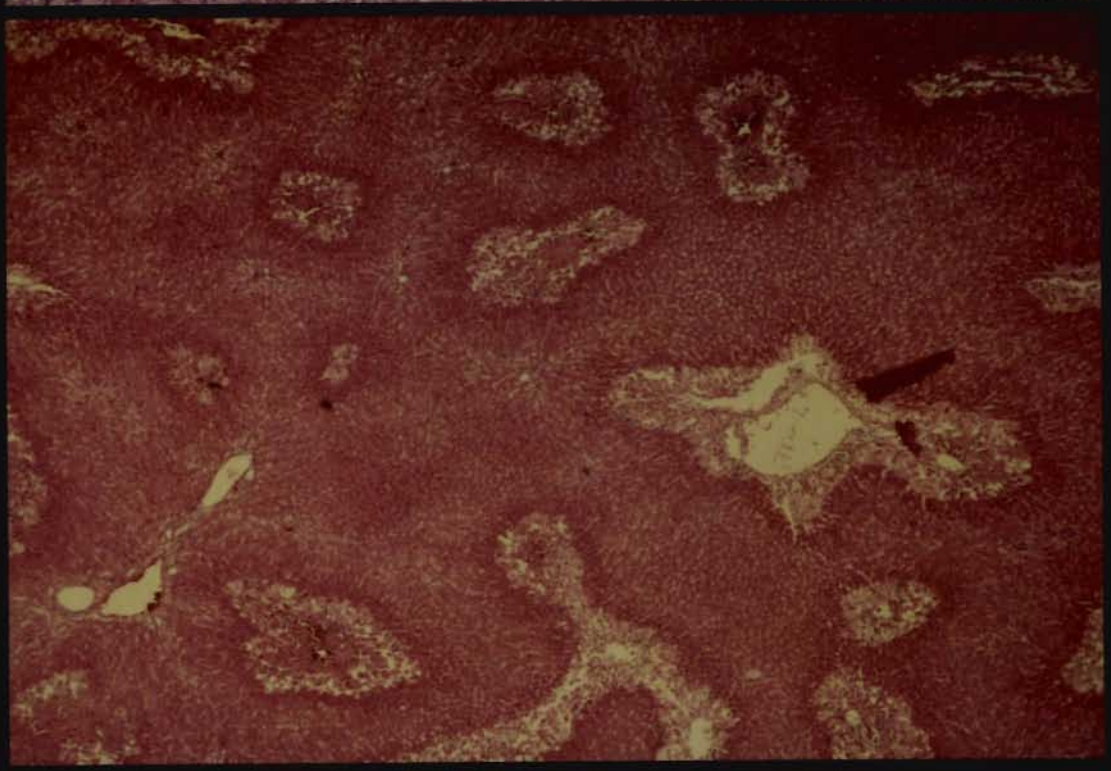
PLATE 9 (middle plate)

This plate shows a histological section of the liver of a rat, 24 hours after an intraperitoneal injection of 47 mg/kg of the methanol extract of the root of *Callilepis laureola*.
The section shows sharply demarcated centrilobular zonal necrosis.
(Mayers haematoxylin and eosin x 50)

PLATE 10 (lower plate)

This high power magnification of the section pictured in Plate 9 shows necrosis of the centrilobular hepatocytes.
(Mayers haematoxylin and eosin x 400)





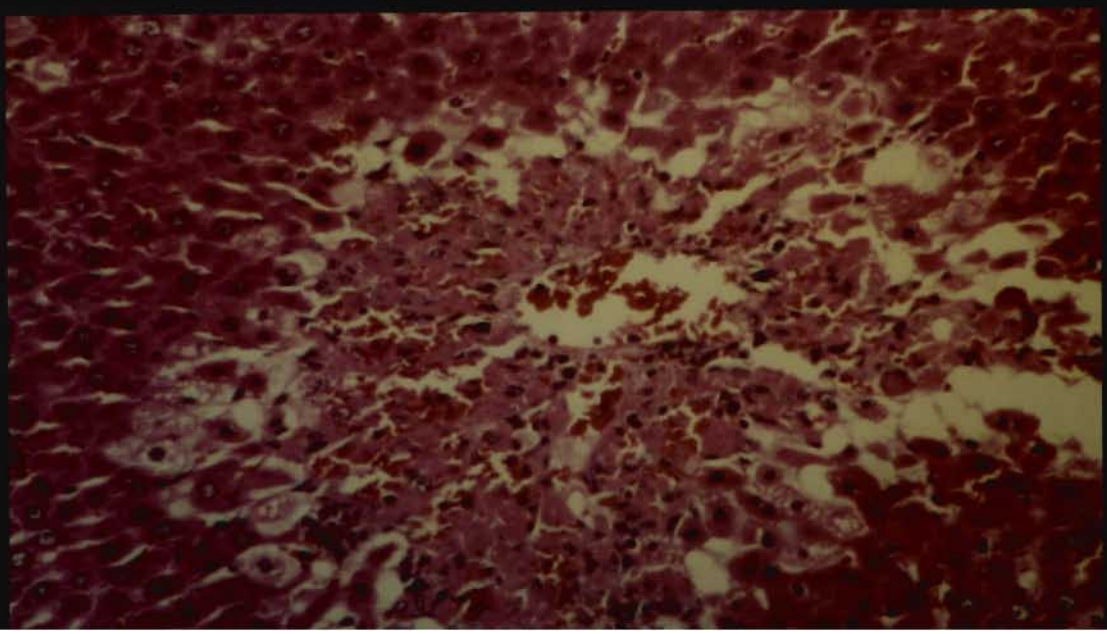


PLATE 11 (upper plate)

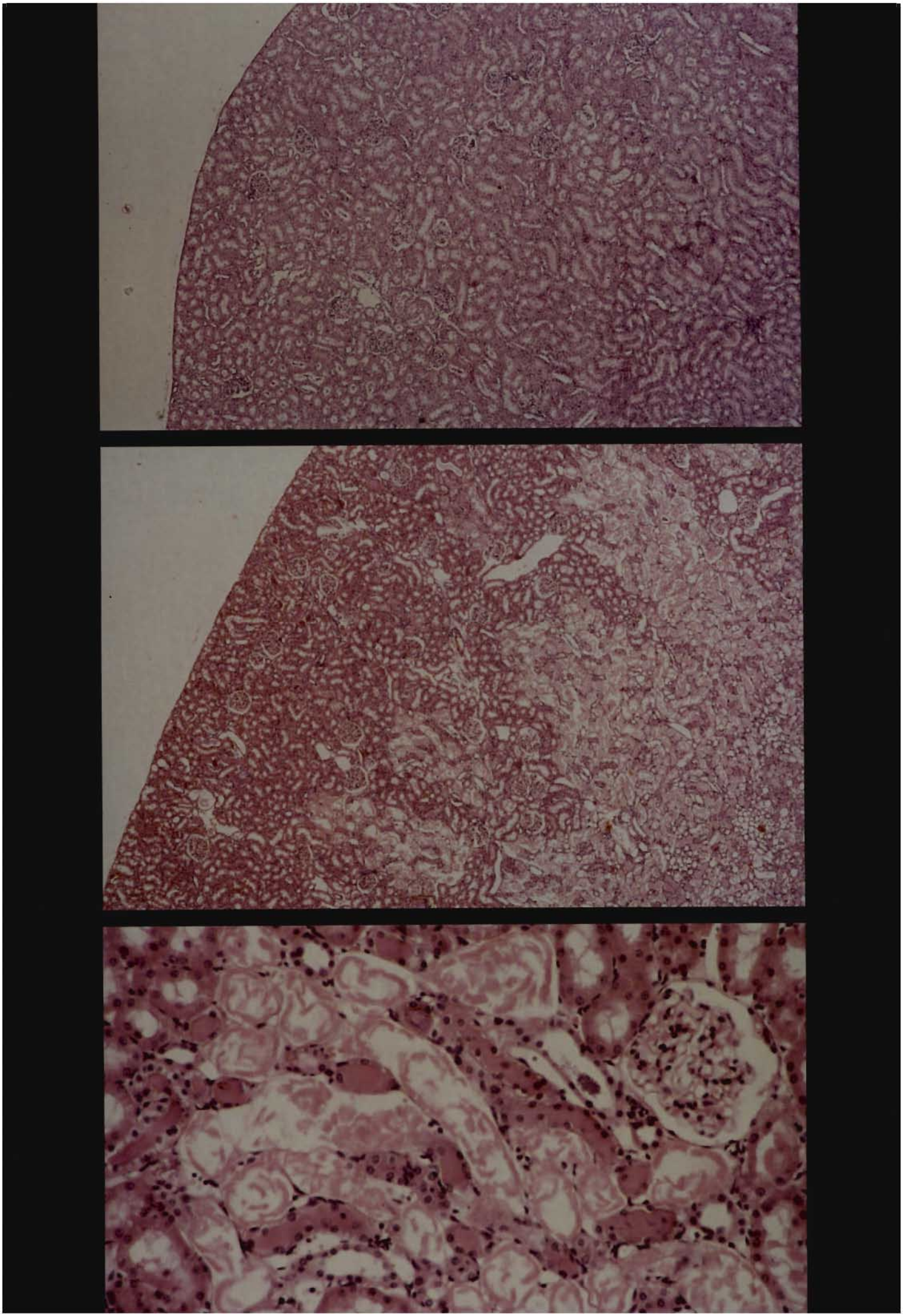
A histological section of a normal rat kidney is shown in this plate.
(Mayers haematoxylin and eosin x 50)

PLATE 12 (middle plate)

This plate shows a histological section of the kidney of a rat, 24 hours after an intraperitoneal injection of 47 mg/kg of the methanol extract of the root of *Callilepis laureola*.
The section shows acute tubular necrosis involving the inner zone of the cortex. The tubular lumina contain desquamated epithelial cells.
(Mayers haematoxylin and eosin x 50)

PLATE 13 (lower plate)

This high power magnification of the section pictured in Plate 12 shows acute tubular necrosis with hyaline, cellular and granular casts in the lumina of the tubules. The intact basement membrane is visible.
(Mayers haematoxylin and eosin x 150)



3. All the rats in the control group were sacrificed after 24 hours. The macroscopic appearance and histological sections of the liver, kidney, heart and lungs were normal in these animals.

The results of Experiment 1 are summarised in Table 1.

EXPERIMENT 2 : SUBSTANCE A

Fifteen pairs of rats were chosen. The experimental rats were injected intraperitoneally with 33 mg, 66 mg and 133 mg/kg body weight (5 rats in each group) of Substance A, dissolved in distilled water. The paired control rats received corresponding volumes of distilled water only.

RESULTS

All the rats in the experimental group and the control group were sacrificed after 24 hours and necropsies were performed. The findings are summarised in Table 2.

EXPERIMENT 3 : COMPOUND B2

The experimental design involved the use of 90 rats which were divided into 3 groups.

Group 1 (30 Rats). These rats were each given intraperitoneal injections of 133 mg/kg of Compound B2 dissolved in distilled water.

Group 2 (30 Rats). These were each injected similarly with 133 mg/kg of synthetic atractyloside (Sigma Chemical Company).

Group 3 (30 Rats) - Control Group. These rats each received intraperitoneal injections of corresponding volumes of distilled water.

Fifteen rats (five from each group) were sacrificed at intervals of

ADDENDUM

EXPERIMENTS: 2, 3 and 7(B)

Previous studies have shown that the intraperitoneal LD_{50} of atractyloside in male albino rats determined according to Litchfield and Wilcoxon (1949) was 143 mg/kg (fiducial limits 153,01 and 133,64 mg/kg).

The minimal LD_{50} dose of 133 mg/kg was therefore used in these experiments. In Experiments 2 and 7(B) subminimal doses were used as well.

References

Litchfield, J.T. and Wilcoxon, F. : "A simplified method of evaluating dose-effect experiments." J.Pharm. Pharmacol. 96 : 99 - 113 (1949)

Carpenedo, F., Luciani, S., Scaraville, F., Palatini, P. and Santi, R. : "Nephrotoxic Effect of Atractyloside in Rats." Arch. Toxicol. 32 : 169 - 180 (1974)

TABLE 1

EXPERIMENT 1 - SUMMARY OF RESULTS

RAT	I.P. INJECTION OF METHANOL EXTRACT mg/kg		RESULTS	
			MACROSCOPIC FINDINGS	MICROSCOPIC FINDINGS
A (5)	26	Sacrificed after 24 hours	No macroscopic or microscopic changes in the liver, kidney, heart and lungs	
B (5)	33			
C (5)	40			
D (5)	47		Liver: Sharply demarcated punctate centrilobular congestion Kidney: Pale, swollen cortices and congested medullae Heart and Lungs: Normal	Liver: Centrilobular zonal necrosis Kidney: Acute tubular necrosis Heart and Lungs: Normal
E (5)	54			
F (5)	61	Died within 8 hours from generalised toxicity	Liver and Kidney: Diffusely congested Heart and Lungs: Normal	Liver and Kidney: No evidence of necrosis Heart and Lungs: Normal
G (5)	68			
			The time course being insufficient to induce hepatic and renal necrosis	

igures in brackets = number of rats in each group

ecropsies on control rats showed no macroscopic or microscopic changes.

