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Influence of Teak (*Tectona grandis;* Family: Verbenaceae) Seed Protein on Some Enzymes and Liver Lipids of Albino Rats

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The influence of protein, isolated from teak *(Tectona grandis)* seed upon albino rats with respect to some of their serum, liver and intestinal enzymes and liver lipids has been studied. The protein in question contains aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, lysine, phenylalanine, histidine and arginine as determined by amino acid analyser. After feeding experiment an increase in body weight including the liver weight was noted in the test animals due to excess protein in the diet. A marked increase was observed in G.O.T., G.P.T. and total lipid of liver, whereas G.O.T. and G.P.T. of serum were decreased. The observed increased concentration of lipid in liver may be due to excess addition of protein in diet. The overall observation is an indication of probable fatty infiltration in liver of test animals.

Key words: Alkaline phosphatase, Aminotransferases, Liver lipids, *Tectona grandis*, Verbenaceae.

It has been reported (6) earlier from some biochemical parameters of albino rats after administration of teak *(Tectona grandis)* seed (a widely available forest agrowaste in India) protein enriched diet that the protein appeared to be non-toxic to test animals. Naturally it would be of considerable interest to observe the influence of the protein enriched diet on aminotransferases of liver and serum, alkaline phosphatase activities of serum, liver and intestine and also on total and phospholipids of liver. The present communication deals with the same object.

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Materials and Methods

Isolation of Protein and amino acid characterization. Protein was isolated (6)

from oil free teak *(T. grandis)* by extraction with aqueous sodium chloride solution at pH 10 followed by precipitation at pH 4.5 — 5.0. It was separated by centrifugation at 5,000 r.p.m. for 5 min, washed well with distilled water and then kept in a refrigerator.

Amino acids of the protein isolated from teak seed were also identified before feeding experiment. For the determination of amino acid composition, hydrolysis of the protein (2 mg) was carried out in an evacuated sealed tube with 3 ml 6 N HCl containing 5 % thioglycolic acid (9) at 110°C for 24 hours. The analysis of amino acids in the protein hydrolysate was then conducted with the aid of Beckmann Multichrome 4255 amino acid analyzer. Following amino acids (g/16 g N), including eight essential amino acids, were present: Aspartic acid (8.00), threonine (2.91), serine (3.63), glutamic acid (25.09), proline (8.73), glycine (10.18), alanine (6.54), valine (6.18), isoleucine (4.73), leucine (8.00), tyrosine (0.73), phenylalanine (4.00), histidine (1.82), lysine (3.64), arginine (5.82).

Trial experiment with teak (T. grandis) *seed protein.* Twelve laboratory inbreed male albino rats were distributed into two groups, i.e., six in control and six in an experimental group. They were housed individually in wire net cages. Animals of the control group were fed a laboratory stock diet consisting of wheat flour and skim milk in the ratio 6:1, sugar (5 g/100 g diet), common salt (2 g/100 g diet), multivitamin (2 g/100 g diet) of Glaxo, India. Each rat of the experimental group was fed with the same diet enriched with 10 % of the teak seed protein.

After 30 days, rats were sacrificed, blood was collected and livers were quickly excised and then homogenized in 0.25 M cold aqueous sucrose solution which was used for the estimation of aminotransferases in liver. Glutamic oxalacetic transaminase (G.O.T.) and glutamic pyruvic transaminase (G.P.T.) of serum and liver were estimated according to the method of REITMAN and FRANKEL (10). Alkaline phosphatase of serum, liver and intestine were measured by the method of KING and ARMSTRONG (8) and verified by the rate of hydrolysis of p-nitro phenyl phosphate as advocated by BESSEY *et al.* (1), phospholipid estimation by the method of FISKE and SUBBAROW (5) and total lipid by DREVON and SCHMIT (4).

Results

After the experiment, gain in body weight, weight of liver, G.O.T., G.P.T. and total lipid of liver of rats of the experimental group were found to be increased significantly, whereas G.O.T. and G.P.T. of serum in the same group decreased significantly in comparison to that of control. In this experiment, a slight increase in phospholipid of liver and a little decrease in alkaline phosphatase of serum and liver of test animals compared to control group were also observed. Moreover, a significant increase in alkaline phosphatase of intestine of test animals was noted in comparison to that of control (Table I).

Discussion

It was previously reported (6) by the same authors that significant increase in body weight of test animals was only due to teak *(T. grandis)* seed protein enriched diet administration. No doubt the significant increase in weight of liver and body weight in experimental rats were due to the excess protein administration.

It is a well known fact that liver is the main site for the different metabolic processes, and aminotransferases are the

Table I. *Effect of teak seed protein enriched diet on some enzymes and liver lipids.*

Biological Indices	Control Group (Mean ± S.E.)	Experimental Group (Mean ± S.E.)
Starting body weight (g)	130.00 ± 0.58	130.00 ± 0.58
Weight gain after experiment (g)	17.50 ± 0.50	29.00 ± 0.35[a]
Liver weight (g)	5.87 ± 0.06	7.32 ± 0.04[a]
G.O.T. of serum (i.u./l of serum/min)	8.74 ± 0.03	7.76 ± 0.01[c]
G.P.T. of serum (i.u./l of serum/min)	4.41 ± 0.02	3.78 ± 0.03[c]
Alkaline phosphatase of serum (K.A. Units)	8.20 ± 0.06	7.98 ± 0.05
G.O.T. of liver (i.u./100 g of liver/min)	4.80 ± 0.07	7.24 ± 0.17[a]
G.P.T. of liver (i.u./100 g of liver/min)	8.22 ± 0.12	8.97 ± 0.14[b]
Alkaline phosphatase of liver (K.A. Units)	10.25 ± 0.08	10.05 ± 0.06
Total lipid (mg/100 g)	125.67 ± 0.15	130.27 ± 0.99[a]
Phospholipid (mg/100 g)	80.25 ± 0.08	81.23 ± 0.80
Alkaline phosphatase of intestine (K.A. Units)	9.98 ± 0.07	11.94 ± 0.12[a]

