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The effect of the fruit pulp of four cultivars of palmyrah on serum cholesterol levels in mice

L. Gayathri Pathberiy and E. R. Jansz
Dept of Biochemistry, University of Sri Jayewardenepura, Nugegoda.

Correspondence to : ***Prof E. R. Jansz***

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Abstract

Four types of the most common palmyrah fruit pulp *(Borassus flabellifer* L*)* were chosen on the basis of morphology of fruit. On feeding to ICR (Institute of Cancer Research) mice at 10% level all types of pulp lowered total cholesterol in ICR mice by 24 - 34 % relative to control. Total cholesterol was lower in all types. The p values of total serum cholesterol were: 0.04, 0.02, 0.0035, 0.0007 for type I, II, III, IV respectively. The effect on HDL cholesterol was widely different for the 4 types of pulp. The mechanism of lowering of cholesterol appears complex, probably involving: dietary fibre, flabelliferin profile and content and perhaps other factors. The well-known cholesterol reducing agents in plants, which are the free phytosterols, are not present in palmyrah fruit pulp.

Keywords: *Borassus flabellifer,* flabelliferins, hypocholesterolaemic, ICR mice, Palmyrah, β sitosterol

1. Inroduction

Palmyrah (*Borassus flabellifer* L) is a palm, which is found in the arid zones of South and South East Asia and Africa[1]. Palmyrah fruit pulp (PFP) though used for many purposes is still underutilized[2]. The PFP is a yellow to orange pulp enmeshing 1-3 seeds and there are 4 common distinct types of fruits called type I, II, III, Iv[3]. PFP has considerable bioactivity. This is mainly on account of the presence of saponins of β sitosterol (flabelliferins)[4]. Flabelliferin-II (FII) is known to reduce weight gain in mice[5] due to inhibition of intestinal ATP^{ase} [5] thus reducing glucose uptake[6]. One report suggests that PFP shows hypocholesterolaemic and antioxidant activity[7], however the sources of PFP and flabelliferins profile were not specified in that report[7]. PFP also has bioactivity in that some carotenoids are bioconvertible to vitamin A[8].

The objective of this study was to:

(1) Determine if all 4 types of PFP reduce cholesterol levels.
(2) Determine if this is a significant variation depending on the fruit type, their flabellferin contents and profiles and also attempt to obtain some insight into the mechanism(s) of lowering of cholesterol by PFP.

2. Materials & Methods

Collection of palmyrah

Palmyrah fruits (n=80) were collected from Kalpitiya in the North-West of Sri Lanka and divided into 4 common types[3] given below, bagged separately and transported to Nugegoda (nearly 200km away) where they were stored at -20°C.

Type I: colour, black; pericarp, rough with brown longitudinal striations; distal side, black[3]

Type II: colour, black; pericarp, smooth and no striations; distal side, three orange spots[3]

Type III: colour, black and orange longitudinal stripes; pericarp, smooth; distal side, black and orange stripes[3].

Type IV: colour, orange; pericarp, smooth[3]

Extraction of PFP

Within one week, PFP was extracted from fruits manually[5] using water in the ratio of 2:1 (v/w) with respect to PFP. The pulp was stored in polythene bags at -20°C.

Determination

For all 4 types of pulp, moisture content was determined using the AOAC, Dean and Stark method[9]. Flabelliferins were extracted by methanol extraction, carotenoids removed by petroleum ether extraction (60-80°C) and sugar removed by acetone extraction followed by evaporation and dry cellulose chromatography[10]. Total flabelliferins were determined by weighing isolates after this step. Flabelliferin profile was determined after lie separation and anisaldehyde spray followed by scanning tic - densitomctry[11].