TABLE 2

EXPERIMENT 2 - SUMMARY OF RESULTS

RAT	INTRAPERITONEAL INJECTION SUBSTANCE A	SACRIFICED AFTER:	RESULT
A (5)	33 mg/kg	24 hours	No macroscopic or microscopic lesions were found in the liver, kidney, heart and lungs
B (5)	66 mg/kg	24 hours	
C (5)	133 mg/kg	24 hours	

Figures in brackets = number of rats in each group

Necropsies on control rats showed no macroscopic or microscopic changes.

1, 3, 5, 24, 48 and 72 hours. Necropsies were performed and tissue taken for histological examination.

RESULTS

Group 1 - Compound B2 (after 1, 3, 5, 24, 48 and 72 hours)

At necropsy rats injected with Compound B2 did not present any distinct morphological changes in the liver. The kidneys, on the other hand appeared swollen with varying degrees of pallor of the cortex and congestion of the medulla. Histological sections of the liver in all the rats in this group showed no evidence of necrosis. Sections of the kidney, however, showed in:

1 Hour Specimens: evidence of mild tubular damage. The cytoplasm of the cells of the proximal convoluted tubules was eosinophilic and the nuclei were pyknotic and densely staining. A few tubules showed definite evidence of necrosis of the epithelium which had begun to slough into the lumen (Plate 14). The necrosis was confined to the proximal convoluted tubules. The distal convoluted tubules and collecting ducts appeared to be spared. The glomeruli and vessels were normal.

3 Hour Specimens: evidence of tubular necrosis was more widespread with more tubules being involved (Plate 15). The tubules were filled with proteinous and cellular casts and granular debris. The glomeruli, vessels and distal convoluted tubules appeared normal.

5 Hour Specimens: showed frank widespread necrosis of the proximal convoluted tubules. The affected tubules were lined by basement membrane and were denuded of cells (Plate 16). Some tubules were filled with proteinous and cellular casts and granular debris. The glomeruli, vessels and distal convoluted tubules were normal.

24 Hour Specimens: tubular necrosis was well established. Large numbers of proteinous, granular and red cell casts were present in

PLATE 14 (upper plate)

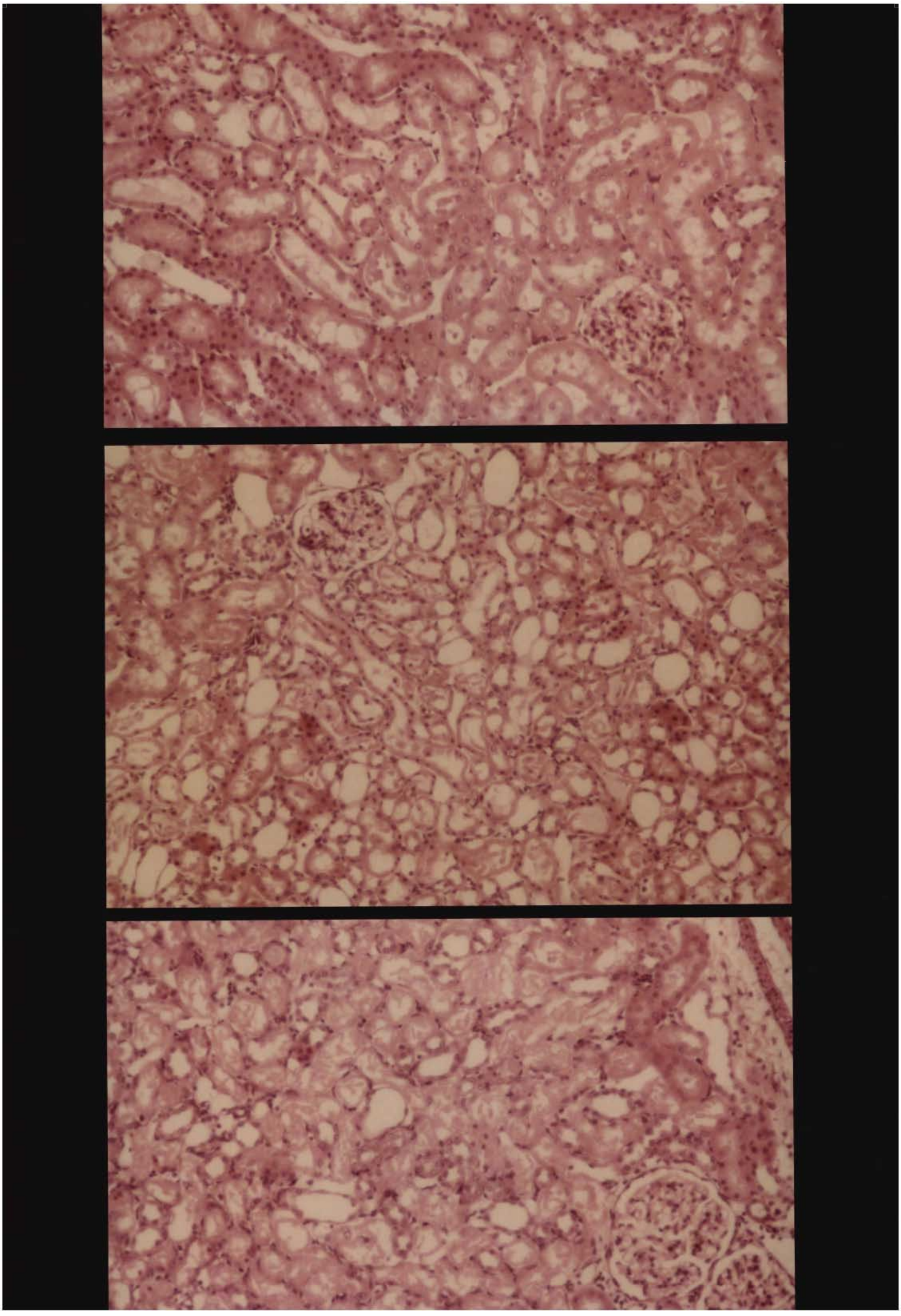
This plate shows a histological section of a rat kidney, 1 hour after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside).
The section shows early tubular necrosis, the tubular epithelial cells appear homogeneously eosinophilic with pyknotic nuclei. Desquamated epithelial cells fill the lumina of the tubules. A few tubules contain granular casts.
(Mayers haematoxylin and eosin x 100)

PLATE 15 (middle plate)

A histological section of a rat kidney, 3 hours after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside) is shown in this plate. Tubular necrosis is more widespread with more tubules being involved.
(Mayers haematoxylin and eosin x 100)

PLATE 16 (lower plate)

This plate shows a histological section of a rat kidney, 5 hours after an intraperitoneal injection of Compound B2 (atractyloside): 133 mg/kg.
The section shows frank widespread tubular necrosis. The affected tubules are lined by basement membrane denuded of cells.
(Mayers haematoxylin and eosin x 100)



the lumina of the tubules (Plate 17). The glomeruli and vessels were normal.

48 Hour Specimens: The necrosed tubules showed intact basement membranes. The tubular lumina contained desquamated epithelium (Plate 18). Some tubules showed evidence of regeneration. No changes were noted in the glomeruli and vessels.

72 Hour Specimens: Many of the necrosed tubules showed evidence of regenerative activity and were lined by basophilic staining cells with granular cytoplasm and vescicular nuclei (Plate 19). Scattered mitotic activity was present and some tubules showed syncitial masses of cells with grouped nuclei (Plate 20). No changes were noted in the glomeruli and vessels.

The heart and lungs showed no macroscopic or microscopic lesions in this group of experimental animals.

Group 2 - Synthetic Atractyloside (Sigma) (after 1, 3, 5, 24, 48 & 72 hours

The macroscopic appearance of the liver and kidney and histological findings in this group of experimental rats, injected with synthetic atractyloside (Sigma) were the same as those seen in rats injected with Compound B2 (Group 1). No lesions were found in the heart and lungs. The renal lesions are illustrated in Plates 21 - 27.

Group 3 - Control Group

The macroscopic and microscopic appearance and histological sections of the liver, kidney, heart and lungs were normal in this group of rats.

The results of Experiment 3 are summarised in Table 3.

PLATE 17 (upper plate)

This plate shows a histological section of a rat kidney, 24 hours after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside).
Tubular necrosis is well established. Cellular debris and proteinous and granular casts are present in the tubular lumina.
(Mayers haematoxylin and eosin x 100)

PLATE 18 (upper middle plate)

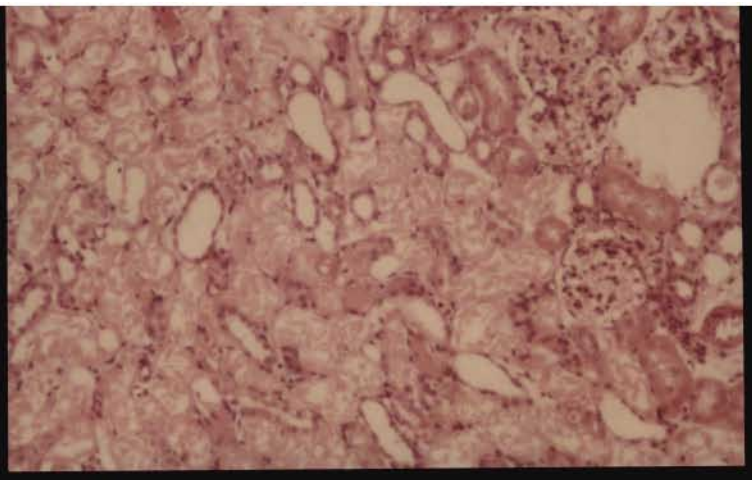
A histological section of a rat kidney, 48 hours after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside) is shown in this plate. The intact basement membrane of the tubules is visible; tubular lumina contain desquamated epithelium.
(Mayers haematoxylin and eosin x 100)

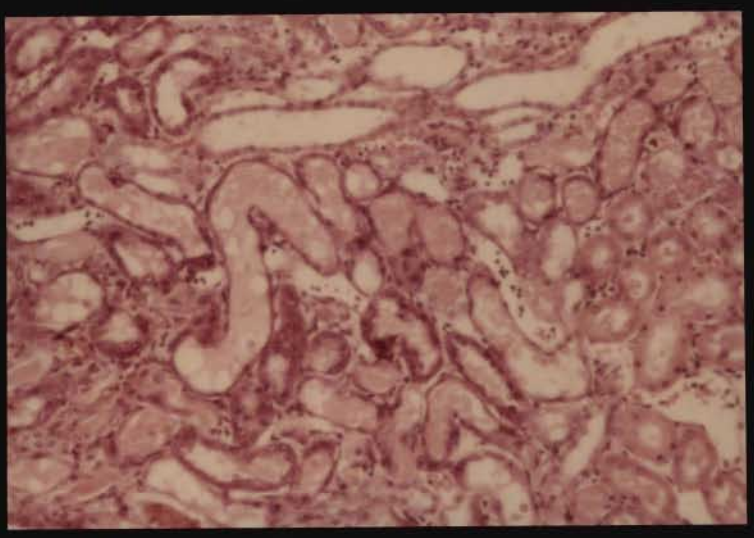
PLATE 19 (lower middle plate)

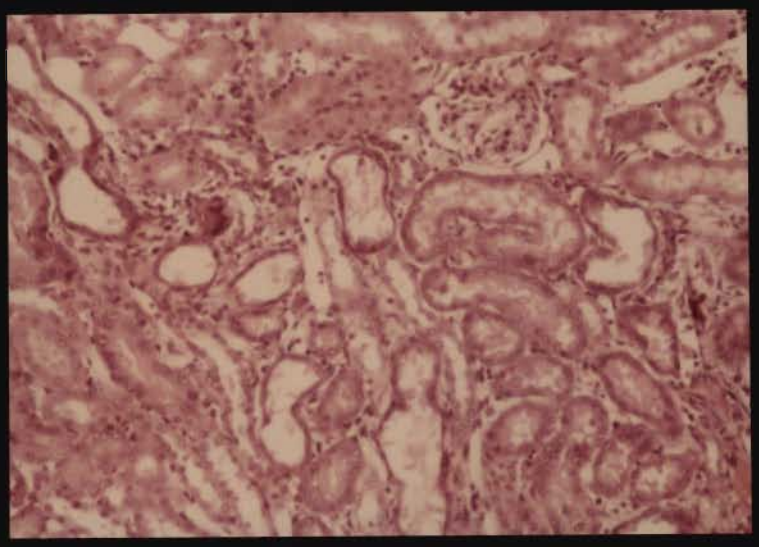
This plate shows a histological section of a rat kidney, 72 hours after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside).
The tubules show regenerative activity and are lined by basophilic staining cells with granular cytoplasm and vescicular nuclei.
(Mayers haematoxylin and eosin x 100)

