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**SOME FACTORS AFFECTING THE NEUROTOXIC EFFECT OF
PALMYRAH FLOUR**

BY

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ABSTRACT

Since 1971, many toxicities have been reported in animal studies particularly using Wistar rats fed on unboiled palmyrah flour (odiyal). The studies mainly contained reports on neurotoxicity and hepatotoxicity. In an attempt to duplicate pioneer studies done in 1971 with Wistar rats, showed that the animals did not consume 100% flour. Therefore the studies were commenced using a mixture of WHO recommended rat feed and palmyrah flour (50:50 and 30:70). Neurotoxic symptoms were visible within five days. This was accompanied by an elevation of AST ($p= 0.040$) but not an elevation in ALT ($p= 0.396$). Studies showed that toxicity varied from site of sampling (Kalpitiya > Mannar > Jaffna). Further, Wistar rats (effect shown in 5 days) seemed to be affected before than ICR mice (symptoms in 8 days). The symptoms were the same as those observed in the past studies, such as ruffled coat, muscle incoordination, characteristic fits, coordinated spasms, falling over backwards, immobility of hind limbs, and finally death. In addition to this, it was observed in this study that the animals were subject to hyper-excitation to touch and pharaphymosis.

The next part of the study was to determine how palmyrah flour could be detoxified. The flour from boiled Kottaikilengu (Plukodiyal) or other wet heat processes (steaming) did not

eliminate the toxicity. Although the toxin is very soluble in water, washing of odiyal did not eliminate toxicity, but rather reduced it. These studies were conducted using traditional palmyrah food processing methods. However dry heat at 80°C for 45 min removed the toxicity (both hepatotoxicity/ neurotoxicity). Hepatotoxicity was judged by histopathology on liver by Haematoxylin & Eosin staining and Oil Red O' staining. There was periportal fatty degeneration and hepatocellular hydropic degeneration at 40°C heat treatment on light microscopy but not in the 80°C treated sample.

The water extract of palmyrah flour produced neurotoxic symptoms, hepatotoxic symptoms (histopathology) and elevation of AST value to 104.3 ± 23.1 ($p=0.0261$). However methanol extract did not extract the toxin. The water: methanol extract showed only sub-clinical symptoms and AST value 95.5 ± 22.9 , ($p=0.044$)

The next line of the study was to purify the toxin. Fold purification by Medium Pressure Liquid Chromatography was 375. The activity appeared only in the water fraction. Although the dose was theoretically high, the unabsorbed eluate obtained from cation exchange resin showed neurotoxic effects in very low intensities. This indicated that either some of the toxin is lost during separation or both fractions (unabsorbed and absorbed) should be present giving a synergistic effect. Thin layer chromatography of the toxic fraction showed spots at $R_f=0.15$ in addition to the normal sugars of palmyrah. The spot at the $R_f 0.15$ was absent in the non-toxic fraction. It appears possible that this may be one of the compounds that contribute to toxicity.

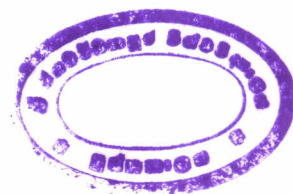
The histopathology of rats provided evidence for toxicity to the liver and this would explain the hepatotoxic effect. Other workers had shown that the mitochondria are

affected. This will explain the elevation of AST levels but not ALT levels. No lesions in the brain or spinal cord were observed when viewed macroscopically. It is hypothesized that the toxin is a mitochondrial toxin that gives both the hepatotoxic effect and the neurotoxic effects as both the muscle and the nerve have the highest number of mitochondria that would be one explanation for the neurotoxic effect. However, this could not be confirmed due to the non-availability of electron microscopy to show mitochondrial damage in muscle and nerve tissue. Another interpretation is that the neurotoxic effect is an extreme manifestation of the hepatotoxic effect. Whatever the case it seems that the neurotoxin and hepatotoxin are whole or part of the same molecule. This explanation requires that the neurotoxic effect is a result of the hepatotoxic effect; brought about as a result of mitochondrial damage to liver mitochondria, which could affect many pathways including the urea cycle.

TABLE OF CONTENTS

	Page No
I. LIST OF TABLES	viii
II. LIST OF FIGURES	ix
II. LIST OF PLATES	x
IV. ABBREVIATIONS	xii
V. ACKNOWLEDGMENTS	xiv
VI. ABSTRACT	xvi
1. INTRODUCTION	
1.1 General introduction	1
1.2 Justification and scope of study	5
2. LITERATURE REVIEW	
2.1 General description of the palmyrah palm	6
2.2 Distribution of palmyrah palm	6
2.3 Production of palmyrah palm	9
2.4 Source of palmyrah flour	9
2.5 Odiyal flour	10
2.5.1 Composition	10
2.5.2 The microelement composition of odiyal flour	10
2.5.3 The amino acid composition of odiyal flour	10

