The number and affinity of platelet insulin receptors in non-insulin dependent diabetes mellitus were significantly reduced. The receptor number in parentheses shows the quantity of binding sites of the low-number–high-affinity receptor class; less than one-third of the control value could be detected. The reduction of the affinity constant and half maximal displacement values demonstrate the decreased affinity of platelet insulin receptors in diabetes mellitus (type 2).

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Emblica officinalis reduces serum, aortic and hepatic cholesterol in rabbits

C.P. Thakur

Patna Medical College, Patna–800004 (India), 16 February 1984

Summary. Emblica officinalis reduced serum cholesterol ($p < 0.001$), aortic cholesterol ($p < 0.001$) and hepatic cholesterol ($p < 0.001$) significantly in rabbits. *Emblica officinalis* did not influence euglobulin clot lysis time, platelet adhesiveness or serum triglyceride levels.
Key words. Emblica officinalis; cholesterol; atherosclerosis, rabbits.

Emblica officinalis (family Euphorbiaceae) grows in homeyards and deciduous forests[1]. The fruit (pulp and seeds), bark, roots, flowers and leaves of this plant have been used as ayurvedic medicines. Its pulp contains 13 separable tannins and colloidal complexes and is a rich source of vitamin C[2,3]. In ayurvedic literature it is mentioned that amla *(E. officinalis)* forms one of the important contituents of a drug (Chavanpras) which prevents ageing. This encouraged us to test the anticholesterolaemic effect of the dried pulp of the amla. It was shown in this laboratory that *E. officinalis* reduced serum cholesterol in rabbits[4]. We further showed that the hypocholesterolemic effect of *E. officinalis* was not entirely due to its vitamin C content and we suggested that there might be other substance(s) responsible for that action. We have extended this study further to examine the effect of *E. officinalis* on cholesterol contents of liver and aorta in experimentally produced atherosclerosis in Indian albino rabbits.

Materials and methods. 70 Indian albino rabbits (half male, half female), weighing 1.1–1.6 kg, and aged 8–10 months, were fed daily on 25 g of Bengal gram, 15 g of maize flour, green grass and drinking water ad libitum. Pre-experimental fasting blood samples for estimation of serum lipids, euglobulin clot lysis time and platelet adhesiveness were collected from an ear vein. Each rabbit was fed with cholesterol 0.1 g/kg b.wt in addition to the routine diet to start with for two weeks. On the basis of preliminary cholesterolemic response, b.wt, age and sex, 50 out of the 70 rabbits were randomly allocated into two groups, A and B. Any rabbit showing a very high or low cholesterolemic response was excluded. The animals were treated as follows:

In addition to the diet, group A received cholesterol 0.3 g/kg b.wt and group B received cholesterol in the above dosage plus *E. officinalis* 1 g/kg b.wt (table 1). The pulp separated from the fresh fruits was dried, powdered and then used.

Serum cholesterol[6] and triglyceride[7] were estimated fortnightly. The experiment lasted for only 16 weeks. At the end of the experiment the blood was taken out for estimation of cholesterol, triglyceride, euglobulin clot lysis time[8] and platelet adhesiveness[9]. The rabbits were killed with an overdose of pentobarbitone, and the aortas were examined. The aorta was opened longitudinally from the aortic valve to the iliac arteries and was stained with a mixture of sudan IV and sudan III. After 10–12 min the aorta was washed with tapwater. Atherosclerotic lesions appeared as sudanophilic areas. Sudanophilic areas were traced on to paper and then the tracings from the whole surface were transferred to graph paper and the percentage involvement was calculated. The aortas of five rabbits from each group were kept for histopathological examination and the aortas of 20 rabbits from each group were kept for cholesterol estimation. The lipids were extracted by the method of Folch et al[11]. Paraffin sections were stained with H & E and van Gieson. Frozen sections were cut on a cryostat and stained with a mixture of sudan III and IV. Atherosclerotic involvement was graded according to the WHO recommendations[10]. Livers were removed and weighed, and an aliquot of liver was homogenized in chloroform-methanol (2:1)[11] and the extract used for determination of total cholesterol. The aortas were similarly homogenized in chloroform-methanol for extraction[11]

Table 1. Dietary regime, initial and final b.wts of animals

Groups	Number	Diet	Initial b.wt (g ± SD)	Final b.wt (g ± SD)	Mean weight gain (g)
A	25	Cholesterol 0.3 g/kg b.wt	1553 ± 190	1580 ± 180	27
B	25	Cholesterol in above dosage + *E. officinalis* (Amla) 1 g/kg b.wt	1540 ± 130	1579 ± 120	38.9

Table 2. Mean serum cholesterol and triglyceride levels during the experiment and their significance (mg ± SD)

