Food Chemistry **20** (1986) 69–78

Technical Note

Biochemical Dynamics of Hypocholesterolemic Action of *Balanites Aegyptiaca* Fruit

ABSTRACT

Whole and extracted pulps of Balanites aegyptiaca *fruits were tested as a hypocholesterolemic agent using adult albino rats. Food intake, gain in body weight, food efficiency ratio and liver/body weight ratio were determined. The addition of 10% whole or extracted pulp to the diet at the expense of starch caused a highly significant decrease in the serum and liver cholesterol and serum protein levels when compared with a positive control (hypocholesterolemic diet). This means that the pulp contains a powerful hypocholesterolemic agent. Phytochemical screening of alcoholic extracts of pulp and kernel shows that sterols, terpenes and saponins are the predominant natural compounds in both pulp and kernel, while tannins, alkaloids and resins appear in slightly lower amounts. Serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) activities were estimated before and after the addition of whole and extracted pulp. Both activities were inhibited by the hypocholesterolemic diets but the decreased values were higher than that of control animals.*

INTRODUCTION

It is well known that many plants contain hypocholesterolemic agents; among these, onion bulb (Youssef *et al.*, 1981), onion seeds (Hanaa Doas, 1984) and many other plants such as garlic (Jain & Vyas, 1977; Sharma & Sharma, 1979 and Youssef *et al.*, 1981) have been cited. Fenugreek mucilage also decreases the serum cholesterol level in blood (Ghali *et al.*, 1981).

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Several investigators have shown that serum and lever cholesterols are decreased by dietary saponin (Tayeau & Nivet, 1956; Paul & Hans, 1958; Newman *et al.*, 1958).

For these reasons the present investigation was carried out to study the relationship between *Balanites aegyptiaca* fruits (as uncommon foodstuffs containing a high amount of saponin) and cholesterol levels in serum and liver, as well as the liver function of albino rats.

MATERIALS AND METHODS

Samples

Whole pulp fruit was extracted with 80 % ethanol in a Soxhlet apparatus for 6 h. The residue was air dried and kept for rat feeding. In another experiment the whole pulp fruit was used for rat feeding.

Experimental animals

Forty male albino rats were fed on a basal diet for 8 days, then divided into five groups (eight rats each). The first group was fed during the experimental period on the basal diet and considered as a negative control. The remaining four groups were fed on a hypercholesterolemic diet. The duration of the experiment in the first stage was 2 weeks. Each rat was weighed every 2 days, gain in body weight, food intake and food efficiency being determined. At the end of this period, one group of hypercholesterolemic rats was killed and serum cholesterol, liver cholesterol and liver/body weight ratios were determined. In the second stage, which was also prolonged for 2 weeks, all of the above-mentioned parameters were determined. Whole and extracted pulps of *Balanites aegyptiaca* fruit were added to the hypercholesterolemic diet (10 % at the expense of starch). The percentage compositions of the diets used are shown in Table 1. Cholesterol was determined according to the method of Henly & Zake (1957). Total protein was determined as described by Gornall *et al.* (1949). Transaminases (GOT and GPT) were determined according to the method of Ritman & Frankel (1957). Saponin was estimated according to the method of Brand (1924) modified by Kofler & Adam (1927). Flavonoids were estimated according to the method of Snell & Snell (1954). Phenols were determined according to the technique

TABLE 1
Diet Compositions (%)

Component	*Diet*			
	Negative control[a]	*Positive control*[b]	*Whole pulp*	*Extracted pulp*
Casein	15	15	15	15
Lard	15	15	15	15
Cellulose	5	5	5	5
Salt mixture[c]	4	4	4	4
Vitamin mixture[d]	1	1	1	1
Cholesterol	—	1	1	1
Bile salts	—	0·25	0·25	0·25
Starch	60	58·75	48·75	48·75
Whole pulp	—	—	10	—
Extracted pulp	—	—	—	10

[a] Negative control: basal diet.
[b] Positive control: hypercholesterolemic diet.
[c] Hegested *et al.* (1941).
[d] Campbell (1961).

of Swain & Hillis (1959). Phytochemical screenings were carried out for the presence of tannins and flavonoids according to the procedures of Wall *et al.* (1954), for sterols and terpenes (Fiesher, 1949, for alkaloids (Robinson, 1955), and for saponin and resins (Trease, 1961). Statistical analysis was evaluated using the analysis of variance (*F*-test) (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

The results of food intake, weight gain and food efficiency of rats fed on normal and hypercholesterolemic diets for the first 2-week period are reported in Table 2. It is clear that the addition of cholesterol and bile salts to the basal diet caused a decrease in the average daily food intake, daily gain in body weight and, consequently, food efficiency ratio. It seems probable that glucose residues of the starch may form a certain complex with the bile salts and cholesterol which prevents its absorption.

