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EFFECT OF *Canavalia gladiata* SEED MEAL ON IMPREGNATED ICR MICE

Sagarika Ekanayake[a,b], E. R. Jansz[a] and Sharmini Jayasekera[b]

[a] Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

[b]Medical Research Institute, Borella, Sri Lanka.

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Abstract

The effect of raw Canavalia gladiata (sword bean; awara – Sinhala; avarai - Tamil) whole seed meal on impregnated ICR (Institute of Cancer Research) mice was tested since it is believed that consumption of this variety of Canavalia cause abortion in cattle. It is also not advised as a food for pregnant women. The female ICR mice, in pro-estrous stage were individually paired with a fertile male of same age. Twenty percent of the protein content of control diet was substituted with Canavalia whole seed flour. The test diet was given to two groups (during the first seven days to one group and from day seven until parturition to the other) of impregnated mice. The feed intake of the mice in test groups was not significantly different ($p \leq 0.05$) to that of the control. The gestation period of the group fed Canavalia diet during the first trimester shortened significantly ($p = 0.016$) when compared to the control. However, the litter and dam were in good condition. Survival rate when compared with control did not show a significant difference ($p \leq 0.05$) in either group. The organs examined for any abnormalities and the weights recorded, showed that Canavalia had no significant ($p \leq 0.05$) short-term toxicological effects on the organs. Raw Canavalia seed meal has no effect on impregnated mice and on litter at 20% substitution (protein) level.

keywords :ICR mice, impregnated, gestation period, litter, sword beans

1 Introduction

The legume *Canavalia gladiata* considered as an "old world tropic" belongs to genus *Canavalia* of the Leguminosae family [1]. The plant is believed to have originated in the Asian continent and spread throughout the tropics. This is now cultivated on a limited scale throughout Asia, the West Indies, Africa and South America and has been introduced into tropical parts of Australia [2][3].
Temperature for cultivation is fairly high (15 - 30^o C) and requires moderately high evenly distributed rainfall, about 900-1500 $mm/annum$. Average yield ranges from 720-1500 kg/ha which can be compared with a soybean yield of 600-1000 kg/ha [4]. For use as a vegetable, the pods can be harvested in 3 to 5 months. Mature seeds are produced in 6 to 10 months [5]. The mature seeds of *Canavalia gladiata* were originally consumed by people of ancient India, and are now consumed even by the urbanized population [6][7]. Sword beans are advocated to be a good source for extending protein since the protein quality is similar to most edible food legumes [4] with desirable agro-climatic conditions suitable for cultivation in the tropics.
The young pods are extensively utilized in Asia as a green vegetable. The mature dry beans may be cooked and eaten as food, but require careful preparation because of the antinutritional factors present [5]. In other parts of Asia, beans are often soaked in water overnight, boiled in water to which a small quantity of sodium bicarbonate has been added, rinsed, boiled, pounded and used in curries, or as a substitute for mashed potato [3]. According to Herklots [2], only a few should be eaten on the first occasion and if there are no harmful effects, (headaches of diarrhea) the amount can be increased on subsequent occasions. The roasted and ground beans have been used as a coffee substitute [4]. In Sri Lanka, the immature pods are made into a dish directly or sometimes after boiling the pods with water to remove the anti-nutritional factors.
The seeds are underutilized in Sri Lanka, though rich in protein and other nutrients [8][9]. This could be due to the common belief that consumption of *C. gladiata* causes abortion in cattle and also the fact that it is not given to humans during early pregnancy. Therefore to confirm if the edible portion (pod or seeds) contains any abortive or implantation inhibiting compounds, this study was carried out using ICR (Institute of Cancer Research) mice bred at the Medical Research Institute (MRI), Borella, Sri Lanka.

2 Materials and methods

2.1 Sample preparation

Mature sword beans (*Canavalia gladiata; awara in Sinhala*) originally obtained from Galle, in south of Sri Lanka, (where the red seeded, white flowered variety known

as *rathu awara* is common), was cultivated in an experimental plot in Kandy and were utilized for the study.

The seeds were removed from the mature pods, air dried and stored at 4 ^{o} C until required for analysis. Prior to analysis, seeds were washed with tap water, rinsed with distilled water and oven dried at 50 ^{o} C overnight (12 *hours*). The whole seeds and cotyledons were ground to a flour of particle size 40-60-*mesh* using a standard mill (Cyclotec 1093, Tecator, Sweden). Flour samples were stored in a desiccator until required for analysis.