Animal model

Inbred homogenous Institute of Cancer Research weanling male mice (initial weight =40±2, age = 6-8 weeks) were obtained from the Medical Research Institute, Colombo, Sri Lanka. The mice were grouped so that mean weight of was approximately constant. Controls were fed on WHO recommended rat and mouse breeding feed[11]. In test feeds on PFP on dry weight (l00g) was substituted in that diet in place of l00g of the 300g maize per kg feed (this gives 10% PFP). The feeds were isocaloric. Controls n=10 and test mice n=6 each were caged separately (one per cage), fed for four weeks with water *ad libitum* and food intake and weight gain recorded[5]. After four weeks the mice were sacrificed after being anaesthetized with diethyl ether and blood (l-2ml) recovered by cardiac puncture. The rest of the animal model was as previously reported[5].

Tests for cholesterol

Serum was separated at 30°C immediately after collection in the morning after 28 days feeding by centrifuging at 1000g and HDL cholesterol and total cholesterol were determined using appropriate kits (CHOD- PAP and REACTIF PRECIPITANT, BIOLABO S.A.,Gardenne, France)[12,13] on the same day.

Statistical Analysis

All the results are presented as mean±SD. Statistical analysis was carried out in Microsoft Excel. The significance was tested by Student's t-test. A probability level of $p<0.05$ was chosen as the criterion of statistical significance

3. Results

Flabelliferin content and profile

As expected all TLC plates did not show any traces to indicate the presence of free steroids (β sitosterol in particular). It is noted that free sitosterols are not normally present in fresh PFP[4,10]. Results given in Table 1 showed that total flabelliferin content was highest in type II. All the flabelliferin profiles were markedly different. Type I had many flabelliferins while the bitter inhibitor of glucose uptake flabelliferin II-tetraglycoside, MW, 1030 dominated. Types II and IV were dominated by the anti-microbial flabelliferin triglycoside MW, 868(F_B). F_D and F_E are diglycosides and F_F and F_G are monoglycosides[3] and the total of $F_D+F_E+F_F+F_G$ did not show much variation. Type III had the flabelliferin triglycoside F_N (MW, 886) which is very uncommon[3].

Table. 1 Content of flabelliferin of 4 types of fruits

	Crude Flabelliferin ($mg100g^{-1}$ DW)	Flabllifrin content (mg)							
		F-II	F-B	F-C	F-D	F-E	F-F	F-G	F-N
Type I	842	480	156	34	89	60	11	09	-
Type II	1085	391	532	-	163	-	-		-
Type III	761	259	270	91	125.5	-	-		14
Type IV	782	-	649	36	96	-	-	-	-

Flabelliferins were separated by Tic[14] and visualized by anisaldehyde spray[4,10] and scanned using two densitometers[10]. The ratio of each flabelliferin was calculated from peak area relative to total peak area. Since the flabelliferin content of total peak area is known, flabelliferin content in mg was calculated

Each densitometry scan was done twice on samples (25g each drawn from bulk PFP of 800g to 2kg.)

Feed intake and weight gain

Feed intake of type II, III, IV and I were lower than the control but not significantly so (Table 2). Weight gain was also slightly lower in the types I, II, III while weight gain in type IV was higher (Table 2). Weight gain in type IV was significantly higher than type 1 (p=0.005) and type II (p=0.03)

Table 2. Feed intake and Weight gain of ICR mice fed with 4 types of Palmyruh fruit pulp

	Feed intake($gday^{-1}$) Mean ± SD	p value with respect to control	Weight gain (g 4 $weeks^{-1}$) Mean ± SD	p value with respect to control
Control	4.56±0.6	-	7.5 ±3.5	-
Type I	3.91±0.37	0.04	3.3 ±1.4	0.15
Type II	3.75 ±0.52	0.09	4.9 ±1.4	0.37
Type III	3.70 ±0.35	0.058	5.9 ±2.1	1.0
Type IV	4.36 ±0.32	0.28	8.5 ±2.3	0.13

n=10 control : n=6 for each type
Type IV weight gain is slightly higher than control. The other types are less.