Table 3 shows that whole and extracted pulp, when mixed with the

TABLE 2

Average Daily Gain in Body Weight, Daily Food Intake, Food Efficiency, Liver/Body Weight Ratio, Liver Weight, Serum and Liver Cholesterol of Rats on a Hypercholesterolemic *Diet*

Diet	*Daily increase in body weight (g)*	*Daily food intake (g)*	*Food efficiency*	*Average liver weight (g)*	*Liver/body weight ratio*	*Serum cholesterol (mg/100 ml)*	*Liver cholesterol (mg/100 ml)*
Negative control	3·4	9·5	0·360	6·23	0·37	82·5	195
Standard deviation	±1·21	±3·81	±0·21	±2·67	±2·13	±12·7	±17·6
Hypercholesterolemic diet as a test period	3·18	9·0	0·353	6·50	0·047	230	324
Standard deviation	±2·01	±4·20	±0·11	±3·11	±0·02	±18·1	±22·1

TABLE 3

Means[a] of Daily Gain in Body Weight, Daily Food Intake, Food Efficiency[b], Liver/Body Weight Ratio, Serum and Liver Cholesterol of Hypercholesterolemic Rats after Adding Whole and Extracted Pulp of *Balanites Aegyptiaca* Fruits Compared with Positive and Negative Control

Diet	*Daily gain in body weight (g)*	*Daily food intake (g)*	*Food efficiency*	*Liver/body weight ratio*	*Cholesterol in serum (mg/100 ml)*	*Cholesterol in liver (mg/100 g)*	*Liver weight (g)*
Negative control (Normal control)	3·4	10·0	0·340	0·037	82·5	195	6·23
Positive control (hypercholesterolemic diet only)	2·8	8·66	0·323	0·047	252	402	6·5
Hypercholesterolemic diet and whole pulp	2·5	9·0	0·277	0·040	130	304	5·97
Hypercholesterolemic diet and extracted pulp	2·06	8·66	0·233	0·042	148	336	6·11

[a] Average of eight rats. [b] Gain in body weight/food intake.

hypercholesterolemic diet, caused a decrease in the average gain in body weight; this decrease was more pronounced in the case of extracted pulp than in the case of whole pulp. The possibility of an inhibitory effect of *Balanites aegyptiaca* fruits is discarded from the fact that, in the case of the extracted pulp, the decrease of gain in body weight was more than that observed for whole pulp when compared with negative or positive controls.

Values of food intake for rats fed the hypercholesterolemic diet were slightly more altered than those fed the normal diet, while neither hypocholesterolemic diets caused much alteration when compared with the positive control. These results support our hypothesis that the digestion of starch may be inhibited somehow by the addition of bile salts because food consumption decreased in the case of both hyper- and hypocholesterolemic diets and gain in body weight was also less. The present results are in agreement with those of Menon & Kurup (1976).

Results obtained for rats fed the hypercholesterolemic diet showed a slight increase in the average fresh liver weight by 4% when compared with the negative control group. Also, the liver/body weight ratio was increased in both groups. These results did not alter more than those of hypocholesterolemic animals.

Liver and serum cholesterol

Liver cholesterol for animals fed normal, hyper- and hypocholesterolemic diets are presented in Tables 2 and 3. The present data show that the increase in liver cholesterol was about 106% in the case of the hypercholesterolemic diet but 56% and 72% for rats fed the hypocholesterolemic diet (whole and extracted pulp, respectively) compared with the negative control. It is clear that the pulp of *Balanites aegyptiaca* fruits contains a powerful hypocholesterolemic agent. The effect of pulp in decreasing the cholesterol levels was probably partially due to fibre content and mucilage (Ghali *et al.*, 1981).

