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Effect of Palmyrah *(Borassus flabellifer L.)* fruit pulp on weight gain of mice

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Abstract

Palmyrah *(Borassus flabellifer L.)* fruit pulp (PFP) contains a group of steroidal saponins called flabelliferins. One of these flabelliferins (a tetraglycoside) causes a bitter flavour in PFP, which detracts from the free use of PFP as a food. Techniques of debittering have been developed but these have suffered from the criticism that debittering could produce new saponins that may cause adverse nutritional effects. In this study, trials were conducted using outberd ICR mice. Experiments carried out with 6 weeks old (growing) mice with bitter PFP showed a statistically significant ($p=0.029$) lowering of weight gain compared to the control feed (standard rat/ mouse breeding feed) although food intake was nearly equal. Debittering of PFP with naringinase reversed the effect, in that there was no statistically significant difference between control and debittered PFP ($p=0.88$). Weight gain in the case of debittered PFP was higher than the bitter PFP ($p=0.023$). The study indicates that debittering does not produce adverse nutritional effects affecting weight gain but rather improves nutritional value of bitter PFP as judged by the weight gain. This may be due to, as revealed by a recent study, the bitter principle having an effect on active transport of nutrients.

KEY WORDS : Palmyrah Fruit Pulp, Flabelliferins, ICR Mice

1. Introduction

Very little work has been reported on the steroidal saponins of PFP from Sri Lanka. Studies by Jayaratnam (1986) showed that the fruit pulp, the annual production of which is about 15-20 k tons, contains a steroid, spirost-

5en-3β-ol (25R) and its monoglucoside and monorhamnoside. The main problem of the use of palmyrah fruit pulp as a food is that it contains a bitter principle. This bitter principle was identified by Jansz *et al.* (1994) as tetraglycoside of the steroid isolated by Jayaratnam. Jansz *et al.* (1994) also showed that the saponin can be debittered by the enzyme preparation naringinase. Debittering causes the disappearance of the natural flabelliferin spots seen on TLC (thin layer chromatography) plates and the formation of two new spots at a higher Rf value (Jansz *et al.*,1994). The debittering process is significant as the bitterness of the PFP limits its 1 wide use This debittering technique (hydrolysis) of the saponins may be adverse nutritionally. This study could help to dispel the criticism which had been hypothesized without evidence.

It has been shown that purified natural flabelliferins have bioactivity; (I) typical saponin type activity including haemolysis of red blood cells (Nikawala *et al.*, 1998a) (II) inhibition of the Na^+/K^+ATP^{ase} (Nikawala *et al.*, 1998b) and (III) inhibition of yeast and bacteria (Nikawala *et al.*, 1998c). The most potent flabelliferin was a saponin triglycoside of M. W. 868 termed F_B. Debittering causes the loss of all the natural flabelliferins (Nikawala *et al.*, 1998a).

The basic objective of this study was to determine if bitter PFP or naringinase debittered PFP has any adverse nutritional effect towards mice as judged by the the weight gain. This study was deemed necessary to determine if debittering of PFP produces an anti-nutritional effect in the end product in order to enhance wider commercial use.

2. Materials and Methods

PFP and debittering

PEP was extracted manually with water in the ratio of 1:1. Debittering was conducted under sterile conditions using naringinase ($1mg.g^{-1}$PFP) at 37^0C and at pH 5 for 24 hours on the extracted PFP. The pulp was concentrated under vacuum and then freeze dried. To confirm of debittering, the crude bitter and the debittered extracts (Nikawala *et al.*, 1998b) were run on TLC using butanol, ethanol and 0.88sp.gr. NH_3 in the ratio of 7:2:5 on silica gel G60 plates and visualized by spraying with anisaldehyde (Jansz *et al.*, 1994). The debittered product had no bitterness to taste and contained two saponins at Rf 0.42 and 0.83 instead of the saponins at Rf 0.35, 0.38, 0.41 and 0.49 as seen in the the bitter PFP extract. This showed that naringinase hydrolyses natural flabelliferins and therefore debitters the PEP.

