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Neurotoxic effect traditional cooking of wistar rats fed with recipies of palmyrah flour

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Abstract

Palmyrah *(Borassus flabellifer L.)* shoot has been proved to give a neurotoxic effect. This study using male Wistar rats shows that the time for advent of neurotoxic symptoms is affected by the location from which the seed shoot (which gives rise to this flour) is collected. Studies show that the advent of neurotoxic symptoms can be delayed but not eliminated by washing and steaming the flour and using boiled shoots to prepare traditional foods.

The neurotoxic effect was always accompanied by a significant elevation of serum aspartate aminotransferase (AST) but not alanine aminotransferase (ALT). Showing that there is no evidence, even sub-clinically, for the hepatotoxic effect previously reported.

The findings are alarming as these traditional foods are widely consumed in North-east Sri Lanka. The only mitigating factor is the possibility of the neurotoxic effect being species dependent.

Keywords: Palmyrah flour, Neurotoxicity, Biodiversity, Reduction of toxicity.

1. Introduction

Kottaikilengu, is the leaf scale of the shoot from a germinating palmyrah seed (Thirulinganathan, 1992). It appears to be a good source of carbohydrate and fiber but is limited in both fat and protein (Mason and Henry, 1994). The shoot, which is hard, dry and tuberous is consumed by humans. The flour obtained from boiled and unboiled dry palmyrah shoot is called "Odiyal" and "Plukodiyal" respectively. It is used to prepare many recipes for human consumption.

Neurotoxic and hepatotoxic effects of palmyrah flour on Wistar rats have been reported previously (Arseculeratne *et al.,* 1971,1982). The neurotoxic effect was confirmed in a study (Sumudunie,unpublished results), which also showed that dry heating (in an oven) of palmyrah (odiyal) flour at 80°C for 45 mm destroyed the neurotoxic effect.

The objectives of this study were to determine whether the neurotoxic effect of palmyrah flour is reduced or eliminated by traditional methods of cooking, and to determine whether there is diversity among palmyrah flour samples with respect to neurotoxins with respect to the locality in which the trees had been growing.

2 Materials and Methods

Raw Material

Flour from boiled and un-boiled palmyrah shoots were obtained from the Palmyrah Development Board. The flour was stored in sealed polythene bags at room temperature. The flour had been prepared specially from shoots collected separately from Kalpitiya in the North West of Sri Lanka and, Mannar and Jaffna in the North of Sri Lanka. The flour was used to prepare feed pellets comprising (50:50) mixture with WHO recommended rat and mice feed (Sabourdy, 1988). For all experiments unless otherwise specified one homogenous batch of flour from Kalpitiya was used to prepare pellets from the pittu feed and palm posha feed and other palmyrah flour controls.

Feed pellets

Preparation of 50% palmyrah feed

Pellets of dimension 2.0cm x 1.5cm were prepared manually using a mixture of WHO recommended rat and mice breeding feed (Sabourdy, 1988) and palmyrah flour *(50:50)*. These pellets were air-dried at 40°C.

(b) Preparation of "pittu" palmyrah pellet feed

Washed odiyal flour (un-boiled) was prepared by soaking in water for 2-3h and washing well and "pittu" mixture (un-boiled palmyrah 500g, prawns 250g, scraped coconut 200g, drumstick leaves 48g, and salt 2g) was prepared by steaming for 15-20 min according to the traditional recipe. Washed and non-washed flour was used separately in two test experiments. Pellets were dried at 40°C.

(c) Preparation of "palm posha" pellet feed

Boiled odiyal flour (715 g) was mixed with scraped coconut (l41.5 g) and sugar (141.5 g) according to traditional recipes. Hot water was added to prepare palm posha and the mixture was dried and pellets made as above.