Table 4 shows the phytochemical screening of alcoholic extracts of pulp and kernel of *Balanites aegyptiaca* fruits. In both pulp and kernel, sterol, terpenes and saponin were the predominant natural compounds, while tannins, alkaloids and resins appeared in slightly lower amounts. Moreover, saponin, flavonoids and phenolic compounds were also quantitatively determined in the pulp (Table 5). Flavonoids are present in high amounts in the pulp, but further study to identify the type and

TABLE 4
Phytochemical Screening of Pulp and Kernel of *Balanites Aegyptiaca* Fruits

Components	*Pulp*	*Kernel*
Tannins	+	+
Sterols and terpenes	+ + +	+ + +
Flavonoids	+ +	+
Alkaloids	+ +	+
Saponin	+ + + +	+ + +
Resins	+	+ +
Phenols	+ +	+ +

\+ traces.
\+ + low concentration.
\+ + + medium concentration.
\+ + + + high concentration.

structure of these flavonoids must be undertaken. It could be concluded that the hypocholesterolemic agents of pulp of *Balanites aegyptiaca* fruits are principally contained in the alcoholic extracts. This was confirmed by the findings of the hypocholesterolemic power of alcohol-extracted pulp which was lower when compared with whole pulp (Table 3). They may be active agents such as certain thiocompounds, saponins and flavonoids. These compounds have already been reported to act as cholesterol lowering agents (Kiyoshi, 1969; Delic *et al.*, 1973).

Effect of *Balanites aegyptiaca* fruits on serum protein

Serum protein contents, as well as serum enzyme activities, of normal, hypercholesterolemic and both hypocholesterolemic diet-fed rats are recorded in Table 6.

TABLE 5
Total Phenols, Flavonoids and Saponin Content in the Pulp of *Balanites Aegyptiaca*

Constituents	*Percentage*
Total phenols	1·73
Total flavonoids	1·24
Total saponin	5·10

TABLE 6
Means and Standard Deviations of Serum Protein, GOT and GPT Activities in Hypercholesterolemic Rats

Rats	*Serum protein*	*GOT*	*GPT*
Negative control	6·97 ± 0·50	29·0 ± 2·66	11·6 ± 1·48
Positive control	11·0 ± 1·01	45·9 ± 2·98	20·8 ± 2·10
Whole pulp	9·14 ± 0·88	33·3 ± 2·93	14·2 ± 1·11
Extracted pulp	9·73 ± 1·00	37·0 ± 3·29	16·6 ± 2·00

Means: Average eight rats in each group. Serum protein: mg per 100 ml GOT and GPT IU (International Units).

The data clearly indicate that hypercholesterolemia elevated the protein level in rat serum when compared with the negative control. In this connection both hypocholesterolemic diets decreased the elevated protein level but they were still higher than that of normal diet-fed rats. Rats of the positive control and the *Balanites aegyptiaca* groups had enzyme activities higher than that of the negative control (normal rats). Hence the hyper- and hypocholesterolemic diets had a significant effect on the GOT and GPT activities. The present findings appear to show that hypercholesterolemic feeding might divert a higher percentage of the carbon moieties of amino acids toward lipogenesis. Hence lipogenesis is more accelerated in rats fed hypercholesterolemic diets than rats eating normal or hypocholesterolemic diets. It seems that, in hypercholesterolemic feeding, the animal cannot assimilate the amino acids of the diet with greater efficiency for protein anabolism. Hence, some of the absorbed amino acid is deaminated and the amino nitrogen excreted as urea. On this basis, a relationship might exist between protein metabolism and the increased body fat content of the rats fed the hypercholesterolemic diet. Our findings of serum protein (Table 6) confirm the transaminases (GOT and GPT) results. The serum protein contents were elevated in hypercholesterolemic rats but the hypocholesterolemic agents of *Balanites aegyptiaca* fruits reduced these levels.

REFERENCES

Brand, W. (1924). *Pharm. Ztg.*, **69**, 204 (*cf* Ghanem El-Hossary (1970). A pharmacognostical study of certain saponaria and valeriana species grown in Egypt. PhD Thesis, Fac. of Pharm., Cairo Univ.)

Campbell, J. A. (1961). *Methodology of protein evaluation.* RAG Nutr. Document R. 101 add. 37, June meeting, New York.

