Proc. Indian natn. Sci. Acad. **B48** No. 5 pp. 596-602 (1982)

Nutritional Evaluation of the Protein Isolate Obtained from the Seed of *Eucalyptus kirtoniana* in Albino Rats—A Comparative Study

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(Received 8 April 1982)

Nutritional evaluation of protein of defatted *Eucalyptus kirtoniana* seed was carried out with albino rats and comparisons were also made with casein and soybean protein. Amino acid composition of the protein isolate showed that it contained 17 amino acids including 10 essential amino acids but was deficient in methionine, isoleucine and valine as compared to casein. Quantitative variation of common consituents of blood like haemoglobin, urea, total lipids, phospholipids, total protein and albumin contents and some serum enzymes, namely, GOT, GPT and alkaline phosphatase were studied. All the values were within the normal range and showed no significant variation in different dietary protein groups. Experimental protein isolate and soybean protein exerted a hypocholesteramic effect but only the protein isolate increased the blood sugar level as compared to the two other proteins used in the investigation. The results of this study indicate that the protein isolate is not toxic.

Key Words: *Eucalyptus kirtoniana*, **Casein, Soybean protein, Nutrition in rats**

Introduction

Recently, Eucalyptus is being widely planted in different forests of India for its rapid growth and valuable pulpwood. Seeds are available in large quantities in different local forests. The nutritional potentialities of such forest resources have not been adequately studied, a factor that limits their utilization as animal food ingredients though they are rich in protein. High prices and shortage of protein stimulated such studies on forest resources as a source of protein. In this context, only a few studies on sal (Mulky et al. 1975, Mulky 1979), Karanja (Singh 1966) have been carried out in India

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during the last few years and these studies showed that the defatted seed rich in protein or crude protein isolate may be utilized for meeting the nutritional requirement of animals.

Based on the surveys conducted by the Indian Central Oil Seed Committee and the Hindustan Liver Ltd (Anonymous 1973), it has been shown that hardly 7% of the total production of non-edible oilseeds with Eucalyptus is being utilized, which shows an wastage of large quantities of those oil seeds. Therefore further research work is needed for exploitation of such forest resources for edible purposes as a source of supplementary protein.

The percentage of nitrogen in defatted *Eucalyptus kirtoniana* seed was 2.1 and the protein content varied from 13.5 to 14.2 g% (Mandal, unpublished data). The protein contains 17 amino acids including 10 essential amino acids and the balanced amino acid composition indicates its high nutritive value. In the present study, rat bioassay procedure was used to assess the quality of the protein isolate and comparison with casein and soybean protein was also performed. The biological indices measured were haemoglobin, sugar and urea content of blood and cholesterol, total lipids, phospholipids, total proteins, albumin and some important enzymes namely GOT, GPT and alkaline phosphatase of serum. Food-intake and growth-response of rats given supplemented diet with different proteins were also studied.

Materials and Methods

Eucalyptus kirtoniana seeds were collected from the local forest of Burdwan and taxonomy was done by the Botany Department, Burdwan University. The seeds were powdered and completely defatted by solvent (petroleum ether) at 60–80°C extraction method in a soxhlet apparatus for 72 hr.

Preparation of the Protein Isolate

Protein was isolated from the defatted seeds following the method of Felker and Bandurski (1977) with slight modification. For the extraction of protein, defatted powdered seeds were suspended in cold dilute sodium hydroxide solution (*p*H 12) and stirred for 1 hr. The resulting solution centrifuged at 15,000 g for 15 min and the protein in the supernatant was precipitated by adding 10% solution of trichloroacetic acid. The process was repeated once and brown coloured protein was finally collected after washing three times with cold distilled water. All the above processings were done in ice-cold condition and freeze-dried protein was stored in deep freeze (—4°C) until use.