Weeks on diet	Group A Cholesterol	Triglyceride	Group B Cholesterol	Triglyceride
Initial	74.4 ± 5.7	68.5 ± 2.2	82.4 ± 5.6	72.4 ± 6.4
12th week	593 ± 98.8	81.4 ± 10.8	241.6 ± 20.8	81.4 ± 7.8
16th week	632.8 ± 158	88.2 ± 15.4	116.8 ± 18.6	72.8 ± 14.8
Cholesterol 16th week: A:B $p < 0.001$				

Table 3. Liver and aortic cholesterol contents, aortic sudanophilia, euglobulin clot lysis time and platelet adhesiveness of the rabbits at the end of experiment

Groups	Liver weights (g mean ± SD)	Cholesterol content of liver (mg/100 g ± SD)	Cholesterol content of aorta (mg/100 g ± SD)	Euglobulin clot lysis time (h)	Aortic sudanophilia (%)	Platelet adhesiveness (mean percentage)
A	38.4 ± 6.5	616.4 ± 99.8	102 ± 21.3	1.18	34%	46.3
B	36.2 ± 5.8	52.4 ± 19.6 $p < 0.001$	35.4 ± 18 $p < 0.01$	1.28	6% $p < 0.001$	48.3

and the cholesterol content of the extract was determined. The serum, aortic and liver cholesterol were measured by the method of Sperry and Webb[6]. Statistical analyses were performed and Student's t-test was used to determine the significance[12]. The 5% level of probability was accepted as indicating a significant biological difference. In another experiment the dosage-response curve was obtained by feeding the rabbits with a graded dosage of *Emblica officinalis;* 500 mg, 300 mg, 200 mg, 100 mg and 50 mg/kg b.wt along with cholesterol 0.3 g/kg b.wt for four weeks.

Results. Animals of both the groups grew well and there was no significant difference between weights of rabbits of the two groups at the end of the experiment (table 1).

Serum lipids. Serum cholesterol continued to rise up to the 16th week in group A but declined after the 12th week in group B. The serum cholesterol level of group B rabbits remained at a lower level compared to group A rabbits throughout the experiment. At the end of the experiment there was a significantly higher level of serum cholesterol in group A compared to group B ($p < 0.001$) (table 2). There was no significant difference in serum triglyceride levels between the two groups at the end of the experiment.

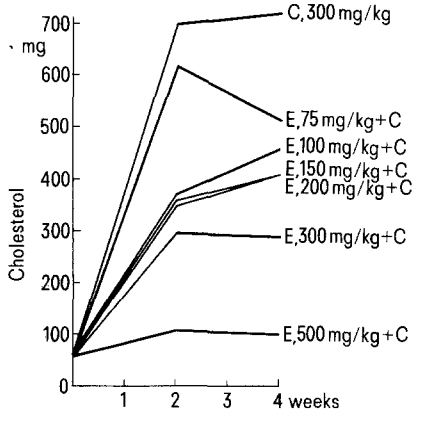
Aortic sudanophilia and cholesterol content of aorta. The aortas of group A showed significantly higher levels of sudanophilia than group B ($p < 0.001$). The cholesterol content of aortas of group B ($p < 0.001$) (table 3) was significantly lower than that of group A.

Liver weight and cholesterol content of liver. There was no significant difference between the weights of the livers of rabbits in the two groups. But the appearance was yellowish in group A and normal in group B. The cholesterol content of the liver of group A was significantly higher than the cholesterol content of the liver of group B ($p < 0.001$).

Platelet adhesiveness and euglobulin clot lysis time. There was no significant difference between the two groups with respect to these parameters (table 3).

Dosage-response curve (fig.). The minimum inhibitory dose was 100 mg/kg b.wt and the hypocholesterolemic effect increased with increasing dosage.

Discussion. The animals of both the groups grew well and there was no significant gain in weight in group B rabbits (*E. officinalis*-cholesterol fed) compared to rabbits of group A (only cholesterol fed). It was suggested by previous workers that *E. officinalis* caused a gain in weight in rabbits[3], but this study did not support this view as there was no difference in the weights of the two groups of rabbits at the end of the experiment. The *E. officinalis* reduced the serum cholesterol as well as the cholesterol content of liver and aorta. It was shown in previous experiments that this action of *E. officinalis* was not entirely mediated through its vitamin C content[5]. Vitamin C has been shown to reduce serum cholesterol in rabbits[13,14] and man[15], although others have failed to find a significant influence of ascorbic acid on serum cholesterol levels in rabbits[16,17] and man[18]. We think that there might be some other hypocholesterolemic and antiatherogenic agent(s) present in *E. officinalis,* but the mechanism of the hypocholesterolemic action of this plant, and the chemical identification of the substance responsible for the hypocholesterolemic action, need to be studied further. *E. officinalis* is a cheap fruit and is used for the preparations of various food items like sweets, pickles and chutneys, which are eaten by healthy persons without any untoward effects. Its hypocholesterolemic effect in human beings needs to be investigated.



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