The Immunosuppressive Effect of Palmyrah *(Borassus flabellifer)* Flour Is Not Associated with Its Neurotoxic Fraction*

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SUMMARY **The humoral and cell-mediated immune competence of rats fed a 75%-palmyrah (*Borassus flabellifer* L) flour diet was examined. The humoral immune response was evaluated by determining haemagglutinating antibody titres and haemolytic plaque-forming cell counts in the spleen following immunisation with sheep red blood cells (SRBC). The cell-mediated immune response was evaluated by the uptake of tritiated thymidine by peripheral blood and splenic lymphocytes following stimulation by phytohaemagglutinin (PHA). A significant depression of the lymphoproliferative response (LPR) of peripheral blood lymphocytes but not splenic lymphocytes was observed after feeding of the flour diet for one week. The depressed LPR was not associated with the partially purified neurotoxin extract from the flour as the equivalent amount of the toxin given once via the oral route had no such adverse effect. Unlike LPR, the humoral immune response of animals fed a 75% flour diet for one week was not significantly depressed.**

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Flour obtained from the young shoot of the palmyrah palm *(Borassus flabellifer* L*)* which is consumed by humans in some Asian and African countries has been previously reported to have a wide range of toxic effects in rats, including hepatotoxic and neurotoxic effects and the development of malignant lymphomas.[1-3]

We reported earlier[4] that this flour had immunodepressive effects in rats which were maintained on a 25%-flour diet for seven weeks as indicated by a reduced anti-SRBC haemolytic plaque-forming cell count in the spleen and the *in vitro* lymphoproliferative response of peripheral blood lymphocytes to PHA stimulation. Although other plant products have been shown to be immunosuppressive, palmyrah flour is of interest to us as it is consumed extensively by some human population groups.

In attemping to characterise the factor(s) responsible for these toxic effects, the neurotoxic fraction has recently been partially purified.[3] In this paper we report that while high doses of flour (75%) are demonstrably immunosuppresive when fed to adult rats for approximately one week, this effect is not associated with the neurotoxic fraction of the flour.

MATERIALS AND METHODS

Palmyrah flour. The flour was prepared by grinding boiled and sun-dried young shoots which were in the form in which they are normally consumed by humans. The flour had no detectable contamination with aflatoxins, nitrosamines, pyrrolizidine alkaloids or commonly used agro-chemicals (e.g., chlorinated hydrocarbons, pyridine (paraquat) and organophosphorus compounds). Each 100 g of flour contained 340 Kcal, 24 mg Mg^{++}, 1.1 mg Zn^{++} and traces of Fe^{++} and Cu^{++} (analysed by the Institute of Nutrition, Mahidol University). The flour, stored at 4°C when not in use, was mixed with powdered rat diet (F.E. Zuellig, Bangkok, Thailand) in a ratio of 3:1 by weight (75% flour diet).

Neurotoxin. The neurotoxic fraction was extracted from the palmyrah flour by using Method B of Greig

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et al.[3] It was partially purified by repeated chromatography on Amberlite CG-120 resin (NH_4^+ form). The potency of this partially purified neurotoxic fraction, which represented 0.23% w/w of the original flour, was confirmed on 50-g rats after oral intubation of a single dose equivalent to 20 g of flour. The neurotoxin fraction had no demonstrable mitogenic activity nor cytotoxicity when tested *in vitro* using lymphocytes from normal rats (see below).

Animals. Randomly bred male albino rats weighing 150-200 g were obtained from the Animal Centre, Faculty of Science, Mahidol University. The rats were kept individually in stainless steel cages at approximately 28°C with light provided 12 hours/day. All animals were given regular rat pellets and tap water *ad libitum* for two weeks prior to experimentation.

Feeding of animals. Flour-fed rats were each given 20 g of the 75% flour-pellet mixture per day. A fresh stock of the flour mixture was added each day after the residual diet was weighed and discarded. The flour-fed rats consumed progressively less of the flour mixture each day and, on the average, the total intake was approximately 20 g of flour component per week. The pair-fed controls was given only the powdered pellet component in amounts equivalent to that consumed by the flour-fed group.

The partially purified neurotoxin solution equivalent to 20 g of flour was given by oral intubation on day 1 while the rats were fed on the powdered pellet diet in amounts equal to that fed to the control animals. The neurotoxin-fed rats were sacrificed and tested on the third post-treatment day.