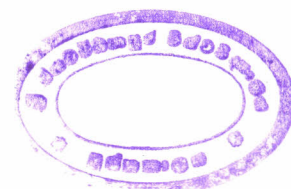
2.5.4	The metal ion content	14
2.6	The products of palmyrah palm	15
2.6.1	Palmyrah juice (Sap)	15
2.6.2	Toddy and sweet toddy	15
2.6.3	Timber	16
2.6.4	Leaf	16
2.6.5	Fruit	16
2.6.6	Root	17
2.6.7	Folk medicine	17
2.6.8	The bitterness of palmyrah fruit	18
2.6.9	Flabelliferins	18
2.6.10	Steroids	19
2.6.11	Dammaranes	19
2.6.12	Others	20
2.7	Reported toxicities	20
2.7.1	The neurotoxic effect	20
2.7.2	The hepatotoxic effect	23
2.7.3	The immunosuppressive effect	25
2.7.4	Mutagenic effect	27
2.7.5	Clastrogenic effect	27



2.7.6	Anti-microbial activity effect	28
2.7.7	Other bioactivities caused by palmyrah flour	29
3.	MATERIALS AND METHODS	
3.1	Materials	31
3.1.1	Water	31
3.1.2	Solvents	31
3.1.3	Special chemicals	31
3.1.4	Enzymes	31
3.1.5	Animals	32
3.1.6	Palmyrah flour/ Odiyal/ Plukodiyal	32
3.1.7	Animal feed	33
3.2	Methods	35
3.2.1	Determination of the components of palmyrah flour	35
3.2.1(1)	Determination of moisture content	35
3.2.1(ii)	Determination of digestible carbohydrate	36
3.2.1(iii)	Determination of the fibre content	38
3.2.1(iv)	Determination of protein content	41
3.2.1(v)	Determination of fat content	42
3.2.2	Animal model	43
3.2.3	Separation of neurotoxic principal	46

3.2.3(i) Crude extractions	46
3.2.3(ii) Preparation of extractives for oral administration	47
3.2.3(iii) Medium pressure liquid chromatography (MPLC)	47
3.2.3(iv) Preabsorbing the sample	47
3.2.3(v) Column packing	48
3.2.3(vi) Solvent gradients	50
3.2.3(vii) Preparation of MPLC fractions for oral administration	50
3.2.4 Further separation of neurotoxic fraction by Ion-exchange resin	52
3.2.4(i) Resin desalting	52
3.2.4(ii) Purification of resin	52
3.2.4(iii) Sample preparation	52
3.2.4(iv) Fractionation	53
3.2.4(v) Preparation of ion-exchange resin fraction for oral administration	53
3.2.5 Variation in palmyrah content	53
3.2.5(i) Preparation of 100% palmyrah feed pellet	53
3.2.5(ii) Preparation of 70% palmyrah feed pellet	53
3.2.5(iii) Preparation of 50% palmyrah feed pellet	54
3.2.6 Variation of Source of palmyrah flour	54
3.2.7 Imitation of traditional methods of cooking	54

of palmyrah recipes	
3.2.7(i) Preparation of "Pittu" from washed palmyrah flour	54
3.2.7(ii) Preparation of "Pittu" from non washed palmyrah flour	54
3.2.7(iii) Preparation of "Palm Posha" feed pellet	55
3.2.8 Variation of heat treatment	55
3.2.9 Studies on species variation	55
3.2.10 Analysis of parameters	55
3.2.10(i) Collection of blood and separation of serum	55
3.2.10(ii) Enzyme assays	55
3.2.11 Statistical analysis	57
3.2.12 Examination of organs	57
3.2.13 Histopathological examination	57
3.2.13 (i) Collection and fixation of tissues	57
3.2.13(ii) Processing, embedding and cutting of tissues	58
3.2.13(iii) Haematoxylin and Eosin (H & E) staining	58
3.2.13(iv) Oil Red O' method	59
3.2.14 Monitoring of fractions of MPLC	60
3.2.14(i) Thin layer chromatography (Tlc)	60
3.2.14(ii) Preparation of Tlc plates	60
3.2.14(iii) Solvent system	61



3.2.14(iv) Spray reagents	61
3.2.14(v) Treatment after spray	61
3.2.14(vi) Scheme for spraying	62
4. RESULTS	
4.1 Proximate composition of palmyrah flour	63
4.2 Preliminary studies	63
4.2.1 Effect of 100%, 70% and 50% palmyrah feed pellets on Wistar rats	63
4.2.2 Effect of 50% palmyrah flour on Wistar rats	63
4.2.3 Effect of 50% palmyrah flour on Alanine aminotransferase and Aspartate aminotransferase	65
4.2.4 Toxicity and locality of collection of palmyrah shoots	66
4.2.5 Effect of extractives	66
4.2.6 Chemical studies on extractives	72
4.3 Species effect	72
4.4 Treatments of palmyrah flour	72
4.4.1 Effect of processed palmyrah flour in pittu	72
4.4.2 Effect of boiled palmyrah flour (plukodiyal) in palm posha	73
4.4.3 Effect of dry heat treatment	74

4.5	Hitopathological examination of effect of heat treatment	78
4.6	Toxic effects of water extracts in the rat	79
4.7	Bioactivity directed separation of toxic fraction	79
4.7.1	Separation of toxic fraction by MPLC method	79
4.7.2	Histopathological examination	80
4.7.3	Further separation of toxic fraction	103
4.7.4	Tlc studies on fractions	104
5.	DISSCUSSION	106
6.	REFERENCE	118