Analytical Procedures

Nitrogen percentage of the isolated protein fraction was obtained by micro-kjeldahl method. Standard methods of moisture and ash analysis as described by the Association of Official Analytical Chemist (1970) were employed. Amino acid composition of the isolated protein was determined by column chromatography in a Beckman model 120-B amino acid analyser (Beckman Instruments, Palo Atto, Calif. USA) using M-72 type resins. 5 mg protein was loaded into a heavy wall glass tube followed by 5 ml of 6 N hydrochloric acid, frozen in cold condition and sealed under vacuum. The hydrolysis was conducted at 110°C for 24 hr. The hydrolysate was then filtered and excess acid was removed by repeated evaporation under reduced pressure. For the determination of sulphur-containing amino acids, the sample was first oxidised with performic acid for 18 hr.

Animals and Diets

Except the protein isolated from Eucalyptus seedmeal, other two proteins used in the present study were casein and soybean, both of them contained about 60g moisture/kg and nitrogen content (g/kg dry matter) was 145.1 and 144.2 respectively. Thirty-six male albino rats of inbread strain(in our own laboratory) weighing about 130–150 g were divided into three groups. The animals were housed individually in wire net cages under controlled temperature and humidity conditions. Twelve animals of each of the three groups (T_1, T_2 and T_3) received diets (composition given below) which were similar except protein. The only source of protein in the diet of rats of T_1, T_2 and T_3 groups was casein, soybean protein and protein isolate (isolated from Eucalyptus seedmeal) respectively.

Composition of diet	(g/100 g diet)
Starch	60
Cellulose powder	10
Protein	10
Coconut oil	5
Sucrose	10
Salt mixture*	4
Vitamin mixture**	1

*Salt mixture contained (g/kg salt mixture): NaCl, 105; KCl, 120; KH_2PO_4, 310; $Ca_3(PO_4)_2$, 149; $CaCO_3$, 210; $MnSO_4$, 0.2; $MgSO_4$, 90; $K_4SO_2Al_2$ $SO_4)_2$ .24 H_2O, 0.09; $FePO_4$ $4H_2O$, 14.7; $CuSO_4$. 5 H_2O, 0.39; $ZnCl_2$, 0.38; NaF, 0.57; KI, 0.05; $COCl_2$, 0.04

**Vitamin mixture contained (mg/100 g diet): thiamin, 0.8; riboflavin, 0.8; pyridoxine, 0.6; niacin, 5.0; calcium pantothenate, 4.0; inositol, 20; choline chloride, 200; folic acid, 0.4; B_{12}, 2μg, biotin, 20μg; retinyl acetate, 1000 IU; ergocalciferol, 150 IU; α-tocopherol, 12; and menaquinone, 0.3.

The animals were fed on the experimental diets for 30 days with water *ad libitum*. Food intakes and body weight were recorded. At the end of 30 days rats were sacrificed, blood was collected and haematological and biochemical estimations were carried out. Haemoglobin content was estimated by the method of Drabkin and Austin (1932). Blood-sugar was estimated according to the method of Somogyi (1945). Blood-urea estimation was done according to Netelson (1957). Serum lipids were extracted and washed according to the method of Folch et al. (1957) and analysed for cholesterol (Sperry & Webb 1950), phospholipid (Fiske & Subbarow 1925) and total lipid was measured by evaporating the measured amount of extract. Estimation of total protein and albumin of serum was carried out by folin-phenol method (Lowry et al. 1951). Glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) of serum were measured according to the method of Reitman and Franknel (1957). Alkaline phosphatase activity of serum was estimated by the method of King and Armstrong (1934) and verified by determining the rate of hydrolysis of p-nitrophenyl phosphate according to the method of Bessey et al. (1946). Statistical analysis was done by using student's 't' test.

Result and Discussion

Table 1 summarises the results obtained for moisture, total ash, total nitrogen content and amino acid profile of the protein isolated from Eucalyptus seedmeal. It is obvious from the amino acid profile that the amount of methionine, isoleucine and valine is less whereas arganine is exceptionally high in the protein isolate as compared to casein.