2.2 Feeding trial

Healthy 2 month old ICR virgin female mice (weight 26-33 g) from MRI, were used. They were housed in plastic cages (5 *mice/cage*) under standard animal house conditions (temperature 22-24^{o} C; photoperiod 12 *hours* light and 12 *hours* dark; relative humidity 70-75%) for 10-12 days, during which they had free access to pelleted mice breeding feed [10] and water.

The test diet was prepared by substituting 20% of the protein in standard mice diet with *C. gladiata* whole seed flour. The diet was prepared by taking the Net Protein Utilization (NPU) from the nutritional evaluation study into account (13.8% for whole seed flour) [9].

The composition of the test diet and the control (Table 1) showed that both diets had similar protein and fat percentages (important during pregnancy) and were iso-caloric. The protein content in the test diet was high, since the utilisable or absorbable protein content from whole seed flour was very low and corrected for NPU.

Table 1: Nutrient composition of control and test (*Canavalia*) diets fed to mice (g/100 g on dry matter basis)[a]

Component	Diet	
	Control[a]	Test[a]
Moisture(FW)	7.00	7.40
Crude protein	18.00	23.43
Fat	5.05	5.51
Ash	9.03	4.86
Crude fibre	3.76	4.40
Carbohydrate (by difference)	57.16	55.40
Energy(kJkg^{-1}DM)	19026.00	19278.00

FW-fresh weight basis

[a]Values are expressed as the average of duplicate analysis.

Estrous cycles of each female were observed by vaginal smearing (lavage method) [11]. After the acclimatization period, the females in pro-estrous stage were individually paired with a fertile male of same age. Successful matings were confirmed by the presence of a copulation plug (a coagulum of semen) and spermatozoa in the vaginal smears (> $5x10^6$ */ml*) on the following morning, designated as sperm day one. These mice were randomly divided into three groups (Control - 5 *mice/group*, Test 1- 5 *mice/group* and Test 2 - 5 *mice/group*). The control group was fed the normal mice breeding diet from sperm day 1 until the sucklings were five days old. The test group 1 (Test 1) was fed the mice breeding diet for seven days from sperm day 1 and *Canavalia* diet from day 7 until the sucklings were 5 days old. The 2^{nd} test group (Test 2) was given *Canavalia* diet for the first 7 days and then the mice breeding diet until the 5^{th} day after parturition. Water was provided *ad libitum*.The food intake and the weight gain of each mouse was monitored daily. After parturition the litter were checked for any macroscopic malformations. The survival rate of the litter of each group was observed until the 5^{th} day. The dams were dissected on the fifth day following parturition and the macroscopic appearance of organs and weights were recorded to test for any possible short-term toxicological effects.

2.3 Statistical analysis

The standard deviations and significant differences between means by Student's t test at 95% confidence level were determined using Microsoft Excel-97.

3 Results and Discussion

A preliminary diet trial was carried out with *C. gladiata* whole seed flour substituting 20% of the protein in the normal mice diet. The substitution was done by taking only the crude protein content of *Canavalia* whole seed (29%) flour into account. This resulted in the dam cannibalizing the litter. This was the case for all the five mice that were made pregnant and then fed the above diet during the gestation period. The cannibalism continued up to the fifth day. This possibly indicates that protein substitution at 20% level (solely from *Canavalia* seeds), led to the utilizable protein content in the diet being inadequate for breeding. It was interpreted that this was due to the low NPU value of the *Canavalia* protein. Therefore the isocaloric test diet with which the following experiment was carried out was prepared by taking the NPU (13.8%) into account [9].

The gestation period, average weight gain and feed intake during 1^{st}, 2^{nd} and 3^{rd} trimesters are presented in Table 2. The feed intake in the first trimester in the three groups showed no significant difference ($p \leq 0.05$), which indicates the *Canavalia* diet is similarly palatable as the control. The mice did not show an aversion to the *Canavalia* diet. This is also reflected in the weight gain in the 1^{st} trimester, where

Table 2: Gestation period, average weight and feed intake of mice during 1^{st}, 2^{nd} and 3^{rd} trimesters.