PLATE 20 (lower plate)

A histological section of a rat kidney 72 hours after an intraperitoneal injection of 133 mg/kg of Compound B2 (atractyloside) is shown in this plate.
This section shows evidence of regeneration with mitotic activity and syncitial masses of cells with grouped nuclei.
(Mayers haematoxylin and eosin x 200)







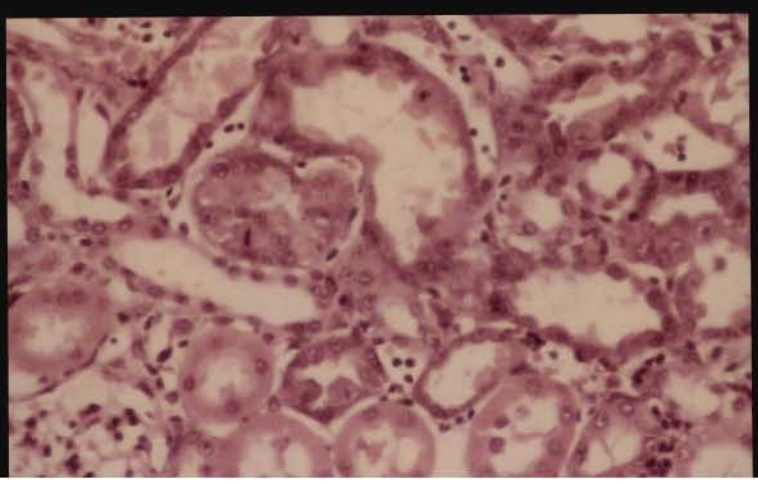


PLATE 21 (upper plate)

This plate shows a histological section of a rat kidney, 1 hour after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside.
The section shows early tubular necrosis. There is desquamation of the epithelium in some of the tubules. A few granular casts are present.
(Mayers haematoxylin and eosin x 100)

PLATE 22 (middle plate)

A histological section of a rat kidney, 3 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside is shown in this plate. The section shows more extensive tubular necrosis.
(Mayers haematoxylin and eosin x 100)

PLATE 23 (lower plate)

This illustration shows a histological section of a rat kidney, 5 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside.
The section shows frank widespread tubular necrosis. The tubules contain cellular and granular casts.
(Mayers haematoxylin and eosin x 100)

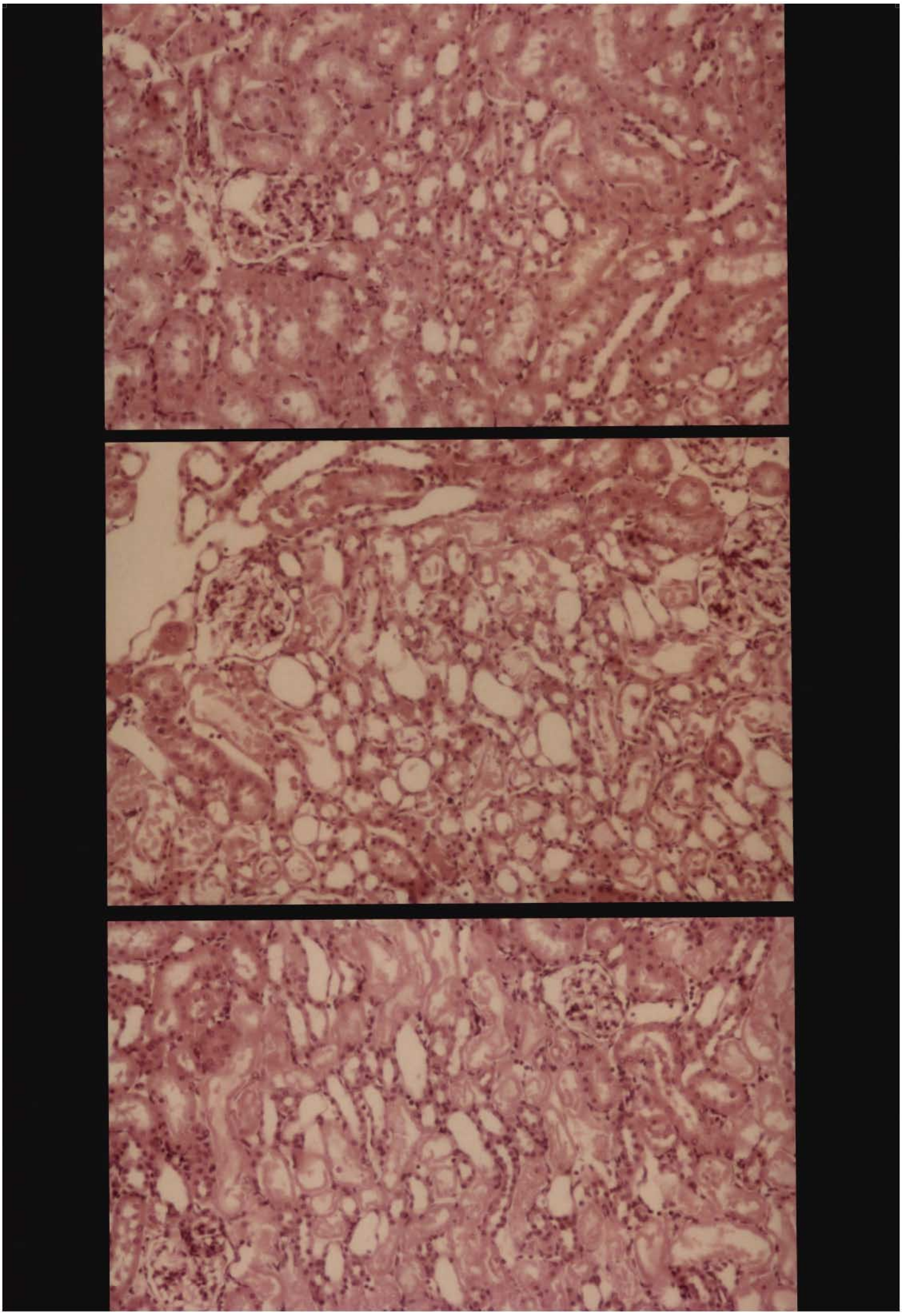


PLATE 24 (upper plate)

This plate shows a histological section of a rat kidney, 24 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside. The section shows well established tubular necrosis with cellular debris and granular casts filling the tubular lumina.
(Mayers haematoxylin and eosin x 100)

PLATE 25 (upper middle plate)

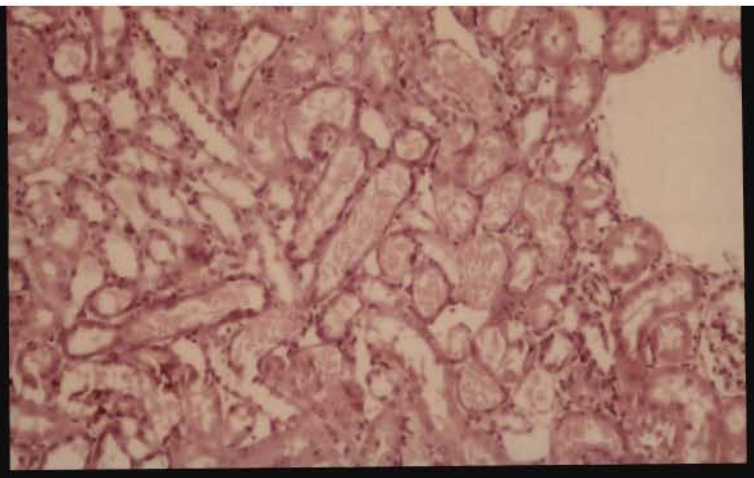
A histological section of a rat kidney, 48 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside is shown in this plate. The tubules are lined by basement membrane denuded of cells. Tubular lumina contain desquamated epithelium. Some tubules show regenerative activity.
(Mayers haematoxylin and eosin x 100)

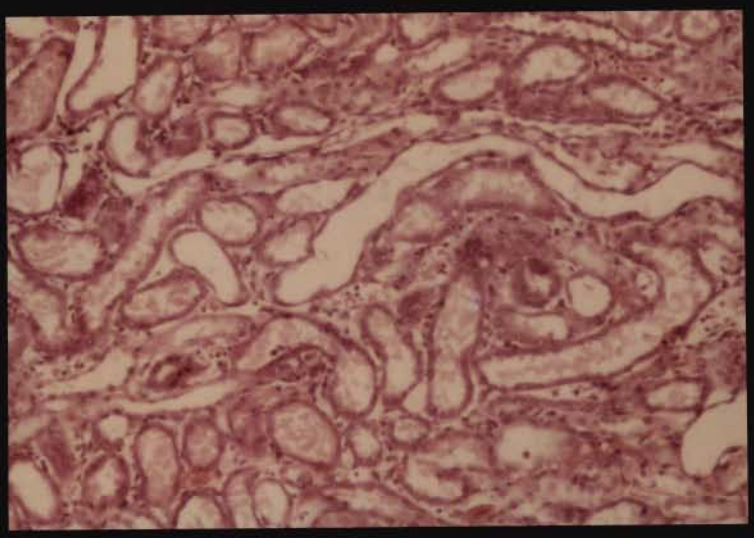
PLATE 26 (lower middle plate)

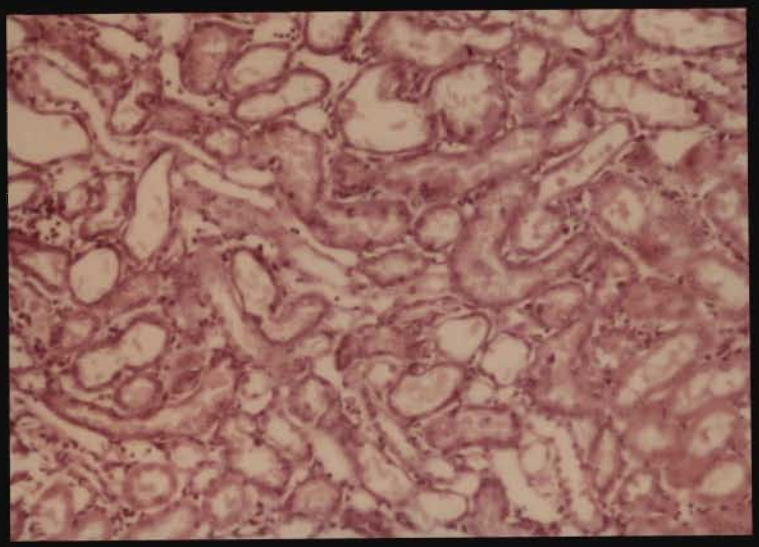
This plate shows a histological section of a rat kidney, 72 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside. Tubular regenerative activity is evident. The tubules are lined by basophilic cells with granular cytoplasm.
(Mayers haematoxylin and eosin x 100)

PLATE 27 (lower plate)

This illustration shows a histological section of a rat kidney, 72 hours after an intraperitoneal injection of 133 mg/kg of synthetic atractyloside. This high power magnification shows tubular regeneration with mitotic activity and syncitial masses of cells with grouped nuclei.
(Mayers haematoxylin and eosin x 200)







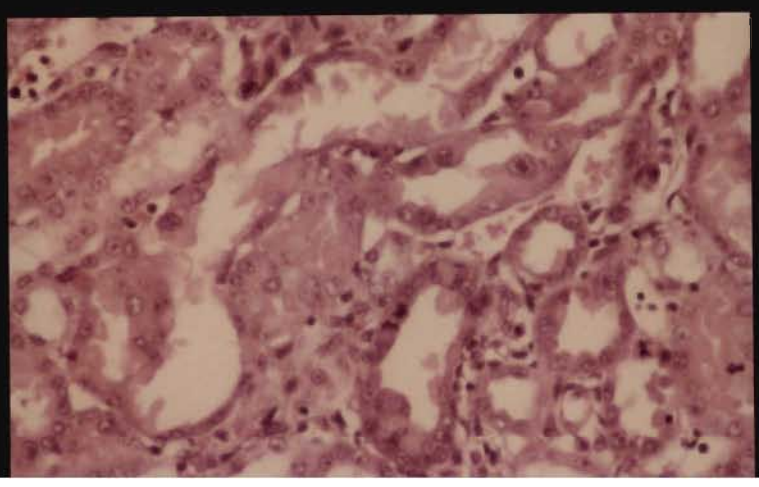


TABLE 3

EXPERIMENT 3 - SUMMARY OF RESULTS

RAT	GROUP	SACRIFICED AFTER:	RESULTS	
			MACROSCOPIC FINDINGS	MICROSCOPIC FINDINGS
A(5)	Group 1 I.P. Injection of Compound B2 133mg/kg	1 hour	Liver: No morphological changes. Kidney: Varying degrees of pallor of the cortex and congestion of medulla. Heart and Lungs: Normal	Liver: No microscopic lesions were found in specimens taken after 1, 3, 5, 24, 45 and 72 hours. Kidney: Early necrosis involving cells of proximal convoluted tubules
B(5)		3 hours		Early tubular necrosis but more widespread
C(5)		5 hours		Frank widespread tubular necrosis. Proteinous and cellular casts in collecting tubules
D(5)		24 hours		Necrosis well established. A few regenerating tubules.
E(5)		48 hours		Some tubules showed evidence of regeneration.
F(5)		72 hours		Large number of tubules show regenerative activity. Heart and Lungs: Normal
A(5)	Group 2 I.P. Injection of Synthetic Atractyloside 133mg/kg	1 hour	as above	as above
B(5)		3 hours		
C(5)		5 hours		
D(5)		24 hours		
E(5)		48 hours		
F(5)		72 hours		
A(5)	Group 3 I.P. Injection of Distill Water	1 hour	No morphological changes in the organs	No histological lesions in lung, liver, heart and kidneys
B(5)		3 hours		
C(5)		5 hours		
D(5)		24 hours		
E(5)		48 hours		
F(5)		72 hours		

