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SOME FACTORS AFFECTING THE NEUROTOXIC EFFECT OF PALMYRAH FLOUR

By

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M. Phil. 2002

DECLARATION BY THE CANDIDATE

"The work described in this thesis was carried out by me under the supervision of Professor E.R. Jansz (Head of the Department, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Professor S.M.D.N. Wickramasinghe (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any University for another Degree/Diploma"

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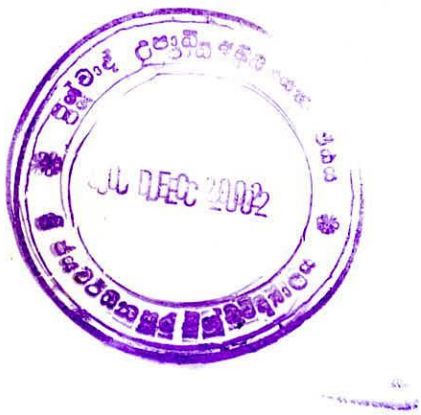
"We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation."



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KURUWITA ARACHCHIGE VINDIKA SUMUDUNIE

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ABBREVIATIONS

PFP	Palmyrah Fruit Pulp
MPLC	Medium Pressure Liquid Chromatography
Tlc	Thin Layer Chromatography
AOAC	Association of Official Analytical Chemists
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
WHO	World Health Organization
PMA	Phospho Molybdic Acid
DRG	Dragendrof Reagent
PDB	Palmyrah Development Board
H&E	Haematoxylin and Eosin
ΔA	Absorbance difference
F-I	Flabelliferin tertraglucoside in Kalpitiya
F-II	Bitter flabelliferin tetraglycoside
F_B	Anti- microbial flabelliferin triglycoside
F_C	Inactive flabelliferin triglycoside
F_D	Flabelliferin diglycoside
F_E	Flabelliferin diglycoside
F_F	Flabelliferin diglycoside
R_f	Retardation factor
Kg	Kilogram

UV	Ultra Violet
ml	Millilitre
^{o}C	Centigrade
MW	Molecular Weight
l	Litres
ICR	Institute of Cancer Research
DNS	3 | 5 | Dinitro Salicylic Acid
g	Grams
IU ml^{-1}	International Units per millilitre
min	Minutes
h	Hour

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SOME FACTORS AFFECTING THE NEUROTOXIC EFFECT OF PALMYRAH FLOUR

KURUWITA ARACHCHIGE VINDIKA SUMUDUNIE

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.