Animal Model

All feeding trials were conducted using outbred ICR (Institute of Cancer Research) mice obtained originally from Charles river, USA and the colony was bred and maintained at the animal centre of the Medical Research Institute (MRI), Sri Lanka for over 6 years. The mice were fed on WHO

recommended breeding feed (Sabourdy, 1998) (Table -I). Male mice aged 6 weeks were separated into 3 groups (1control and 2 tests), 10 mice in each group, caged separately. Mice were 26-33g in weight and the groups were selected such that average weight in each group was similar. They were given food and water *ad libitum*. The control group was given WHO standard breeding feed (Table - I). The test bitter PFP group was given 10% bitter PFP incorporated control feed and the test debittered PFP group was given 10% debittered PFP incorporated control feed (Table - I). Attempts were made to maintain the protein and the caloric content at similar levels in the test and the control diets. The composition of PFP is given in Table-II Here maize was substitited with PFP at 25% level so that the feed contained 10% PEP. The feeds were was isocaloric. The only difference between bitter and debittered PEP was that sucrose was converted to glucose and fructose and flabelliferin II (F-II), the bitter compound and F_B, the antimicrobial compound had been hydrolyzed to smaller glycosidic saponins.

Table - I : Feed formula (WHO recommended rat and mice breeding feed)

Ingredients	Control (g)	Test (g)
PEP (bitter/ debittered)	-	100
Maize	405	303.2
Brown rice	100	100
Rice bran	25	25
Wheat bran	20	20
Wheat flour	135	135
Fish meal	80	80
Soya meal	80	80
Milk powder	60	60
Soya oil	20	17.7
Grass powder	30	30
Bone meal	15	15
Mineral mix	4	4
Vittamin mix	2.4	2.4
NaCl	2.0	2.0
Beta mix E 50	0.2	0.2
DL methionine	0.5	0.5
B complex	5 tablets	5 tablets

The three groups of mice were housed in six separate cages, 5 in each cage of dimensions 30x22x13cm (medium size mice cage) made out of polypropylene which contained a stainless steel lid with a feed hopper and a bottle holder. Temperature was maintained at 24±1°C. Cages were opened everyday and the small cut pieces of paper and solid material at the bottom of the cage were removed and new paper introduced each day. Faeces, residual feed and paper separated and the residual feed was weighed. The urine (absorbed on the paper) was discarded.

Table - II : Composition of palmyrah fruit pulp

COMPONENT	* FRESH WEIGHT	** FREEZE DRIED WEIGHT
Moisture	79%	10%
Protein	2.8%	12%
Sugar	14%	60%
Minerals	4.3%	18.41%
Pectin	4.4%	18.81%
Fibres	0.3%	1.28%
Lipids	0.2%	0.86%
Amino acids	0.3%	1.28%
Carotenoids	1-10mg	4.3-42.9mg
Vitamin C	28mg. $100g^{-1}$	120mg. $100g^{-1}$
Steroids	15mg. $100g^{-1}$	64.3mg. $100g^{-1}$

* Jayaratnam (1986).
** By calculation.

The feed was given as pellets and the test period was 28 days. Mice were weighed separately at weekly intervals and the amount of feed consumed per cage per day was calculated taking into account the spillages. The mice were sacrificed and the liver, kidney and the small intestines were examined macroscopically. Blood of the three groups was obtained by cardiac puncture and pooled separately which was freeze dried and extracted into methanol. Blood was also used for the RBC counts.

Test for flabelliferins in blood

Concentrated methanol extract was spotted on TLC plates and chromatographed with butanol, ethanol and 0.88sp.gr. NH_3 in the ratio of 7:2:5 and sprayed with anisaldehyde spary reagent, using the same procedure as for the isolated flabelliferins from bitter and debittered PFP (Nikawala *et al.*, 1998a).