Figures in brackets = number of rats in each group

TABLE 4

EXPERIMENT 4 - SUMMARY OF RESULTS

RAT	I.P. INJECTION OF COMPOUND C2		RESULTS	
			MACROSCOPIC FINDINGS	MICROSCOPIC FINDINGS
A (5)	2 mg/kg	sacrificed after 24 hours	No macroscopic or microscopic lesions noted in the liver, kidney, heart and lungs	
B (5)	4 mg/kg	sacrificed after 24 hours	Liver: congested	Liver: no evidence of necrosis
C (5)	8 mg/kg	died 8 hrs after injection	Kidney: mild pallor of cortex	Kidney: early tubular necrosis
D (5)	16 mg/kg	died 6 hrs after injection	Heart and Lungs: normal	Heart and Lungs: normal

Figures in brackets = number of rats in each group

Paired control rats sacrificed after 24 hours showed no macroscopic or microscopic lesions.

ADDENDUM

The intraperitoneal LD_{50} of carboxyatractyloside (Compound C2) in male albino rats determined according to Lichtfield and Wilcoxon (1949) was 2,9 mg/kg (fiducial limits 3,33 and 2,52 mg/kg).

The minimum and maximum LD_{50} as well as higher doses of 8 and 16 mg/kg of Compound C2 (carboxyatractyloside) were used in this experiment.

References are given on Page 102a.

TABLE 5

EXPERIMENT 5 - SUMMARY OF RESULTS

RAT	I.P. INJECTION OF COMPOUNDS D AND E	SACRIFICED AFTER:	RESULTS
A (5)	1 ml 2% solution ($^{w}/_{v}$)	24 hours	mildly congested liver but no evidence of liver cell necrosis or renal tubular necrosis Heart and lungs were normal
B (5)	1,5 ml 2% solution ($^{w}/_{v}$)	24 hours	
C (5)	2 ml 2% solution ($^{w}/_{v}$)	24 hours	

Figures in brackets = number of rats in each group

Paired control rats sacrificed after 24 hours showed no macroscopic or microscopic changes.

TABLE 6

EXPERIMENT 6 - SUMMARY OF RESULTS

R_f FRACTIONS SUBMITTED BY DR. MALAN

Toxicity tests involved the use of paired rats. One rat was injected intraperitoneally with the R_f fraction submitted by Dr. Malan and the other rat was given an equivalent volume of distilled water only.

The experimental design and results of these tests are tabulated below.

RAT	INTRAPERITONEAL INJECTION		SACRIFICED AFTER:	RESULTS	
				TOXICITY	
	R_f-FRACTION	DOSE		LIVER	KIDNEY
A	0,73	28 mg/kg	24 hours	None	Mild
B	0,65	76 mg/kg	24 hours	None	Mild
C	0,59	54 mg/kg	24 hours	None	Mild
D	0,56	103 mg/kg	24 hours	None	Mild
E	0,54	85 mg/kg	24 hours	None	Severe
F	0,41	116 mg/kg	24 hours	None	Severe
G	0,36	174 mg/kg	50 hours	None	Severe
H	0,30	90 mg/kg	24 hours	None	Moderate
I	0,22	185 mg/kg	50 hours	None	Severe
J	0,16	97 mg/kg	24 hours	None	Severe
K	0,08	107 mg/kg	Died after 4 hours	None	Severe
L	Residue from column	175 mg/kg	24 hours	None	Mild
				No changes in the heart and lungs	

Control rats showed no macroscopic or microscopic changes.

ADDENDUM

Because the chemical analysis was not repeated and because only small amounts of the various R_f fractions were available, it was decided by the author to administer the total amount of each fraction into one animal. Despite these shortcomings, the experiment does show that none of the fractions isolated, although administered in different dosages, produced hepatic necrosis.

During this study Dr. Malan also prepared methanol extracts of the (a) flowers and (b) leaves and stems of *Callilepis laureola*. These extracts were submitted to the author for toxicity tests.

EXPERIMENT 7

A) METHANOL EXTRACT OF FLOWERS

A dose of 133 mg/kg of the extract, dissolved in distilled water was injected intraperitoneally into 5 rats. Paired control rats were given equivalent amounts of distilled water only. All rats were sacrificed after 24 hours and necropsies were performed.

RESULTS

At necropsy, in the experimental animals, the kidneys appeared swollen with marked pallor of the cortex. No macroscopic changes were noted in the liver, heart and lungs. Histological sections showed severe acute renal tubular necrosis but liver cell necrosis was not seen. The heart and lungs were normal. The paired control rats showed no macroscopic or microscopic abnormalities.

The results indicate that while a methanol extract of the root of *Callilepis laureola* produced both liver cell necrosis and renal tubular necrosis an extract of flowers displayed only nephrotoxic activity. This suggested that the nephrotoxin (atractyloside) was being concentrated in the flowers of the plant.

B) METHANOL EXTRACT OF LEAVES AND STEMS

Ten paired rats were selected. The extract, dissolved in distilled water, was injected intraperitoneally into the experimental rats in doses of 66 mg/kg and 133 mg/kg body weight (5 rats in each group). The paired control rats were given equivalent amounts of distilled

water. All the rats were sacrificed after 24 hours and necropsies were performed.

RESULTS

No macroscopic organ chances were noted in the experimental animals. Histological sections showed no evidence of hepatic or renal toxicity. The control animals were normal. The results are summarised in Table 7.

TABLE 7

EXPERIMENT 7 (A AND B) - SUMMARY OF RESULTS

RAT	INTRAPERITONEAL INJECTION METHANOL EXTRACT OF FLOWERS	SACRIFICED AFTER:	RESULTS
A (5)	133 mg/kg	24 hours	Swollen kidneys with pale cortex. No changes in liver, heart, lungs. Acute renal tubular necrosis. Liver, heart, lungs showed no micro-scopic lesions

Figures in brackets = number of rats

RAT	INTRAPERITONEAL INJECTION METHANOL EXTRACT OF LEAVES & STEMS	SACRIFICED AFTER:	RESULTS
A (5)	66 mg/kg	24 hours	No macroscopic or micro-scopic lesions in liver, kidney, heart and lungs
B (5)	133 mg/kg	24 hours	

Figures in brackets = number of rats in each group

All the paired control rats in Experiment 7 A and B showed no organ changes.

B. HYPOGLYCAEMIC ACTION OF COMPOUND B2 AND SYNTHETIC ATRACTYLOSIDE

This section describes experiments designed to demonstrate and compare the hypoglycaemic action of a) compound B2-isolated from rootstock of *Callilepis laureola* and b) an authentic sample of synthetic atractyloside supplied by Sigma Chemical Company.

MATERIAL AND METHODS

The experiments involved the use of 65 male albino rats (Wistar), aged 4 - 5 months and weighing 250 - 350 grams. All the rats were subjected to an overnight fast of 15 hours and for experimental purposes divided into four groups. The experimental design and the mean weight of rats in each group are shown in Table 8. The design was adapted from that used by Kupiecki et al (1974) who assayed the hypoglycaemic activity of a compound isolated from the seeds of xanthium strumarium L (compositae).

Group A FASTING GROUP (5 rats)

The rats in this group, subjected to an overnight fast of 15 hours, were bled from the left ventricle after pentobarbitone sodium anaesthesia (6 mg/100 grams body weight).

Group B CONTROL GROUP (20 rats)

After an overnight fast of 15 hours these rats were given an intraperitoneal injection of 2 ml of distilled water followed immediately with subcutaneous injection of glucose (1 g/kg body weight).

Group C COMPOUND B2 GROUP (20 rats)

These rats subjected to an overnight fast of 15 hours were injected

TABLE 8

EXPERIMENTAL DESIGN TO DEMONSTRATE THE HYPOGLYCAEMIC ACTIVITY OF A) COMPOUND B2 ISOLATED FROM ROOTSTOCK OF *CALLILEPIS LAUREOLA* AND B) SYNTHETIC ATRACTYLOSIDE (SIGMA)

	NUMBER OF RATS	MEAN WEIGHT OF RATS
Group A : Fasting Group	5	303 grams

TIME SPAN IN HOURS	1	2	3	5	MEAN WEIGHT OF RATS
Group B : Control Group 2 ml distilled water I.P. followed by subcutaneous injection of glucose (1 g/kg body weight)	5	5	5	5	311 grams
Group C : Compound B2 Group 133 mg/kg in 2 ml distilled water I.P. followed by subcutaneous injection of glucose (1 g/kg body weight)	5	5	5	5	306 grams
Group D : Synthetic Atractyloside Group 133 mg/kg in 2 ml distilled water I.P. followed by subcutaneous injection of glucose (1 g/kg body weight)	5	5	5	5	302 grams

All the rats were subjected to an overnight fast of 15 hours.

intraperitoneally with 133 mg/kg in 2 ml of distilled water, of compound B2 followed by subcutaneous injection of glucose (1 g/kg body weight).

Group D SYNTHETIC ATRACTYLOSIDE GROUP (20 rats)

This group, after an overnight fast of 15 hours, was injected intraperitoneally with 133 mg/kg in 2 ml of distilled water, of synthetic atractyloside (Sigma) followed by a subcutaneous injection of glucose (1 g/kg body weight).

Rats from Groups B, C and D were bled from the left ventricle after intraperitoneal anaesthesia (pentobarbitone sodium - 6 mg/100 gram body weight), at intervals of 1, 2, 3 and 5 hours as shown in experimental design (Table 8).

Blood glucose was measured using an automated analyser by modification of the ferricyanide procedure of Hoffman (1937).

RESULTS

The fasting blood glucose values of rats in Group A are shown in Table 9. They varied from 1,03 to 1,16 mmol/litre with a mean of 1,10 mmol/litre. The experiments relating to the blood glucose estimations for control (Group B), Compound B2 (Group C) and synthetic atractyloside (Group D) are illustrated in Figure 1 and detailed values are tabulated in Table 9 and statistical data annotated in Tables 10A and 10B.

The time course of blood glucose changes, after the subcutaneous administration of glucose, 1 g/kg body weight, in the control group of rats showed substantial rise over fasting values, increasing for two hours and then subsequently falling towards the fasting values.