Values for the blood biochemical parameters, food-intake and bodyweight-gain of the experimental animals given diets supplemented with different proteins are represented in table 2. In general, food intake was greater in the rats given soybean protein or protein isolate than in those

Table 1 *Composition of the protein isolated from defatted* Eucalyptus kirtoniana *seeds (Mean value of two determinations)*

	(g/kg)
Moisture	50.0
Total ash	15.0
Total nitrogen	142.5
AMINO ACIDS	(g/16 g N)
Glycine	4.21
Alanine	3.15
Threonine	2.46
Serine	4.82
Valine	3.12
Leucine	6.31
Isoleucine	2.81
Proline	2.61
Phenylalanine	2.52
Methionine	0.87
Cystine	0.42
Tyrosine	3.21
Histidine	2.83
Arginine	10.42
Lysine	5.22
Aspartic acid	7.03
Glutamic acid	27.02

given casein but the difference was not significant. Results show that protein isolate supplementation in the diet for 30 days produced a gain in body weight which was about 85% of that with the casein diet but it was 96.8% of that of soybean protein diet. This difference in gain in body weight of the animals given different dietary proteins could not be correlated simply to the amount of food intake. Retardation in growth response may be correlated with the deficiency of methionine in the protein isolate. Drouliscos and Malefaki (1980) showed that supplementation with dl-methionine with methionine-deficient protein in the diets of rats elicited a growth response but other essential amino acids had no such effect. It could be of interest to see the growth response to methionine supplemented diet of the protein isolate. In the above study haemoglobin content of all the rats varied from 15.4 to 15.6 g/100 ml blood which were thus within the normal range of variation. Protein-intake caused

Table 2 *Blood biochemical parameters, food intake and gain in body weight of albino rats given diets supplemented with different proteins* (n = 6, ± Standard error)

Biological indices	GROUPS		
	T_I	T_2	T_3
Gain in body weight (g) after 30 days experiment	35.8 ± 2.35	31.5 ± 2.41	30.5 ± 2.61
Food intake (g/day)	12.8 ± 1.41	13.2 ± 0.84	13.4 ± 0.82
Blood haemoglobin (g/100 ml)	15.6 ± 0.25	15.4 ± 0.24	15.6 ± 0.21
Blood sugar mg/100 ml)	84.8 ± 2.45	88.6 ± 3.21	92.8 ± 2.01*
Blood urea (mg/100 ml)	41.2 ± 1.64	40.5 ± 1.84	38.6 ± 1.92
Serum total lipid (mg/100 ml)	245.8 ± 5.25	241.5 ± 6.21	240.4 ± 5.82
Serum phospholipid (mg/100 ml)	82.4 ± 1.36	81.3 ± 1.25	81.8 ± 1.12
Serum cholesterol (mg/100 ml)	68.4 ± 1.36	58.3 ± 1.25**	56.8 ± 1.12**
Serum total protein (g/100 ml)	6.1 ± 0.14	5.8 ± 0.21	5.8 ± 0.22
Serum albumin (g/100 ml)	3.6 ± 0.11	3.4 ± 0.12	3.4 ± 0.14

Values were significantly different from those for the corresponding case in group
* $P < 0.05$; ** $P < 0.001$