Lowering of cholesterol

Total cholesterol was lowered significantly with all 4 types of PFP with respect to control (Table 3). There were no significant differences between types. HDL-cholesterol showed significant variation between control and type III and type IV respectively. Type IV showed lower HDL than types I (p=0.0002) type II (p=0.002) and higher values than type III (p=0.02) respectively (Table 4). LDL + VLDL cholesterol (by difference) showed that type I and type II had significantly lower levels than control p=0.02 and p=0.04 respectively. Type I also showed lower LDL + VLDL than type II (p-0.01),.type III (p=0.002) and type IV (p=0.001) respectively. Type II had significantly lower LDL + VLDL cholesterol than type III (p=0.03).

Table 3. Effect on Total cholesterol of 4 types of PFP

	Total cholesterol (mg/dl) Pvlean ± SD	P value compared \with control
Control	243 ± 34.3	-
Type I	176.3±22.8	0.04
Type II	184.6±17.4	0.01
Type III	164.8±20.5	0.0035
Type IV	159.8±28.8	0.0007

n=10 control, n=6 for each type. Total cholesterol contents between types is not significant.
Each estimation was carried out in duplicate.

Table 4. Effect of 4 types of PFP on LDL+VLDL cholesterol & HDL cholesterol

	LDL+VLDL Cholesterol (mg/dl) Mean ± SD	P value compared to control	**HDL** cholesterol (mg/dl) Mean ± SD	P value compared to control
Control	192.3±48.1	-	51.5±35.4	-
Type I	85.6 ±16.3	0.018	83.6±9.0	0.49
Type 11	114.6±10.1	0.04	71.8±11.1	0.84
Type III	149.6±18.8	0.412	9.2±1.5	0.008
Type IV	141.8±28.0	0.244	19.6±5.6	0.014

Control n=10 mice, Types n=6 mice
Each determination was carried out in duplicate.
LDL by difference (Total-HDL)

4. Discussion

As expected the 4 fruit types had different flabelliferin profiles. In view of the theories involving cholesterol lowering, note was made on the content of F_{II}, F_B and the smaller carbohydrate moiety flabelliferins F_D, F_E, F_F and F_G As expected[5] types I, II and III with higher F_{II} resulted in reduced weight gains. Reduced weight gain was approximately proportional to F_{II} content of PFP. This is expected, as F_{II} is a known inhibitor of glucose uptake in 1CR mice[6]. Type IV did not have F_{II} and showed slightly higher weight gain than control.

It is expected that lowering glucose uptake will lead to reduce acetyl coenzyme A, which is well known to be the main precursor of cholesterol biosynthesis and thus could manifest lowering of serum cholesterol, but this relationship was not observed (Table 3). However incorporation of PFP of all types (including type IV) at 10% level into feed still lowered total cholesterol.

The extent of lowering total serum cholesterol in type IV, indicates that there are some other factors involved on cholesterol reduction apart from the effect caused by F_{II}. Phytosterols lower cholesterol by interfering with (a) cholesterol uptake from micelles and (b) bile salt uptake[14]. However, free sitosterol is not present in PFP[4, 10] and not formed until the flabelliferins reach the colon (Pathberiya and Jansz 2004, unpublished results). It was thought possible that the flabelliferins, which are saponins of sitosterol, especially those with small carbohydrate moiety (F_D, F_E, F_F and F_G) may be sufficiently non-polar to mimic β-sitosterol action but this could not explain the difference of lowering of cholesterol by the 4 types of PFP as the total content of these small carbohydrate moiety flabelliferins do not vary much from type to type of PFP. Flabelliferins are not absorbed[15] and therefore cannot interfere

with cholesterol synthesis. The other constituents of PFP that could lower cholesterol are fibre (10-12%), which includes pectin (5-6%)[2]. As the total content of fibre does not show much variation[2], perhaps a detailed analysis of non-sugar carbohydrates of PFP varieties may provide the answer; but this is a major project in itself.

5 . Conclusion

PFP can lower serum total cholesterol in mice. The HDL content is affected differently on feed containing the different types of PFP. The extent of change varies with fruit type. The content of F_{II} cannot explain cholesterol lowering. Fibre may play a bigger part in this effect together with perhaps some unidentified minor constituents also being involved.

6. Acknowledgments

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