TABLE 9

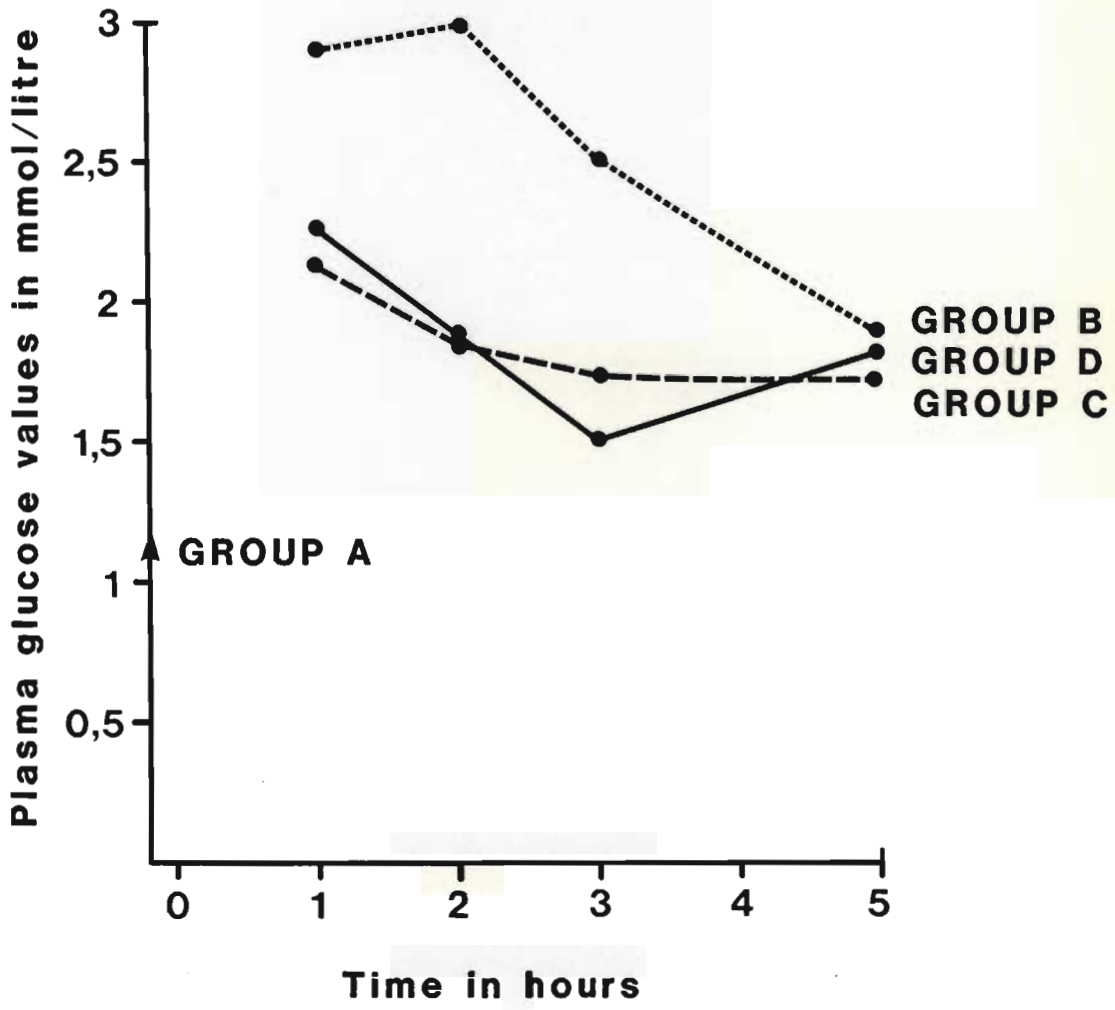
PLASMA GLUCOSE VALUES IN GROUPS A, B, C AND D

Group A Fasting	1,12
	1,04
	1,14
	1,03
	1,16
Mean	1,10

Values expressed in mmol/litre.
Results expressed per 100 grams body weight.

TIME SPAN	1 HOUR	2 HOURS	3 HOURS	5 HOURS
Group B Control	2,53	2,89	2,68	2,22
	2,60	3,64	2,35	1,90
	2,58	3,00	2,55	1,96
	3,35	3,30	2,11	1,50
	3,46	2,10	2,83	1,83
Mean	2,90	2,99	2,50	1,88
Group C Compound B2	1,70	2,03	1,79	2,00
	1,94	2,03	1,47	1,33
	1,68	1,86	1,38	1,73
	2,63	1,16	2,00	1,79
	2,75	2,11	2,00	1,77
Mean	2,14	1,84	1,73	1,72
Group D Synthetic Atractyloside	2,27	2,00	1,51	2,30
	2,41	3,34	1,94	1,57
	2,50	1,18	1,18	1,67
	1,51	1,10	1,26	1,68
	2,56	1,72	1,59	1,86
Mean	2,25	1,87	1,50	1,82

FIG. 1 PLASMA GLUCOSE VALUES IN GROUPS A, B, C, & D



GROUP A : Fasting

GROUP B : Control

GROUP C : Compound B2

GROUP D : Synthetic Atractyloside

TABLE 10A

STATISTICAL DATA ON PLASMA GLUCOSE ESTIMATIONS SHOWN IN TABLE 9

PAIRED AND STATISTICAL COMPARISON

COMPARING GROUPS B AND C				
TIME COURSE IN HOURS	GROUP B MEAN	GROUP C MEAN	P-VALUE	
1	2,90	2,14	< 0,05	Significant
2	2,99	1,84	< 0,005	Significant
3	2,50	1,73	< 0,01	Significant
5	1,88	1,72	< 0,20	Not significant

COMPARING GROUPS B AND D				
TIME COURSE IN HOURS	GROUP B MEAN	GROUP D MEAN	P-VALUE	
1	2,90	2,25	< 0,025	Significant
2	2,99	1,87	< 0,025	Significant
3	2,50	1,50	< 0,0005	Significant
5	1,88	1,82	< 0,35	Not significant

COMPARING GROUPS C AND D				
TIME COURSE IN HOURS	GROUP C MEAN	GROUP D MEAN	P-VALUE	
1	2,14	2,25	< 0,35	Not significant
2	1,84	1,87	< 0,475	
3	1,73	1,50	< 0,45	
5	1,72	1,82	< 0,3	

TABLE 10B

STATISTICAL SUMMARY OF DATA ON PLASMA GLUCOSE ESTIMATIONS SHOWN IN TABLE 9

GROUP	TIME COURSE IN HOURS	MEAN	STANDARD DEVIATION
A		1,10	0,06
B	1	2,90	0,46
	2	2,99	0,57
	3	2,50	0,28
	5	1,88	0,26
C	1	2,14	0,51
	2	1,84	0,39
	3	1,73	0,29
	5	1,72	0,24
D	1	2,25	0,43
	2	1,87	0,90
	3	1,50	0,30
	5	1,82	0,29

This time course was significantly modified by Compound B2 and synthetic atractyloside. The initial rise was markedly attenuated and the magnitude of the subsequent fall increased. This effect was transient because at 5 hours the values approached those of control estimations.

The quantitative difference of 0,23 mmol/litre in the time course of Compound B2 and synthetic atractyloside at 3 hours suggests that possibly Compound B2 may require further purification to produce hypoglycaemia of similar potency to that of synthetic atractyloside.

The data presented shows that there is both a hepatotoxin and a nephrotoxin in the rootstock of *Callilepis laureola*. The nephrotoxin, which also has hypoglycaemic action, has been identified as atractyloside. The hepatotoxin has not been isolated. A new preparative high pressure liquid chromatograph has been recently acquired by Professor E. Malan, Department of Chemistry, University of Durban-Westville and further experimental work will be undertaken to try to isolate, purify and identify the hepatotoxin.

SUMMARY

Experiments were designed to assess the biological toxicity of the methanol extract of the rootstock of *Callilepis laureola* and the chemically isolated fractions A, B2, C2, D and E, in male albino rats (Wistar).

1. Intraperitoneal injections of the methanol extract, in doses of 47 mg and 54 mg/kg body weight, consistently produced centrilobular zonal necrosis in the liver and acute renal tubular necrosis. No morphological changes were evident in the heart and lungs.
2. Substance A (a carbohydrate) was found to be non-toxic.
3. Compound B and synthetic atractyloside (Sigma) both produced renal tubular necrosis but no hepatotoxic effect.
4. Compound C2 (carboxyatractyloside) exhibited mild nephrotoxicity contrary to the findings of other workers (Carpenedo et al, 1974); possibly due to small amounts of Compound B2 being present in the sample. Compound C2 produced no hepatic lesions.
5. Compounds D and E failed to cause hepatic or renal lesions.
6. Methanol extract of the flowers of *Callilepis laureola* was nephrotoxic but not hepatotoxic.
7. Methanol extract of the leaves and stems of *Callilepis laureola* produced no effects in the liver or kidney.
8. The hypoglycaemic effect of Compound B2 was demonstrated and compared with the similar action produced by synthetic atractyloside (Sigma).

DISCUSSION

In Natal and other areas of Southern Africa *Callilepis laureola (impila)* has been used for many years as a medicinal plant by the Black population (Maberley, 1905) and its toxic nature was reported by Bryant as early as 1909. However, subsequently it seems to have faded from recognition. Decisive investigation into the nature of the toxicity of *Callilepis laureola* was initiated by Wainwright and Schonland in 1977. Several plants commonly used by herbalists for medication were tested in laboratory rats; of these *Callilepis laureola* was found to be toxic and to produce hepatic and renal lesions resembling those seen in patients who had died after intake of herbal mixtures.

Isolation of toxins

Chemical analysis was undertaken to identify the toxic principles contained in the rootstock of *Callilepis laureola* (Brookes, 1979). Whereas the methanol extract produced both liver and renal toxicity, the isolated compound B2 (atractyloside) was found to have nephrotoxic and hypoglycaemic properties only. As the hepatotoxin has not yet been finally identified, its mode of action cannot be ascertained. Toxins contained in the rootstock of *Callilepis laureola* have been shown in the present study to have a three-fold effect both in human subjects and in laboratory rats.

1. Hepatotoxicity : causing centrilobular zonal necrosis.
2. Nephrotoxicity : causing necrosis of proximal convoluted tubules.
3. Hypoglycaemia.

Atractyloside

Atractyloside, responsible for nephrotoxicity and hypoglycaemia, was

first isolated from the root of atractylis gummifera L (compositae) by Lefranc, an apothecary of the French Army, as long ago as 1868. The thistle atractylis gummifera grows in the countries around the Mediterranean Sea and its toxic properties had been noted as far back as 300 B.C. (Theophrastos) and 1st Century A.D. (Dioscorides). The plant was known as "White Chamaeleon" (Ancient Greek), "el heddah" (Arabian) and "Masticogna" (Sicilian). Lefranc's work was largely disregarded and it was only in 1906 that the plant was reinvestigated owing to many new cases of fatal poisoning in Sicily. However, the chemical structure of atractyloside was only fully elucidated a century after Lefranc's pioneering work.

Chemical Structure of Atractyloside

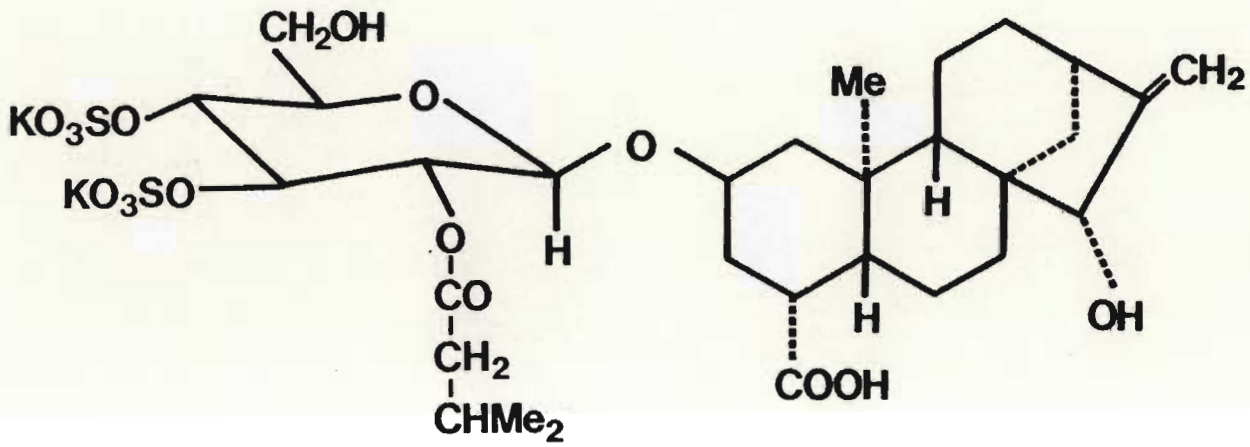
Atractyloside is a glycoside whose aglycone is a diterpene with a perhydrophenanthrenic structure having a residue = CH_2, two oxydrilic and a carboxylic groups.

The glucidic part is made up of a D-glucose molecule with two sulphuric acid residues and a molecule of isovalerianic acid. (Ajello et al, 1960; Piozzi et al, 1967; Brookes, 1979). The chemical structure of atractyloside as elucidated by Brookes is shown in Figure 2. Carboxyatractyloside was isolated from extracts of atractylis gummifera and its chemical structure determined by Danieli et al in 1971. It differs from atractyloside in having a second carboxylic group. Controlled heating of carboxyatractyloside cause decarboxylation with the formation of atractyloside (Brookes, 1979).

Nephrotoxicity of atractyloside

Carpenedo et al (1974) studied the effects of atractyloside and carboxyatractyloside in male albino rats. The significant results

FIGURE 2



CHEMICAL STRUCTURE OF ATRACTYLOSIDE

of these experiments were: The intraperitoneal LD_{50} of atractyloside in male albino rats was determined to be 143 mg/kg (fiducial limits 153,01 and 133,64 mg/kg). After intraperitoneal injection of 10 to 100 mg/kg of atractyloside, the rat liver did not present any morphological changes. The rat kidney however showed acute tubular necrosis, 150 - 180 minutes after atractyloside injection. The lesions were confined to the distal portion of the proximal convoluted tubules. This was accompanied by increase in the volume of urine with proteinuria, glycosuria, ketonuria, increase in potassium excretion and a slight decrease in sodium excretion. The glomeruli appeared intact with no significant change in creatinine clearance. The value of serum urea was increased almost three-fold confirming renal tubular damage. Only a few drugs are known to show such specific nephrotoxicity. Acute renal tubular necrosis of the distal portion of the proximal convoluted tubules similar to that of atractyloside is produced by sublethal doses of uranyl nitrate (Kempcziski and Caulfield, 1968) and mercury bichloride (Rhodin and Crowson, 1962; Taylor, 1965). However, as the dose of uranyl nitrate or mercury bichloride is increased, the proximal portions of the proximal convoluted tubules become involved. Atractyloside even at high doses of 100 to 200 mg/kg is still specific for the distal portion of the proximal convoluted tubule. The renal lesion is believed to originate from inhibition of mitochondrial oxidative phosphorylation, which may be responsible for the nephrotoxic effect, since tubular cells of the distal portion of the proximal convoluted tubules are very rich in mitochondria and therefore particularly sensitive to anoxia.