increase in the haemoglobin content if it was below the normal range. Total serum protein content of different dietary protein groups also did not differ significantly. All the above observations indicate that dietary supplementation of other proteins and protein isolate do not induce any significant effect on the haemoglobin content, it only helps to maintain the dynamic equilibrium of the blood proteins. Blood urea level is usually reflected by the amount of exogenous protein-intake. Normal values for all the groups indicate that the exogenous protein intake was metabolised within the normal limit and the protein isolate does not show any adverse nutritional effects. High blood sugar level was significant ($P<.005$) in the group (T_8) of rats given protein isolate supplemented diet. It is probably due to inhibition of glycolysis by some glycoprotein which is present in the protein isolate. The protein isolate contain a very low percentage of saponin which might have some ill effect on pancreatic β-cells, as a result of which β-cells might show slower release of insulin or its partial destruction. Total lipid and phospholipid content of serum did not undergo any noticeable alterations in the rats given supplemented diet with different proteins. Both the soybean protein and the protein isolate in the diets produced a significantly ($P<0.001$) lower serum cholesterol level than casein. Nagata et al. (1980) showed that the regulatory effect of dietary proteins on serum cholesterol level in rats is easily modified by the type and amount of dietary fat but Carroll, Huff and Roberts (1977) have found that hypocholesteraemic effect of protein is independent of the lipid composition of the diet. Kritchevsky (1979) has, recently, pointed out that the amino acid composition is the factor responsible for bringing change in the serum cholesterol of rats and showed that the ratio arganine: lysine might be the major factor responsible for causing a change in serum cholesterol given different dietary proteins. Arganine : lysine ratio was calculated to be 0.48, 1.12 and 1.9 for casein, soybean protein and the protein isolate respectively. But it is still not known how this difference in the ratio influences the serum cholesterol level. Okenfull and Fenwick (1978) and Potter et al. (1979) ascribed the hypocholesteraemic action of vegetable protein due to saponins remaining in the protein preparation. The soybean protein and the experimental protein isolate used in the present study, contained essentially no saponin (for soybean protein it is about 6 g/kg and 8 g/kg fort he protein isolate). It is unlikely that these low levels of saponins influence the serum cholesterol, since larger amounts of saponins are usually required to produce a significant hypocholesteraemic effects. However, soybean protein or protein isolate often contains approximately 100 g non-proteinous materials/kg which should be considered (Helms 1977).

It is evident from the results of table 3 that the nature of protein had no influence on serum GOT, GPT and alkaline phosphatase activities and the values for different dietary protein groups were almost the same. Elevation of transaminase activity in blood has been used as an indicator of tissue damage. Damaged cells may release transaminase into blood stream. However, as pointed out by Denman et al. (1963) that other factors such as alterations in permeability of cell membrane, increased synthesis or decreased enzyme degradation may also be involved. Normal activities of serum

Table 3 *Serum GOT, GPT and alkaline phosphatase activities of albino rats given diets supplemented with different proteins* (n=6, ± Standard error)*

Enzymes studied	GROUPS		
	T_1	T_2	T_3
Serum glutamic oxalacetic transaminase (SGOT) (i.u/litre serum/min)	10.4 ± 0.81	9.8 ± 0.92	9.4 ± 0.88
Serum glutamic pyruvic transaminase (SGPT) (i.u/litre serum/min)	6.1 ± 0.24	5.9 ± 0.31	5.8 ± 0.34
Alkaline phosphatase (King-Angstorn Unit/ 100 ml serum)	8.1 ± 9.54	8.2 ± 0.56	8.3 ± 0.64

*All values were non-significant compared to corresponding casein group

GOT and GPT of rats of different dietary protein groups indicate the absence of any hepatotoxic injury in the experimental animals, otherwise the activities would increase to some extent (Wroblewski & La-Due 1955, 1956). The elevation of serum alkaline phosphatase, generally, indicates increased activity of osteoblasts and interference with the excretion of bile (Oser 1965). Gutman (1959) and Polin et al. (1962) suggested that alkaline phosphatase may increase due to liver cell injury but our results suggest lack of such activities in different dietary proteins.

On the basis of the present investigation, it may be concluded that protein isolated from *Eucalyptus kirtoniana* seed-meal would be safe for edible use.

Acknowledgements

Authors are greateful to the Principal, Burdwan Medical College for laboratory facilities for this work. Sincere thanks are due to Dr C Dey, B Basak, N Hazra and M Banerjee for their helpful co-operation and to the authorities of the Council of Scientific and Industrial Research, New Delhi for awarding Junior Fellowship to one of the authors (BM).

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