Group	%Average weight gain during gestation			Feed intake (g)			Gestation period
	1^{st} mean± sd	2^{nd} mean± sd	3^{rd} mean± sd	1^{st} mean± sd	2^{nd} mean± sd	3^{rd} mean± sd	days mean± sd
Control	6.67±1.08	23.54±5.79	61.97±6.09	4.94±0.55	5.05±0.76	6.56±1.12	19.0±0.00
Test 1	5.46±2.30	25.49±4.41	47.92±11.15	4.86±0.26	5.42±0.42	6.85±0.50	18.8±0.45
Test 2	6.70±3.28	25.46±3.31	41.18 ±4.68*	4.71±0.36	5.79 ±0.37*	6.88 ±0.39	18.2±0.45*

sd=standard deviation;n=5

Values followed by an asterisk differ significantly ($p \leq 0.05$) from control.

there was no significant difference in weight gain in the three groups.
Percentage weight gain in all three groups during the second trimester also did not show a significant difference ($p = 0.05$) although the food intake was significantly higher in test group 2 than the control. The feed was changed to the normal breeding feed in the second trimester in test group 2 and this could be the reason for the higher food intake.
In the third trimester the feed intake was not significantly different but the weight gain in test group 2 was significantly lower ($p=0.0045$) to that of the control, even after changing the diet to normal breeding feed from the second semester. However, since the final average litter size and litter weight of all three groups were not significantly different, the lower weight gain in the third trimester has had no significant effect (Table 3).

Table 3: Average litter size, weight at birth and 5 days after birth in the control and test groups (litter dam)

	Average litter size		Average litter weight(g)	
	at birth mean ±sd	after 5 days mean ±sd	at birth mean ±sd	after 5 days mean ±sd
Control	14.00±2.0	13.17 ±1.94	1.64±0.10	3.21±0.25
Test 1	14.00 ±2.6	13.00 ±2.45	1.61±0.10	2.98±0.06
Test 2	11.17±1.6	10.83±1.94	1.63±0.08	3.77±0.67*

sd=standard deviation; n=5
Value followed by an asterisk differ significantly ($p \leq 0.05$) from Test land also differs significantly from that of the control at 90%($p \leq 0.1$).

A significant observation was the decrease in the gestation period of test group 2 compared to control ($p = 0.016$), but no such difference exists between control and test group 1 ($p = 0.37$). It appeared that feeding *Canavalia* in the first trimester has an effect on the gestation period. However, this seems to be a short term and the only effect that the material has on pregnant mice. When the test diet is fed from the beginning of the second trimester, the average gestation period becomes less than the control, but the difference is not significant ($p \leq 0.05$).
The average litter size and average litter weight were recorded at birth and after five days (Table 3). There was no significant difference among the three groups at 95% confidence interval. However, it was noted that the litter size of test group 2 was significantly lower than that of the control at 90% confidence interval ($p = 0.0946$). The macroscopic examination of the litter immediately after birth and after five days showed the litter of all three groups to be normal. Survival rate when compared with control did not show a significant difference ($p \leq 0.05$).

The dams of all three groups were dissected after 5 days to investigate any possible short term toxicological effects on the major organs. The organs examined for any abnormalities and the weights recorded (Table 4) showed that there were no toxicological effects on the organs since percent weights of the major organs of the test mice (Test 1 & Test 2) were not significantly different to that of the control ($p \leq 0.05$).

Table 4: Organ weights (%) of the postpartum

Organ	%Average organ weights of different diets fed groups		
	Control mean±sd	Test 1 mean±sd	Test 2 mean±sd
Liver	5.79±0.38	5.91±0.28	6.23±0.40
Spleen	0.48±0.10	0.43±0.07	0.43±0.04
Kidney	1.21±0.07	1.28±0.06	1.20±0.10
Intestine	21.58±1.50	25.61±2.40	22.16±1.22

sd=standard deviation; n=5

The data recorded showed that there were no abortive or other adverse effects due to the introduction of *Canavalia* seed flour in the diet in this proportion. The only effect the seed flour has on impregnanted mice is a shortening of the gestation period when given during the first trimester in this proportions. However, the preliminary experiment with pregnant mice indicated that using *Canavalia* as the sole and principle source of protein is not nutritionally advisable.

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