Statistical Analysis

Comparisons were done by analysis of variance (ANOVA) and the t-test using Epiinto software package.

3. Results

Debittering

After the action of naringinase, the bitter taste of natural PFP disappeared. Further the bitter flabelliferin (F-II) and the antimicrobal fliabelliferin (F_B) was not present as evidenced by TLC. Two new non-bitter flabelliferins appeared at Rf 0.42 and at Rf 0.83.

Pellet feeding trials

In this study, young growing mice were used as it was felt that growing mice would show a clearer effect on weight gain. Results are given in table III. Food intake for the three groups were almost the same (4.90, 5.09 and 4.99g mouse^{-1} day^{-1} respectively) when calculated for the control group, PFP group and the debittered PFP group. The same results were obtained calculating on the basis on percentage weight gain which was 20.70 for bitter PFP group, 24.74 for the debittered PFP group and 25.88 for control group (the difference between the debittered PFP group and the control group was not statistically significant at 5% level)

Table - III : Summary of results

Group	Food Intake Mouse^{-1} Day^{-1}(g)	Average Weight Gain Mouse^{-1} (g)	SD of Average Weight Gain
Control	4.90	7.75	±1.568
Bitter PEP	5.09	6.25	±1.292
Debitttered PEP	4.99	7.65	±1.230

Average weight gain was calculated from the weight at the end of the test period. (Bitter and debittered PEP where incorporation at 10% level in control feed by substituting some maize).

Other results

RBC counts of all groups did not show significant differences. There was no haemolysis. Visual examination of organs including liver, kidney, duodenum, jejunum and ileum did not show differences.

TLC of blood serum from the heat which had a sensitivity of 5μg flabelliferins did not show the presence of flabelliferins. This was despite the fact that a mouse would have consumed 500μg flabelliferin. day^{-1} and this should have been seen on TLC.

The trial indicated that flabelliferins were probably not absorbed. Histopathological studies on the organs were not carried out.

Discussion

In this study the control group was fed with WHO standard rat/ micebreeding feed (Table - I). The tests contained 25% substitution of maize in the control feed by bitter and debittered PEP. Despite the possibility of certain nutritional variations from control and test, there was no significant difference between weight gain of control and debittered test (p=0.88) and thereby indicating the comparability of feeds.

Using 6 weeks old mice, despite food intake being very comparable, a statistically significant difference in weight was shown between the bitter PFP and control. This effect was reversed on debittering of PFP with naringinase. The significantly comparable weight gains of debittered PFP and control feeds I (p=0.88) strongly suggests that debittering by naringinase action was the vital factor. A similar reversal of bioactive effect has been observed in the alcoholic fermentation of PFP where boiled PFP fermented very slowly but rate of fermentation increased on treatment with naringinase (Nikawala and Jansz, 1994).

The explanation for reduced weight gain when bitter PFP is used could be that bitter PFP contains the flabelliferin tetraglycoside (F-11), which is hydrolyzed on debittering by naringinase F-11 has been reported (Jansz et al., 1994) to inhibit the Na^+/K^+ ATP ^{ase} of ghost red blood cells (Nikawala et al., 1998b). If the ATP^{ase} of the intestine is also inhibited, it will effect the uptake of glucose and amino acids (which are transported by active transport). This could lead to lesser nutrient absorption and thus explain the reduced weight gain, despite food intake being similar.

It appears possible that the flablliferins are not absorbed as they could not be located on testing of blood, despite the sensitivity of the test. The most important result is that debittering with naringinase was that debittering does not release products that produce adverse efffects on the growth of mice and therefore there is no evidence that debittering of PFP releases any additional growth retarding substances. This is significant commercially, from the standpoint of making a consumer acceptable end product that can be used as a beverage. It is also noted that the antimicrobial flabelliferin, F_B was also lost during debittering and this could be useful in commercial alcoholic fermentation. (Nikawala and Jansz, unpublished results).

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