Lymphoproliferative response (LPR) to phytohaemagglutinin stimulation. Control and flour-fed rats were sacrificed and tested when the latter showed typical signs of palmyrah intoxication. Transformation of peripheral blood and splenic lymphocytes following stimulation by phytohaemagglutinin (PHA-P, Difco Laboratories, Detroit, Michigan, USA) was performed in triplicate by a microassay method as described previously.[4] The quantity of ^{3}H-thymidine incorporated, determined in a liquid scintillation spectrometer, was used for the calculation of stimulation indices (SI) for each of the three mitogen concentrations used (33, 100 and 330 μg/ml). The results presented here correspond to the SI from 100 μg PHA-P/ml since this gave the maximum stimulation in both the control and test groups.

Anti-SRBC antibody response. Animals were immunised with sheep red blood cells (SRBC) and both the levels of haemagglutinating antibody and the number of plaque-forming (antibody-producing) cells were determined as previously described.[4,5] The animals were immunised within 2-3 days after the commencement of the flour diet and tested four days after immunisation.

Test for mitogenic activity of the neurotoxin. The procedure using lymphocytes from normal rats was similar to that described for LPR except that the partially purified neurotoxin (dissolved in RPMI 1640, sterilized by membrane filtration) replaced PHA-P in the test system. The neurotoxin was used at final concentrations of 250, 500 and 1000 μg/ml.

Test of cytotoxic activity of the neurotoxin. Cytotoxicity of the neurotoxin was tested by using the trypan blue dye exclusion method.[5] Peripheral blood lymphocytes from normal rats were cultured for 24 or 48 hours as for LPR in either the presence or absence of the neurotoxin solution, and the viability was determined by cell counts in a haemocytometer chamber.

RESULTS

Within a few days of feeding the animals with a diet containing a high

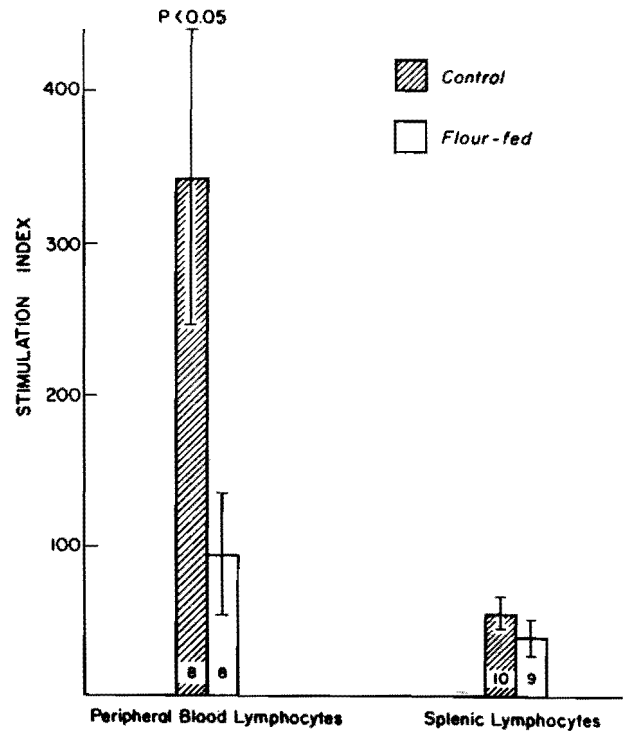

Fig. 1. Stimulation indices (SI) of peripheral blood and splenic lymphocytes from rats fed a 75% palmyrah flour diet for one week. Bars and lines represent mean ± SEM; the number at the base of each bar represents the number of specimens analysed.