[a] $p < 0.001$
[b] $p < 0.002$ Increases significantly.
[c] $p < 0.001$ decreases significantly.

key metabolic enzymes which are involved both in protein catabolism and anabolism. It has been observed that liver cells, being damaged either by drugs or deficiency of choline or methionine in diet, may result in the fatty infiltration of liver. In such cases, aminotransferase activities and alkaline phosphatase are found to be increased (2). In the present study, although the aminotransferase activities of serum decreased in comparison to control but that of liver significantly increased (Table I). This observation is in agreement with those of WROBLEWSKI and LADUE (12, 13), GUTMAN (7) and ROBERTS (11). It may be presumed that fatty infiltration of liver occurred in the experimental group. The increased aminotransferase activity of the liver tissue, with simultaneous decrease in this activity in serum may be explained on the premise that, until there is a damage in the liver tissue or regurgitation from the bile capillary ensue due to biliary obstruction, the reflection of increased liver enzyme activity may not be obtained from serum. Alkaline phosphatase activities of serum and liver slightly decreased, whereas alkaline phosphatase activity of intestine increases significantly in the experimental group in comparison to that of control. Furthermore, it was previously observed (6) that the blood sugar level was higher in the experimental group by this treatment. Thus, increased intestinal alkaline phosphatase activity may be an indication of increased absorption of sugars from the intestinal mucosa, thereby giving rise to increased blood sugar level.

Amino acids, the building block of protein, usually converted to acetyl Co-A through different metabolic pathways, and this acetyl Co-A is usually oxidized through TCA cycle into CO_2 and water. But when this cycle is overloaded with acetyl Co-A, it may be diverted into neolipid synthesis. Addition of excess protein in the diet increases the formation of acetyl Co-A, which in turn converted to fat, with the result of an increase in concentration of lipid in the liver. Insufficient phosphorylation causes little increase (Table I) in phospholipid, which may be due to somewhat deficiency of choline and other lipotropic factors (3).

Hence, probably fatty liver occurred with such increased aminotransferase activities after administration of teak *(T. grandis)* seed protein.

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Resumen

Se estudia la influencia de una proteína aislada de la semilla de teca *(Tectona grandis)* sobre el contenido de distintos enzimas en hígado, suero e intestino de ratas blancas adultas, así como en otros parámetros fisiológicos. Los animales cuya dieta está enriquecida con esta proteína (10 %) experimentan un incremento significativo de su peso corporal y del hígado. A nivel hepático, se encuentra un notable incremento de la transaminasa oxalacética glutámica (GOT), de la transaminasa pirúvica glutámica (GPT), y del nivel de lípidos totales, mientras que no se modifica el contenido de fosfatasa alcalina ni de fosfolípidos. En el suero hay disminución significativa de GOT y de GPT y no se modifica el nivel de fosfatasa alcalina, enzima que se encuentra aumentada en intestino. El incremento observado de lípidos en hígado puede deberse al exceso de proteína en la dieta. Se estudia también el contenido e identificación en aminoácidos de la citada proteína.

References

1. BESSEY, C. A., LOWRY, O. H. and BROCK, M. J.: *J. Biol. Chem.*, **164**, 321-325, 1946.
2. CANTAROW, A. and TRUMPER, M.: In «Clinical Biochemystry» (6th ed.). W. B. Saunders Company, Philadelphia, 1962, pp. 547-550.
3. CHATTERJEE, C. C.: In «Human Physiology» (9th ed.). Medical Allied Agency, Calcuta. India, 1977. Vol. I, pp. 561, 572-573.
4. DREVON, B. and SCHMIT, J. M.: *Bull. Trav. Soc. Pharm. Lyon*, **8**, 173-178, 1964.
5. FISKE, C. and SUBBAROW, Y. P.: *J. Biol. Chem.*, **66**, 375-377, 1925.
6. GHOSH-MAJUMDAR, S., LASKAR, S., BASAK, B. and MAITY, C. R.: *Ind. J. Physiol. Allied Sci.*, **34**, 59-62, 1980.
7. GUTMAN, A. B.: *Am. J. Med.*, **27**, 875-881, 1959.
8. KING, E. J. and ARMSTRONG, A. R.: *Can. J. Med. Ass.*, **31**, 376-379, 1934.
9. MATSUBARA, H. and SASAKI, R. M.: *Biochem. Biophys. Res. Commun.*, **35**, 175-180, 1969.
10. REITMAN, S. and FRANKEL, S.: *Am. J. Clin. Path.*, **28**, 56-62, 1957.
11. ROBERTS, W. M.: *Br. J. exp. Path.*, **2**, 90-94, 1930.
12. WROBLEWSKI, F. and LA-DUE, J. S.: *Ann. Intern. Med.*, **43**, 34-35, 1955.
13. WROBLEWSKI, F. and LA-DUE, J. S.: *Proc. Soc. exp. Biol. Med.*, **19**, 569-572, 1956.