The intraperitoneal LD_{50} of carboxyatractyloside in male albino rats was determined by Carpenedo et al to be 2,9 mg/kg (fiducial limits 3,33 and 2,52 mg/kg) which is 40 - 50 times lower than that of

atractyloside. Carboxyatractyloside showed general toxicity similar to atractyloside as regards metabolic alterations (e.g. hypoglycaemia) but failed to produce nephrotoxicity. After administration of sublethal (2 mg/kg) and lethal (4 mg/kg) doses of carboxyatractyloside these authors found no morphological changes in the kidneys. The failure of carboxyatractyloside to produce nephrotoxicity, although it is very similar to atractyloside in chemical structure (Danieli et al, 1971, 1972), "in vitro" mechanism of action at mitochondrial level (Luciani et al, 1971) and in metabolic alterations, may be explained by the presence of a second carboxylic group in the atractyligenin ring. This may enhance the polarity of carboxyatractyloside thereby reducing its tubular reabsorption.

In the author's experiments, Compound C2, which was characterised as carboxyatractyloside, did produce early renal tubular necrosis when administered intraperitoneally in doses of 4, 8 and 16 mg/kg. Possibly small amounts of atractyloside may have been present in the sample giving rise to the nephrotoxic effect.

Hypoglycaemic effect of atractyloside

Luciani et al (1978) found that intravenous injection of atractyloside (15 mg/kg) given to dogs resulted in an initial rapid rise in blood glucose (marked enough to cause glycosuria) followed by gradual evolution to severe hypoglycaemia. At first, blood lactate levels closely followed the changes in glucose values but in the hypoglycaemic phase the lactate levels rose steadily. During this period the non esterified fatty acid concentration in blood also became elevated. These authors also demonstrated depletion of hepatic glycogen as well as diminution of skeletal sarcoplasmic glycogen stores following sublethal atractyloside dosage administered to rats. Depletion of liver and skeletal muscle (but not of the heart) glycogen (Santi and

Cascio, 1955b), as well as inhibition of glycogen synthesis (Santi et al, 1968) no doubt accounts for the profound hypoglycaemic effect of atractyloside. Although inhibition of oxidative phosphorylation in isolated rat liver mitochondria by atractyloside has been demonstrated (Vignais and Vignais, 1969), unlike the kidney, an impairment of the mitochondrial oxidative phosphorylation in the liver "in vitro" is not accompanied by alteration of hepatic function and structure "in vivo".

In 1958, Santi, Bistocchi and Bruni showed that atractyloside inhibits mitochondrial oxidative phosphorylation. The mechanism of action of atractyloside was further elucidated during the period 1962 - 1978. It was shown that:

1. Atractyloside competitively inhibits adenosine diphosphate (ADP) stimulated respiration of mitochondria (Bruni et al, 1962; Vignais and Vignais, 1964; Bruni et al, 1965).
2. Atractyloside prevents binding of external ADP to mitochondria (Bruni et al, 1965).
3. Atractyloside has no effect on the oxidative phosphorylation of intramitochondrial ADP as compared with its powerful inhibitary effect on the oxidative phosphorylation of external ADP (Kemp and Slater, 1964; Chappel and Crofts, 1965; Heldt et al, 1965).
4. Vignais and Vignais (1969) demonstrated that atractyloside selectively binds to the outer mitochondrial membrane.
5. Carboxyatractyloside, on the other hand, was found to be a non-competitive inhibitor of ADP transport (Vignais et al, 1971; 1973; Luciani et al, 1971).
6. Oxidative phosphorylation in homogenates of liver and kidney taken from rats and guinea pigs pretreated with atractyloside appeared to be severely depressed (Luciani et al, 1978).

7. Atractyloside has been shown to be a powerful inhibitor of the citric acid cycle due to its effect on oxidative phosphorylation (Bistocchi and Bruni, 1959).
8. Increasing toxicity from mouse and rat to dog suggested very high atractyloside toxicity for man (Luciani et al, 1978).

Atractyloside is at present generally recognised as a specific inhibitor of the translocation of adenine nucleotides across the mitochondrial membrane (Luciani et al, 1978).

Effect of atractyloside on heart muscle

Interesting, but not yet clarified, is the different behaviour of atractyloside and carboxyatractyloside on the heart. When added "in vitro" they inhibit oxidative phosphorylation of heart mitochondria (Brierley and O'Brien, 1965; Luciani and Varotto, 1974) but are devoid of any effect when injected in animals. This effect is in agreement with the lack of metabolic alterations in the heart e.g. glycogen depletion (Santi and Cascio, 1955a).

The author found no light microscopical changes in the heart muscle of patients dying of *impila* poisoning. Rats injected with the methanol extract of the root of *Callilepis laureola* and the purified compounds B2 (atractyloside) and C2 (carboxyatractyloside) also showed no cardiac lesions.

CONCLUSIONS

The minimal toxic dose of the methanol extract of the rootstock of *Callilepis laureola* was established as being 47 mg/kg body weight, in male albino rats (Wistar), given by intraperitoneal injection. Doses of 47 mg/kg and 54 mg/kg consistently caused centrilobular liver necrosis as well as renal tubular necrosis, selectively involving the proximal convoluted tubules. Doses of 61 mg/kg or more caused rapid death of the animal and renal or hepatic necrosis did not develop, the time course being insufficient to induce the initial hepatic or renal lesions to be apparent.

Compounds isolated from the methanol extract were tested on laboratory rats. The results of these toxicity tests showed that Compound B2, obtained in a pure state after repeated crystallisation, was found to be a nephrotoxic as well as a hypoglycaemic agent. An intraperitoneal injection of 133 mg/kg produced cellular necrosis which was confined to the proximal convoluted tubules. Early necrosis was seen 1 hour after administration; the necrosis being well established after 24 hours. Parallel toxicity tests using Compound B2 and synthetic atractyloside (Sigma) has confirmed the chemical characterisation of Compound B2 as atractyloside. Neither compound B2 nor synthetic atractyloside produced liver cell necrosis, even 72 hours after administration of a dose of 133 mg/kg.

The hypoglycaemic action of Compound B2 was demonstrated and compared with the similar action of synthetic atractyloside (Sigma), over a time span of 5 hours, after intraperitoneal injections of 133 mg/kg. These experiments show significant fall of blood glucose values in the experimental animals.

Compound C2 which was chemically identified as carboxyatractyloside was found to exhibit mild nephrotoxicity when administered in 1/15 or less than the dose of Compound B2. This was contrary to the findings of Carpenedo et al (1974) who found no light microscopical changes in the kidneys after administration of lethal and sublethal doses of carboxyatractyloside. Possibly the sample of Compound C2 supplied by Mrs. Brookes may have contained small amounts of Compound B2 (atractyloside). Compound C2 (carboxyatractyloside) also failed to produce liver necrosis.

Substance A (a carbohydrate) and Compounds D and E (thymol esters) were found to be non toxic to the liver and the kidney.

A chemical analysis was undertaken by Dr. Malan subsequent to the work of Mrs. Brookes. The various fractions isolated from a methanol extract of the root of *Callilepis laureola* were tested on laboratory rats. None of these fractions showed hepatotoxic activity. During this study it was established that the flowers of *Callilepis laureola* contained the nephrotoxin (atractyloside) but not the hepatotoxin. A methanol extract of the leaves and stems of the plant was devoid of any toxic effects in laboratory rats.

Biological tests in laboratory rats have shown that the rootstock of *Callilepis laureola (impila)* contains lethal hepatotoxic, nephrotoxic and hypoglycaemic activity. While the methanol extract of the root produced both centrilobular zonal liver necrosis as well as acute renal tubular necrosis, the chemically isolated Compound B2 (atractyloside) was found to be a nephrotoxic and hypoglycaemic agent without causing liver cell necrosis.

The experimental evidence therefore suggests that:

a) there may be at least two toxins contained in the rootstock of *Callilepis laureola*, one causing the liver lesion and the other (atractyloside) causing nephrotoxicity and hypoglycaemia; the liver toxin or toxins being lost during the process of extraction and purification; or

b) as the liver necrosis takes up to 24 hours to develop, the delay might indicate a metabolite rather than a toxin itself causing the cell necrosis; or

c) there may be a synergistic effect of two or more compounds leading to the hepatic necrosis.

It is significant to note that, whereas an intraperitoneal dose of 61 mg/kg or more of the methanol extract caused rapid death, rats administered doses of 133 mg/kg of Compound B2 (atractyloside) survived for up to 72 hours. The combination of the hepatotoxic, nephrotoxic and hypoglycaemic activity contained in the rootstock of *Callilepis laureola* would therefore appear to be far more dangerous than the renal toxicity and hypoglycaemia produced by atractyloside alone.

Biological tests in laboratory rats have confirmed the toxic nature of the root of *Callilepis laureola*. The lesions produced in laboratory rats were found to be identical to those seen in patients who died after intake of *impila*, prescribed by witchdoctors and other dispensers of herbal medicines.

SECTION 4

GENERAL DISCUSSION AND CONCLUSIONS

For some years hospital clinicians and pathologists at King Edward VIII Hospital have encountered an illness of sudden onset characterised by gastrointestinal symptoms, hypoglycaemia, uraemia, diminished level of consciousness, convulsions and coma followed by death within a few days. Necropsies in these patients showed centrilobular zonal liver necrosis and acute renal tubular necrosis. For a number of years the aetiology of this "syndrome" remained unknown.

In 1964, Neame and Pillay investigated eleven cases of patients admitted to hospital with hypoglycaemia who did not respond to intravenous dextrose therapy and died soon after admission. Necropsies in these patients showed acute diffuse centrilobular zonal necrosis of the liver. Four patients had acute renal tubular necrosis as well. However, the important finding by these authors was that seven of these patients had taken a herbal remedy immediately before the onset of symptoms. Seedat and Hitchcock in 1971 reported a case of acute renal failure in a patient who had ingested a herbal mixture known to the Zulus as *"impila"* which he had obtained from an *inyanga*.

An investigation of the nature of herbal intoxication among our Black population was initiated by Wainwright and Schonland in 1977. Several roots and plants in common use were identified and extracts of these proved to have no toxic effects on laboratory animals. However, the root of one plant, *Callilepis laureola (impila)* produced toxicity in laboratory rats, very similar to that seen in patients who had died of herbal intoxication. A survey undertaken by a Zulu social worker revealed that 30% of the residents of a Black township (Umlazi) used

or had used *impila* as a medicinal plant.

The present clinico-pathological and biochemical study of the toxicity of *Callilepis laureola (impila)* was undertaken by the author at the suggestion of Professor J. Wainwright. Necropsy records of post mortems performed by the Department of Pathology of the Faculty of Medicine, University of Natal, have shown that over a 20 year period (1958 - 1977), 447 cases of death were due to acute liver necrosis. In 263 of these patients the necrosis was centrilobular in distribution while in 184 cases the necrosis was either massive or submassive. A comparative study of these two groups, as regards clinical presentation, duration of illness, biochemical, gross anatomical and histological findings became necessary in order to establish the clinico-pathological syndrome of *Callilepis laureola (impila)* poisoning a distinct recognisable entity.

This study has shown that there has been an average of 13 deaths per annum, during a period of 20 years, at King Edward VIII Hospital, from *Callilepis laureola (impila)* poisoning. The duration of illness before admission to hospital was usually very short (1 - 2 days) and was characterised by vomiting, epigastric pain and headache. Occasionally there was mild to moderate jaundice. Convulsions and coma were common with rapidly fatal outcome and 91% of patients had died within 5 days. Biochemical investigation showed hypoglycaemia in 71% of patients and a raised blood urea in 72%. Disturbance of liver function was evident in patients surviving several days. Urine analysis showed albuminuria, glycosuria and haematuria. There was marked electrolyte imbalance with hyperkalaemia in 55%, hyponatraemia in 58% and hypochloraemia in 50%.