Animal model

All feeding trials were conducted using out bred Wistar rats originally from the Clea, animal-breeding company, Tokyo, Japan. The colony has been bred and maintained at the animal center of Medical Research Institute, Colombo, Sri Lanka, for over 10 years. The rats had been fed on WHO recommended breeding feed formula. Weanling male Wistar rats aged 4 weeks were separated into three groups (one control and two test groups) with 6 rats in each group (depending on the experiment). The animals (80-100 g) were caged separately and the groups were selected so that the average weights in each group were similar. The test feed pellets was palm posha and pittu. 50% palmyrah pellets (the positive control) was used for the test group while the control was fed on WHO-recommended breeding feed with water *ad libitum*. The caging (one rat per cage) details are according to protocol reported previously (Ariyasena *et al.,* 2000).

Collection of blood and separation of serum

Animals were anesthetized using diethyl ether and blood (1.5 ml) collected by cardiac puncture. Clear non-haemolyzed serum was separated by centrifugation at 3000 rpm for 10 minutes using a micro-centrifuge (Iwaki CFM- 100, Japan).

Enzyme assays

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed (Bergmeyer *et al.,* 1986) separately using reagent kits purchased from DMA (USA). Samples (2.0ml) were added into test and control tubes, and mixed by using a vortex mixer. Sample mixtures were incubated at 30^0C for 30 seconds. Each sample was then introduced into silica cuvettes and readings were taken at 340 nm using a reagent blank using a spectrophotometer (Shimadzu UV-120 1V, Japan).

Statistical Analysis

This was conducted using T-Test from Microsoft Excel packages.

3. Results and Discussion

Toxicity and locality of collection of palmyrah shoot.

When rats were fed on *50%* palmyrah feed, the samples from Jaffna showed symptoms within 12-13 days whereas those fed on Mannar and Kalpitiya flour showed symptoms within 7-8 days and *5-6* days respectively (Table 1). It is concluded that Jaffna sample showed least toxicity and Kalpitiya showed highest toxicity. It was therefore decided to conduct all subsequent trials using one master batch from Kalpitiya for consistency for time of the appearance of symptoms in the control. The symptoms were the same as that observed by Arseculertne *et al.,* 1971, i.e. ruffled coat, muscle in-coordination, characteristic fits, coordinated spasms, falling over backwards, immobility of hind limbs, and finally death. In addition, in this study it was observed that the animals were subject to hyper-excitation to touch, retraction of testicles and pharaphymosis.

Effect of processed palmyrah flour pittu in pellets

The experiment was done using two different pittu pellets from un-boiled palmyrah samples originally obtained from Kalpitiya. This experiment had a positive control (50% palmyrah feed) and a negative control (WHO-recommended rat feed). Both non-washed and washed pittu feed resulted in low feed intake and weight loss and appearance of a neurotoxic effect (Table 2.). When compared with the animals, which were fed on palmyrah flour, which was washed, the time of advent and intensity of the occurrence of symptoms were greater in the case of unwashed flour showing that some of the toxin can leached by water. Using unwashed flour, on the sixth day the rats began to give the symptoms and on the twelfth day one animal died. Symptoms appeared on the12th day for washed flour and no deaths were recorded on that day.

That is the animals were fed on pittu from washed flour also showed neurotoxic symptoms but not so severely as non-washed group. This feed also resulted in low feed intake, weight loss and to give the same symptoms. This indicates that washing and steaming unit operations lower but do not eliminate the yet unknown neurotoxic agent.

Effect of boiled palmyrah flour (plukodiyal) in palm posha

The animals fed on palm posha (which is made of boiled palmyrah flour i.e. plukodiyal.) showed symptoms after 10 days of feeding. It also resulted in low feed intake and weight loss (Table 3). The symptoms appeared on the eighth day in the case of the 50% boiled palmyrah feed. Results show that

the neurotoxin is not eliminated boiling although its effects are delayed. Further increasing nutritional status of feed while preparing palm posha also delayed but did not eliminate the neurotoxic effect.