Delic, I., Vucurevic, N. & Stojanovic, S. (1973). Alfalfa saponin inactivation under *in vivo* conditions in mice. *Acta Vet.* (*Belgrade*), **23**(6), 291–5 (*cf Chem. Abst*, **82**, 71926, 1975).

Fiester, L. F. (1949). *Steroids.* Reinhold Publishing Corp., New York.

Ghali, Y., El-Nahry, F., Abdel-Rahim, E. A. & Wasif, M. M. (1981). *Effect of carbohydrates on cholesterol level in liver and serum. II. Polysaccharides.* First National Cong. of Biochem., Cairo.

Gornall, A. G., Bradawell, G. J. & David, M. M. (1949). Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.*, **177**, 751.

Hanaa, A. Doas (1984). *Biochemical studies on some Egyptian seeds.* PhD Thesis, Fac. of Agric., Cairo Univ.

Hegested, D. M., Mills, R. C., Elvehjem, C. A. & Hart, E. B. (1941). Cholin in the nutrition of chicks. *J. Biol. Chem.*, **138**, 459.

Henly, A. A. & Zake, B. (1957). Determination of serum cholesterol. *Analyst.*, **82**, 286.

Jain, R. C. & Vyas, C. R. (1977). Onion and garlic in atherosclerotic heart disease. *Medikon*, **6**(5), 12–14, 17–18. (*cf Chem. Abst.*, **87**, 116795).

Kiyoshi, K. (1969). Biologically active components in garlic. I. Thioglycoside. *Chem. Pharm. Bull.*, **17**(11), 2193.

Kofler, L. & Adam, Ph. A. (1927). *Arch. Pharm.*, **265**, 625. (Through *Chem. Abst.*, **22**, 478, 1928). (*cf* Ghanem A. El-Hossary). *A pharmacognostical study of certain saponaria and valeriana species growing in Egypt.* PhD Thesis, Fac. of Pharm., Cairo Univ., 1970.

Menon, P. V. & Kurup, P. A. (1976). Dietary fiber and cholesterol metabolism. Effect of fiber-rich polysaccharide from black gram (*Phaseolus mungs*) on cholesterol metabolism in rats fed normal and antherogenic diet. *Biomedicine*, **24**(4), 248.

Newman, H. A., Kummerow, F. A. & Scott, H. M. (1958). Dietary saponin, a factor which may reduce liver and serum cholesterol levels. *Poultry Sci.*, **37**, 42.

Paul, G. & Hans, F. (1958). Dietary saponin and plasma cholesterol in chicken. *Proc. Soc. Expt. Biol. Med.*, **99**, 424.

Ritman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer. J. Clin.*, **28**, 56.

Robinson, R. (1955). *The structural relations of natural products.* Oxford University Press.

Sharma, K. K. & Sharma, S. P. (1979). Effect of onion and garlic on serum cholesterol in normal subjects. *Mediscope*, **22**(7), 134. (*cf Chem. Abst.*, **92**, 127498y).

Snedecor, G. W. & Cochran, W. G. (1967). *Statistical methods* (6th edn), Iowa State Univ. Press., Ames., Iowa, USA, 393 pp.

Snell, F. D. & Snell, G. T. (1954). Colorimetric method of analysis. (3rd edn) D. Van Nostrand and Co., New York.

Swain, I. & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. *Sci. Food Agric.*, **10**, 63.
Tayeau, F. & Nivet, R. (1956). Esterification of cholesterol and serum lipoproteins. *Biochem. Problems Lipids, Proc. Intern. Conf. Second*, Ghent, 363, 1956 (*cf Chem. Abst.*, **52**, 13055 g, 1958).
Trease, G. E. (1961). *Textbook of pharmacognosy* (8th edn), Bailliere Tindall and Ox, London, 596.
Wall, M. E., Kreider, M. M., Kremson, C. F. & Eddy, G. R. (1954). Survey of plants for steroidal saponins and other constituents. *J. Pharm. Soc.*, 43–1.
Youssef, A. K., Aboul-Enein, A. M., Osman, S. A. & Hewedi, F. M. (1981). *Chemical analysis of Egyptian onion and garlic extracts* (*Hypoglycaemic extracts*). Ain Shams Univ., Agr. Sci. Bull., 1968.

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(Received: 19 July, 1985)