The significant necropsy findings were centrilobular punctate congestion in the liver which was often reduced in weight. Marked pallor of the renal cortex was observed in many cases; the kidney was increased in

weight. Histologically, the liver showed a distinct centrilobular zonal necrosis with fatty change in the surviving peripheral hepatocytes. In cases of late death there was centrilobular collapse of reticulin and regenerative activity in the peripheral hepatocytes. The kidneys showed evidence of acute tubular necrosis in 80% of cases. Haemorrhages were often seen in other organs.

The salient clinico-pathological and biochemical features of *Callilepis laureola (impila)* poisoning can be summarised as follows:

1. Short history of illness.
2. History of herbal remedy.
3. Abdominal pain.
4. Vomiting ± diarrhoea.
5. Headache.
6. Disturbed level of consciousness.
7. Convulsions and coma.
8. Hypoglycaemia.
9. Disturbed liver function with mild to moderate jaundice in some cases.
10. Renal failure with uraemia.
11. Electrolyte imbalance.
12. Albuminuria, glycosuria, haematuria.
13. Rapidly fatal outcome.
14. Centrilobular zonal necrosis of the liver.
15. Acute renal tubular necrosis.
16. Haemorrhages in other organs.

By contrast in cases with massive and submassive liver necrosis the duration of illness was longer and characterised by abdominal pain and discomfort, headache and confusion, rapidly increasing jaundice with foetor hepaticus, liver failure, mental changes with rapidly advancing stupor or coma.

At necropsy the liver showed marked reduction in weight, was flabby, deeply icteric and the cut surface showed mottled appearance of red and yellow areas. The kidneys were slightly swollen and bile stained. Haemorrhagic phenomena and gastrointestinal haemorrhage were often encountered. Histological sections showed massive or submassive liver cell necrosis. Sections of the kidneys showed bile nephrosis with tubular degenerative changes in about half the cases.

In summary, the features characterising massive and submassive liver necrosis due either to fulminant viral hepatitis or other causes were:

1. Illness of longer duration.
2. No history of herbal remedy.
3. Abdominal pain and discomfort.
4. Headache, irritability, drowsiness, and confusion.
5. Pyrexia.
6. Nausea, vomiting and anorexia.
7. Rapidly increasing jaundice, marked disturbance of liver function leading to liver failure and coma.
8. Death 1 - 22 days after admission.
9. Massive or submassive liver necrosis.
10. Bile nephrosis.
11. Haemorrhages in organs including massive gastrointestinal haemorrhage.

This study establishes *Callilepis laureola (impila)* poisoning as a separate clinico-pathological entity, distinct from acute massive and submassive liver necrosis due to other aetiological agents. Careful history taking and the clinico-pathological findings presented in this study will no doubt be helpful in the early recognition of this syndrome.

Subsequent investigation into the toxicity of *Callilepis laureola* involved chemical identification of the toxic agents and their effects

in laboratory rats. The chemical analysis was undertaken by Mrs. Brookes of the Department of Chemistry, University of Natal. The tests for toxicity were conducted by the author. Seven compounds were isolated by Mrs. Brookes. One compound (Substance A) was found to be a carbohydrate and non toxic. Four compounds (B1, B2, C1 and C2) were chemically related and were characterised as atractyloside and its analogue carboxyatractyloside. Two compounds were thymol related oils and non toxic.

The crude methanol extract, when injected in minimal toxic doses, in laboratory rats produced centrilobular zonal liver necrosis and acute renal tubular necrosis. These lesions were macroscopically and microscopically identical to those seen in patients who had died of *Callilepis laureola (impila)* poisoning. Toxicity tests using isolated compound B2 (atractyloside), however, while causing nephrotoxicity, failed to produce liver cell necrosis.

Experiments on blood glucose levels in laboratory rats proved atractyloside to be a hypoglycaemic agent. These investigations and the experimental evidence indicates that the herbal mixture prepared from the root of *Callilepis laureola* and administered to "patients" by the herbalists has a threefold effect.

1. Hepatotoxicity - producing centrilobular zonal necrosis of the liver.
2. Nephrotoxicity - causing acute renal tubular necrosis.
3. Hypoglycaemia with marked lowering of blood glucose levels soon after or within a day or two of the administration of the herbal remedy.

The second and third effects are caused by the toxic agent atractyloside contained in the root of *Callilepis laureola*. The nature of the hepatotoxicity remains unknown.

Consultation of traditional "practitioners" such as *inyangas* and *isangomas* is widespread among the Black population. *Callilepis laureola*, an attractive plant found in Natal, Transkei, Swaziland and Eastern Transvaal, has been for many years used as a medicinal plant by the Black population. The extract prepared from the root of this plant is used for a variety of ailments. The tragic aspect of the situation is that about a third of the deaths occur in children under the age of 5 years. Many of these children are well nourished and well cared for and at necropsy many showed no evidence of other illness apart from the hepatotoxic and nephrotoxic effects of *impila* poisoning.

It would appear therefore that conscientious and overprotective Black parents employ herbal medication to ward off illness or evil spirits in their children. Our records have shown that several children in the same family have died from *impila* poisoning. Apart from treatment of illness, the use of herbal medicines is often linked with superstition and ancestral beliefs and parents of deceased children are reluctant to accept the fact that administration of such medicines has caused the death of their child.

It must be pointed out that although herbs, roots and barks are commonly taken by Blacks without apparent ill effect, the use of *Callilepis laureola (impila)* as a medicinal plant should be actively discouraged. To this effect, the author, in an interview with a reporter of a widely distributed newspaper, elaborated on the toxic nature of this plant, giving relevant details including the number of deaths that had occurred from its use as a medicinal remedy (The Sunday Tribune - 11 February 1979).

Following upon this publicity the State Health Department called on all herbalists to destroy their stocks of the root of *Callilepis laureola* immediately. (The Sunday Tribune - 25 February 1979). It became

apparent that many herbalists were unaware that mixtures prepared from this plant had been responsible for many deaths.

A further survey of clinical and autopsy records has shown the following prevalence of deaths from *impila* intoxication. It appears that there was initial delay by the herbalists and the Black community in realising the poisonous nature of *impila*. However, 3 years after the initial announcement, a significant decline in the necropsy prevalence of death from *impila* was noted.

Year	Number of Deaths
1978	5
1979	7
1980	8
1981	6
1982	2
	28

Hopefully, this study, together with the dissemination of knowledge concerning the toxicity of *Callilepis laureola (impila)* will be instrumental in completely eradicating its use as a herbal remedy.

REFERENCES

Ajello, T., Piozzi, F., Quilico, A. and Sprio, V. : "Atractylin and atractyligenin." Atti Acc. naz. Lincei, Rend, cl. sci. fis. mat. e nat. VIII, 38: 545-550 (1960)

Barnes, J.M. and Magee, P.N. : "Some toxic properties of dimethyl-nitrosamine." Brit. J. Indust. Med., 11: 167-174 (1954)

Beaver, D.C. and Robertson, H.E. : "Specific character of toxic cirrhosis as observed in cinchophen poisoning : A review of five fatal cases." Proc. Mayo Clin., 6: 216-218 (1931)

Becroft, D.M.O. : "Syndrome of Encephalopathy and Fatty Degeneration of Viscera in New Zealand Children." Brit. Med. J., 2: 135-140 (1966)

Bistocchi, M. and Bruni, A. : "Inhibition of the citric acid cycle in rat liver homogenates by a vegetal glicoside." It. J. Biochem., 8: 77-89 (1959)

Bobrow, S.N., Jaffe, E. and Young, R.C. : "Anuria and Acute Tubular Necrosis Associated with Gentamicin and Cephalothin." J.A.M.A., 222: 1546-1547 (1972)

Bourgeois, C., Olson, L., Comer, D., Evans, H., Keschamres, N., Cotton, R., Grossman, R. and Smith, T. : "Encephalopathy and Fatty Degeneration of the Viscera : A clinico pathological analysis of 40 cases." Am. J. Clin. Path., 56: 558-571 (1971)

Brierley, G. and O'Brien, R.L. : "Compartmentation of heart mitochondria." J. biol. Chem., 240: 4532-4539 (1965)

Brookes, K.B. : "A chemical study of the rootstock of Callilepis laureola." M.Sc. Thesis: University of Natal, Durban (1979)

Bruni, A., Contessa, A.R. and Luciani, S. : "Atractyloside as inhibitor of energy-transfer reactions in liver mitochondria." Biochim. Biophys. Acta., 60: 301-310 (1962)

Bruni, A., Luciani, S. and Bortignon, C. : "Competitive reversal by adenine nucleotides of atractyloside effect on mitochondrial energy transfer." Biochim. Biophys. Acta., 97: 434-441 (1965)

Bryant, A.T. Ann. Natal Mus., 2: 1 (1909)

Bull, L.B. and Dick, A.T. : "The chronic pathological effects on the liver of the rat of the pyrrolizidine alkaloids heliotrine lasiocarpine and their N-oxides." J. Path. Bact., 78: 484 (1959)

Bywaters, E.G.L. : "Anatomical changes in the liver after trauma." Clin. Sci., 6: 19-31 (1948)

Carpenedo, F., Luciani, S., Scaravilli, F., Palatini, P. and Santi, R. : "Nephrotoxic Effect of Atractyloside in Rats." Arch. Toxicol., 32: 169-180 (1974)

Carr, H.J. Jr. and Knauer, Q.F. : "Death due to hepatic necrosis in a patient receiving zoxazolamine." New Eng. J. Med., 264: 977-980 (1961)

Chappel, J. and Crofts, A. : "The effect of atractylate and oligomycin on the behaviour of mitochondria towards adenine nucleotide." Biochem. J., 95: 707-716 (1965)

Clarke, W.T.W. : "Centrilobular hepatic necrosis following cardiac infarction." Am. J. Path., 26: 249-253 (1950)

Cohen, R., Kalser, M.H. and Thomson, R.V. : "Fatal Hepatic Necrosis Secondary to Isoniazid Therapy." J.A.M.A., 176: 877-879 (1961)

Cook, C.D. and Haggerty, R.J. : "Toxic Hazards. Mycetismus *(Amanita Phalloides)*." New Eng. J. Med., 262: 832-833 (1960)

Danieli, B., Bombardelli, E., Bonati, A. and Gabetta, B. : "Carboxy-atractyloside, a new glycoside from Atractylis gummifera L." Fitoterapia., 42: 91-93 (1971)

Danieli, B., Bombardelli, E., Bonati, A. and Gabetta, B. : "Structure of the diterpenoid carboxyatractyloside." Phytochemistry 11: 3501-3504 (1972)

Davidson, C.S. : "Hepatotoxicity of foods : a consideration of the hepatotoxicity of a few phanerogams and cryptogams; their possible influence in the pathogenesis of cirrhosis and hepatoma." Ann. N.Y. Acad. Sci., 104: 1026-1033 (1963)

Dioscorides, P.A. : De Materia Medica libri quinque III, 93. Max Wellmann ed., Berolini (1906)

Dubash, J. and Teare, D. : "Poisoning by amanita phalloides." Brit. Med. J., 1: 45-47 (1946)

Dyer, R.A. : *The Genera of South African Flowering plants.*" Vol. 1: P.694. (1975). Published by Department of Agricultural Technical Services : Botanical Research Institute, Republic of South Africa.

Ellenberg, M. and Osserman, K.E. : "The Role of Shock in the Production of Central Liver Cell Necrosis." Am. J. Med. XI: 170-178 (1951

Fenech, F.F., Bannister, W.H. and Grech, J.L. : "Hepatitis with biliverdinaemia in association with indomethacin therapy." Brit. Med. J., 3: 155-156 (1967)

Flinn, F.B. and Jarvik, N.E. : "Liver lesions caused by chlorinated naphthalene." Am. J. Hyg. 27: 19-27 (1938)

Frascino, J.A., Vanamee, P. and Rosen, P.P. : "Renal oxalosis and azotemia after methoxyflurane anaesthesia." N. Engl. J. Med., 283: 676 (1970)

Geiling, E.M.K. and Cannon, P.R. : "Pathologic effects of elixir of sulfanilamide (diethylene glycol) poisoning." J.A.M.A., 111: 919-926 (1938)

Gibaldi, M. and Perrier, D. *Pharmacokinetics*.Vol. 1, P.232 (1975) Durgs and the Pharmaceutical Sciences. Published by Marcel Dekker Incorporated. New York.

Githens, T.S. Univ. Pa. Afr. Hdbk. 8: (1949)

Guild, W.R., Young, J.V. and Merril, J.P. : "Anuria due to carbon tetrachloride intoxication." Ann. Intern. Med., 48: 1221-1227 (1958)

Harvey, A. McGehee and Bordley, J. III. *Differential Diagnosis*. 2nd ed. P.981 (1970) Published by W.B. Saunders Company. Philadelphia. London. Toronto.