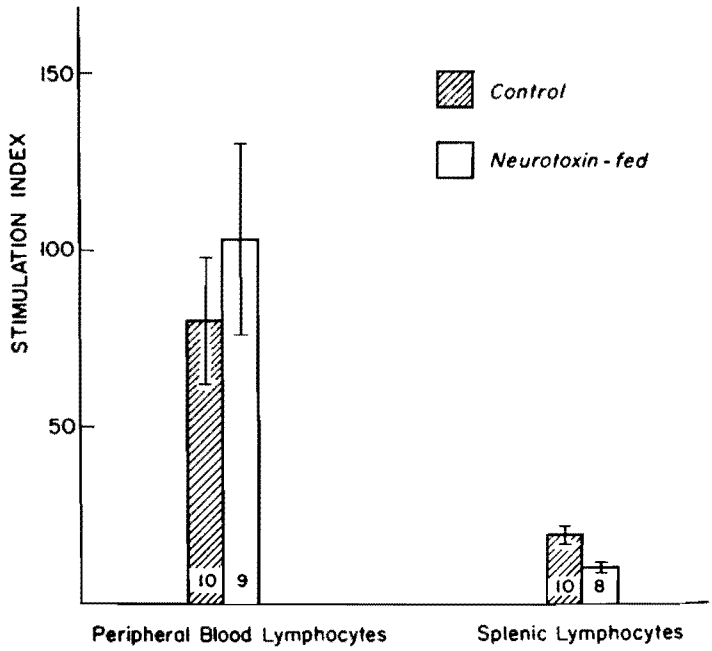

Fig. 2. Stimulation indices (SI) of peripheral blood and splenic lymphocytes from rats given partially purified neurotoxin extract equivalent to 20 g of flour via the oral route. See legend to figure 1 for other explanations.

dose of flour (75%), signs of palmyrah intoxication including piloerection, spasms, hyperexcitability and inertness readily appeared. On the day when these changes were obvious, the flour-fed rats together with controls were sacrificed and LPR for both groups was determined simultaneously. The results shown in Fig. 1 clearly demonstrate that the response of peripheral blood and not splenic lymphocytes from the flour-fed rats was significantly depressed compared with that of the pair-fed controls ($P < 0.05$ by Student's t-test). For each pair of flour-fed and control animals, the response of the flour-fed was always lower than that of the corresponding controls. The magnitude of the depression varied from as low as 8 per cent to as high as 86 per cent of the control values, with an average of 27 per cent for the whole group.

On the other hand, when the peripheral blood lymphocytes were otained from animals fed the neurotoxin by the oral route, no such depression was observed (Fig. 2).

Contrasting this with LPR, the humoral immune response of these flour-fed rats was not different from that of the pellet-fed controls. Data shown in Table 1 demonstrate that the flour had no adverse effects on either the levels of circulating antibody to SRBC or the number of antibody producing cells in the spleen of these animals.

DISCUSSION

In a recent paper,[4] we reported that feeding a low dose (25%) of palmyrah flour for seven weeks resulted in a significant depression of the lymphoproliferative response of peripheral blood lymphocytes of rats to PHA stimulation. The results presented in this paper not only confirm that palmyrah flour possesses immunodepressive activity but also show that the period of feeding could be shortened to 3–6 days if the flour content was increased to 75 per cent. However, the results shown in Fig. 2 indicate that the immunodepressive activity of the flour is not associated with the neurotoxin component of the flour. The failure of partially purified neurotoxin to depress the LPR of animals receiving the neurotoxin via the oral route is probably not due to insufficeint absorption through the gastrointestinal tract because even when the same preparation was given by a parenteral route no detectable depression was noted (unpublished observations). A similar preparation of neurotoxin was previously shown to have neurotoxicity on young rats.[3] An attempt is

Table 1 Humoral immune response of rats fed a 75% palmyrah flour diet for one week*

Immune parameter	Control	Palmyrah fed	P value
PFC/10^6 mononuclear cells	**599 ± 374 (15)	350 ± 236 (12)	$(0.1 > P > 0.05)$
Haemagglutinating antibody titre (log_{10})			
Total	1.5368 ± 0.7374 (16)	1.4898 ± 0.2538 (12)	$(P > 0.5)$
2-mercaptoethanol resistant	1.1022 ± 0.4868 (16)	1.0256 ± 2251 (12)	$(P > 0.5)$

*Rats were fed a 75%-palmyrah flour diet and, within 2-3 days when typical signs of intoxication appeared, all experimental animals together with appropriate controls were immunised with SRBC. Four days later the animals were bled for quantification of circulating anti-SRBC and spleens were removed for plaque-forming cell (PFC) counts. Figure in perenthesis represents the number of samples tested.

**Mean ± SEM.

now being made to isolate other toxic component(s) to be tested for immunodepressive activity.

Although the data currently available are not sufficient to explain the mechanism(s) for immunodepression caused by palmyrah flour, the clastogenic activity exhibited by aqueous extracts of the flour[6] seems to be a good candidate because many chemical clastogens are known to have immunosuppressive activity as well. The differential susceptibility of lymphocytes from the blood and spleen could be based upon differential activity of the flour on various lumphocyte subpopulations. Limited data currently available using passive cell transfer (unpublished observations) suggest that T suppressor cells may be involved. Alternatively, the effect of the flour on lymphocyte traffic should also be considered and should be investigated.

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