Enzyme Levels

The serum of rats that were fed on pittu pellets made from un-boiled palmyrah flour, showed no significant difference in levels of ALT but significant difference in AST levels ($p = 0.00061$, $p = 0.0053$) for washed and unwashed palmyrah flour respectively relative to the control. Although all the rats on the test group showed neurotoxic symptoms, the ALT values were similar in all the rats tested, that is for rats both showing and not showing neurotoxic symptoms (Table 2). As the blood samples were collected at a time, which was not constant, but when the animals gave severe symptoms, it was not possible to compare the AST values of the two test groups. The results show that, when the extent of neurotoxic symptoms is similar, the AST values are also similar ($p= 0.614$). The elevations of AST levels paralleling the neurotoxic symptoms could be an indication of neural on non-specific damage. There was no sub-clinical evidence of liver damage as earlier reported (Arseculeratne *et al.,* 1971) as evidenced by ALT values although mitochondrial damage is supported by AST value.

Rats were fed on palm posha and tested for AST. The AST values (tested on the same day) were significantly higher ($p = 0.0053$, $p = 1.46 \times 10^{-3}$) for palm posha and unboiled palmyrah flour respectively compared to the control feed.

The boiled flour (plukodiyal) is comparable to palmposha feed. Comparison of data shows that boiling of palmyrah delayed (compare with Table 1) but did not eliminate neurotoxic symptoms. Addition of nutrients required for palm posha preparation delayed the advent of symptoms further (Table 3).

Studies using ICR mice with the same flour and the same protocol as Wistar rats showed that the neurotoxic effect appeared in 8 days. This is somewhat slower than Wistar rats *(5* days). This might be an indication of a species effect on toxicity.

4. Conclusion

Preparation of traditional foods with palmyrah flour by well established processing techniques delay but do not eliminate neurotoxic symptoms and increased AST values in Wistar rats. Although there may be a species effect, deleterious effects of regular consumption of palmyrah flour product by humans cannot be ruled out.

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6. References

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Table 1. Effect of toxicity according to locality.

Location	Average feed consumption ($g.rat^{-1}.day^{-1}.$)			Average weight Loss or gain ($g.rat^{-1}.day^{-1}.$)	Time for the first symptoms (Days)
	(0-5) Days	(6-10) Days	(11-14) Days		
Kalpitiya	5.8	2.2	1.4	-1.7	5
Mannar	4.8	3.5	1.0	-1.6	7
Jaffna	5.9	3.4	2.5	-1.5	12
Control	8.9	10.5	12.1	+3.8	-

n=4

Control is standard rat and mice feed

TabIe2. Effect of washed and non-washed palmyrah flour in "pittu" on neurotoxicity

Feed	Average feed Consumption (g.rat-1. day-1.)			Average weight Loss or gain ($g.rat^{-1}.day^{-1}.$)	Time for the first symptoms (Days)	Deaths	ALT (IU.ml)	AST (lU.ml)
	(0.5) Days	(6-10) Days	(11-14) Days					
1. Pittu from washed flour	6.1	2.2	0.4	-0.8	12	-	26.81±7.77	107.69±2
2. Pittu from non-washed flour	3.5	1.6	-	-1.2	6	1 (Day 12)	26.51±8.92	117.73±3
3. Control	13.2	14.1	14.2	+4.2	-	-	30.05± 9.15	46.84±8.4

n=6

Control is standard WHO rat and mice feed

p values for AST = 0.00061(1&3)

p values for AST = 0.0053 (2&3)

p values for AST = 0.6 137 (1&2)

Differences in ALT are not significant

Table 3. Effect of using boiled shoot flour in palm posha on neurotoxicity

Feed	Average feed Consumption (g.rat-1. day-1.)			Average weight Loss or gain ($g.rat^{-1}.day^{-1}$.)	Time for the first symptoms (Days)	Deaths	ALT (IU.ml)
	(0.5) Days	(6-10) Days	(11-14) Days				
1. Palm posha	2.0	2.6	-	-2.2	10	-	68.94±13.16
2. 50% palmyrah (Boiled)	2.7	4.8	-	-1.8	8	1	72.13±4.74
3. Control	10.2	14.3	-	-4.5	-	-	42.42±3.75

n=6

± = Standard deviation

Control is standard WHO rat and mice feed

p values for AST = 0.6053 (l&2)

p values for AST = 0.0053 (1&3)

p values for AST = 0. 1.463×10^{-3} (2&3)