Heldt, H.W., Jacobs, H. and Klingenberg, M. : "Endogenous ADP of mitochondria, an early phosphate acceptor of oxidative phosphorylation as disclosed by kinetic studies with (^{14}C) labelled ADP and ATP and with atractyloside." Biochem. Biophys. Res. Commun., 18: 174-179 (1965)

Hernandez, F., Agulleiro, B., Lujan, E. and Delgado, A. : "Reye Syndrome : Virus or artifact in liver?" Am. J. Dis. Child. 130: 1035-1036 (1976)

Hill, K.R. : "The vomiting sickness of Jamaica." A review. W.I. Med. J., 1: 243-264 (1952)

Hilliard, O.M. : "Composital in Natal." New Library Book. P. 287-288 (1977). University of Natal Press, Pietermaritzburg

Hoffman, W.S. : J. Biol. Chem., 120: 51 (1937)

Hollenberg, N.K., McDonald, F.D., Cotran, R., Galvanek, E.G., Warhol, M., Vandam, L.D. and Merrill, J.P. : "Irreversible acute oliguric renal failure : A complication of methoxyflurane anaesthesia." N. Engl. J. Med., 286: 877 (1972)

Hook, J.B. : "Fluoride and methoxyflurane nephropathy." Anaesthesiology., 35: 238-240 (1971)

Huttenlocher, P.R., Schwartz, A.D. and Klatskin, G. : "Reye's Syndrome : Ammonia intoxication as a possible factor in the encephalopathy." Pediatrics., 43: 443-454 (1969)

Joshi, P.H. and Conn, H.O. : "The syndrome of methoxyflurane-associated hepatitis." Ann. Int. Med., 80: 395-401 (1974)

Kelsey, W.M. and Scharyj, M. : "Fatal hepatitis probably due to indomethacin." J.A.M.A., 199: 586-587 (1967)

Kemp, A. and Slater, E.C. : "The site of action of atractyloside." Biochim. Biophys. Acta., 92: 178-180 (1964)

Kempcziski, R.F. and Caulfield, J.B. : "A light and electron microscopic study of renal tubular regeneration." Nephron. 5: 249-264 (1968)

Klatskin, G. : "Toxic and drug induced hepatitis." *Diseases of the Liver.* 4th ed. P.604-710 (1975) Edited by Leon Schiff. Published by J.B. Lippincott Company. Philadelphia. Toronto.

Krige, E.J. : *"The Social System of the Zulus."* 6th impression. P.297-329 (1974). Published by Shuter and Shooter, Pietermaritzburg

Kupiecki, F.P., Ogzewalla, C.D. and Schell, F.M. : "Isolation and Characterisation of a Hypoglycemic Agent from *Xanthium Strumarium.*" Journal of Pharmaceutical Sciences., 63: No. 7 (1974)

Lefranc, M. : "Sur l'acide atractylique et les atractylates, produits immediats extraits de la racine de l'Atractylis gummifera L." Compt. Rend., 67: 954-956 (1868)

Letty, C. : *"Wild Flowers of the Transvaal"* P.348 (1962). Division of Botany. Department of Agricultural Technical Services. Published by the Trustees : Wild Flowers of the Transvaal Book Fund.

Luciani, S., Martini, N. and Santi, R. : "Effects of carboxyatractyloside, a structural analogue of atractyloside on mitochondrial oxidative phosphorylation." Life Sci., 10: Part II, 961-968 (1971)

Luciani, S. and Varotto, R. : "Factors affecting the binding of carboxyatractyloside to the mitochondrial membrane." Abs. 9th FEBS Meeting, Budapest (1974)

Luciani, S., Carpenedo, F. and Tarjan, E.M. : "Effects of atractyloside and carboxyatractyloside in the whole animal." P.109-122 (1978) *Atractyloside. Chemistry, biochemistry and toxicology.* Edited by R. Santi and S. Luciani. Published by Piccin Medical Books, Italy

Maberley, J. : Rep. Jt. Meeting Brit. Ass. Adv. Sci. and S. Afr. Ass. Adv. Sci., 3: 327 (1905)

Madden, J.W., Gertman, P.M. and Peocock, E.E. Jr. : "Dimethylnitrosamine-induced hepatic cirrhosis : A new canine model of an ancient human disease" Surgery, 68: 260-267 (1970)

Magee, P.N. : "Toxic Liver Injury. The metabolism of dimethylnitrosamine." Biochem J., 64: 676-682 (1956)

Magee, P.N. and Barnes, J.M. : "Induction of kidney tumours in the rat with dimethylnitrosamine (N-Nitrosodimethylamine). J. Path. Bact., 84: 19-31 (1962)

Malan, E. Department of Chemistry, University of Durban Westville. Personal communication, 3rd June 1980

McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R.M. Jr. and Tompsett, R. : "Pyrazinamide-isoniazid in tuberculosis" Am. Rev. Tuberc., 69: 319-333 (1954)

Mendoza, M., Alvira, M.M. and Smith, R.D. : "Viral crystalline arrays in Reye's syndrome." A.J.C.P., 65: 262-263 (1976)

Mokhobo, K.P. : "Herb Use and Necrodegenerative Hepatitis." S. Afr. med. J., 50: 1096-1099 (1976)

Moon, H.D. : "The pathology of fatal carbon tetrachloride poisoning with special reference to the histogenesis of the hepatic and renal lesions." Am. J. Path. 26: 1041-1055 (1950)

Morgenstern, L., Sacks, H.J. and Warmer, M.J. : "Post operative jaundice associated with halothane anesthesia." Surg. Gynec. Obstet., 121: 728-732 (1965)

Moult, P. and Sherlock, S. : "Halothane-related hepatitis." Quarterly Journal of Medicine, 44: 99-114 (1975)

Neame, P.D. and Pillay, V.K.G. : "Spontaneous hypoglycaemia, hepatic and renal necrosis following the intake of herbal medicines." S. Afr. med. J., 38: 729-732 (1964)

Peters, R.L., Edmondson, H.A., Reynolds, T.B., Meister, J.C. and Curphey, T.J. : "Hepatic necrosis associated with halothane anesthesia." Am. J. Med., 47: 748-764 (1969)

Piozzi, F., Quilico, A., Fuganti, C., Ajello, T. and Sprio, V. : "Struttura dell'attrattiloside." Gazz. Chim. Ital., 97: 935-954 (1967)

Popper, H. : "Pathological findings in jaundice associated with iproniazid therapy." J.A.M.A., 168: No. 17, 2235-2242 (1958)

Portmann, B., Talbot, I.C., Day, D.W., Davidson, A.R., Murray-Lyon, I.M. and William, R. : "Histopathological changes in the liver following a paracetamol overdose : correlation with clinical and biochemical parameters." J. Path., 117: 169-181 (1975)

Powell, S.J. : "The serum protein pattern and liver function tests in the Natal African." S.A. Journal of Laboratory and Clinical Medicine., 4: 273-282 (1958)

Powell, W.J. Jr., Koch-Weser, J. and Williams, R.A. : "Lethal Hepatic Necrosis After Therapy With Imipramine and Desipramine." J.A.M.A., 206: 642-645 (1968)

Reye, R.D.K., Morgan, G. and Baral, J. : "Encephalopathy and fatty degeneration of the viscera. A disease entity in childhood." Lancet, 2: 749-752 (1963)

Rhodin, A.E. and Crowson, C.N. : "Mercury nephrotoxicity in the rat. I. Factors influencing the localisation of the tubular lesions." Am. J. Path., 41: 297 (1962)

Safran, A.P. and Schaffner, F. : "Chronic passive congestion of the liver in man. Electron Microscopy Study of Cell Atrophy and Intralobular Fibrosis." Am. J. Path., 50: 447-452 (1967)

Santi, R. and Cascio, G. : "Ricerche farmacologiche sul principio attivo dell'Atractylis gummifera. I. Azione generale." Arch. ital. Sci. farmacol., 5: 354-363 (1955a)

Santi, R. and Cascio, G. : "Ricerche farmacologiche sul principio attivo dell'Atractylis gummifera. II. Azione sulla glicemia e sulle riserve di glicogeno." Arch. ital. Sci. farmacol., 5: 364-372 (1955b).

Santi, R., Bistocchi, M. and Bruni, A. : "Inhibition of citric acid cycle by a vegetal glucoside." Proceed. IV Int. Congress of Biochemistry. Wien. P.68. (1958) Pergamon, London

Santi, R., Luciani, S. and Santi-Soncin, E. : "Atractyloside." Fitoterapia., 4: 118 (1968)

Schreiner, G.E. and Maher, J.F. : "Toxic Nephropathy" Am. J. Med., 38: 409-439 (1965)

Seedat, Y.K. and Hitchcock, P.J. : "Acute Renal Failure from *Callilepis Laureola*." S. Afr. Med. J., 45: 832-833 (1971)

Seedat, Y.K. and Reddy, J. : "The clinical pattern of hypertension in the South African Black population : a study of 1000 patients." Afr. J. Med. med. Sci., 5: 4 (1976)

Selzer, G. and Parker, R.G.F. : "Senecio Poisoning Exhibiting as Chiari's Syndrome." Am. J. Path., 27: 885-900 (1951)

Smetana, H. : "Nephrosis Due to Carbon Tetrachloride." Arch. Intern. Med., 63: 760-776 (1939)

Spilsbury, B.H. : "Toxic Jaundice in Munition Workers and Troops." Brit. Med. J., 1: 156 (1917)

Stewart, M.J. : "Atrophy of the liver." Brit. Med. J., II: 584-585 (1920)

Stuart, K.L. and Bras, G. : "Veno-occlusive disease of the liver." Quart. J. Med., 26: 291-315 (1957)

Tanaka, K. and Isselbacher, K.J. and Shih, V. : "Isovaleric and α-Methylbutyric Acidemias Induced by Hypoglycin A : Mechanism of Jamaican Vomiting Sickness." Science, 175: 69-71 (1972)

Tang, T.T., Siegesmund, K.A., Sedmak, G.V., Francois, B. and McCreadie, S.R. : "Reye's syndrome : A correlated biochemical, virologic and electron microscopic observation." A.J.C.P., 65: 262 (1976)

Taylor, N.S. : "Histochemical studies of nephrotoxicity with sublethal doses of mercury in rats." Am. J. Path., 46: 1 (1965)

The Sunday Tribune : "Deadly Muti Kills 263 Blacks." February 11, 1979

The Sunday Tribune : "Burn the killer impila NOW!" February 25, 1979

Theophrastos, E. : *De Historia plantarum*, Cap. XIII, libro nono, 1552, Lugduni, Amstelodami. (1644)

Vander Veer, J.B. and Farley, D.L. : "Mushroom Poisoning (Mycetismus)" Report of four cases. Arch. Intern. Med., 55 : 773-791 (1935)

Vignais, P.V. and Vignais, P.M. : "Effect of ADP on the inhibition of oxidative phosphorylation by potassium atractylate." Biochem. Biophys. Res. Commun., 14: 559-564 (1964)

Vignais, P.V. and Vignais, P.M. : "Atractyloside binding to mitochondrial membranes." Biochem. Biophys. Res. Commun., 37: No. 1: 72-79 (1969)

Vignais, P.V., Vignais, P.M. and Defaye, G. : "Gummiferin, an inhibitor of adenine-nucleotide translocation." FEBS-Lett., 17: 281-288 (1971)

Vignais, P.V., Vignais, P.M. and Defaye, G. : "Adenosine diphosphate translocation in mitochondria." Biochemistry, 12: 1508-1519 (1973)

Wainwright, J. and Schonland, M.M. : "Toxic Hepatitis in Black Patients in Natal." S. Afr. Med. J., 51: 571-573 (1977)

Wainwright, J., Schonland, M.M. and Candy, H.A. : "Toxicity of *Callilepis laureola*." S. Afr. Med. J., 52: 313-315 (1977)

Watson, A.R., Coovadia, H.M. and Bhoola, K.D. : "The Clinical Syndrome of *Impila (Callilepis laureola)* Poisoning in Children." S. Afr. Med. J., 55: 290-292 (1979)

Watt, J.M. and Breyer-Brandwijk, M.G. *Medicinal and Poisonous Plants of Southern and Eastern Africa.* 2nd ed., P.208 (1962) Published by E & S Livingstone, Edinburgh

Willmot, F.C. and Robertson, G.W. : "Senecio Disease, or cirrhosis of the liver due to senecio poisoning." Lancet., 2: 848-849 (1920)

Wood, J.M. : *Natal Plants* (1898-1912) Bennett and Davies Printers, Durban

Wróblewski, F. : "Distribution of LDH Isoenzymes in Human Organs." Progr. Cardiovasc. Dis., 6: 63 (1963)

Zelman, S. : "Liver Cell Necrosis in Chlorpromazine Jaundice (Allergic Cholangiolitis). Am. J. Med., 27: 708-729 (1959)

Zimmerman, H.J. and Ishak, K.G. : "Hepatic injury due to drugs and toxins." *Pathology of the Liver.* P.342 (1979). Ed. MacSween, R.N.M., Anthony, P.P. and Scheuer, P.J. Published by Churchill Livingstone, Edinburgh, London, New York