

THE EFFECTS OF VARIOUS FRACTIONS OF GUM GUGGUL ON EXPERIMENTALLY PRODUCED HYPERCHOLESTRAEMIA IN CHICKS

By

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Atherosclerosis is manifested by the effects of occlusion of the circulation, giving rise to coronary heart disease, cerebrovascular disease and certain forms of peripheral vascular disease. The recognition by pathologists that lipid deposition is a characteristic feature of atherosclerosis, has emphasized the relationship between atherosclerosis and lipid metabolism. Whilst, there is enough circumstantial evidence implicating rise in serum lipids as a least one contributing cause, there is no definitely established pathogenesis for atherosclerosis in man. There is also no direct evidence as yet that lowering serum lipid levels will influence the progress of atherosclerosis or the prognosis of patients suffering from it. Any how, one approach to the treatment of atherosclerosis has been concerned with attempts to decrease the concentration of lipids in tissues and blood by lowering the serum cholesterol. To achieve this end, a large number of drugs like estrogens, thyroid hormone, triparanol, nicotinic acid, nicotinamide, sitosterol, neomycin and ethyl chlorphenoxyisobutyrate and plant sterols have been introduced in the past decade or so.

It has been mentioned in Sushruta that an old sample of guggul is highly effective in reducing the fat in the body and it has also been shown by Satyavati (1966) that guggul lowers the serum cholesterol, the phospholipid level and also the C/P ratio in hypercholestraemic rabbits. It was, therefore, thought worthwhile to see the effect of different fractions of guggul on male chicks rendered hypercholestraemic.

MATERIALS AND METHODS

Chicks were chosen for this study for two reasons. Firstly, because chicks can be made hypercholestraemic in seven days and have good resistance; secondly, to have work on additional species with this agent.

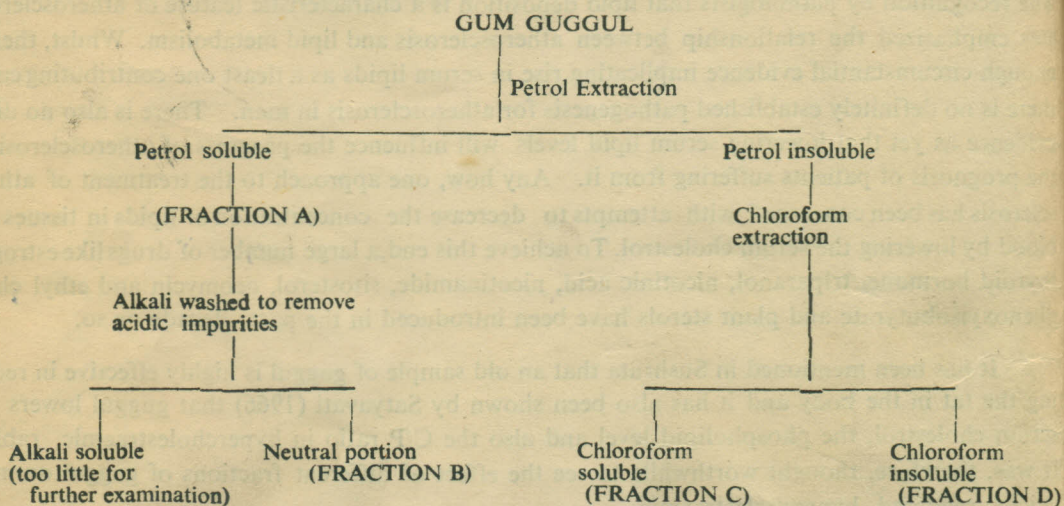
Healthy eight week old male white leg-horn chicks were selected for the present study to see the effect of different fractions of gum guggul on hypercholestraemia. The birds were acclimatized for one week before starting the experiment and the serum cholesterol and serum phospholipid levels were measured before the actual start of the experiment.

In the first set of experiments the birds were divided into five groups of six each. Hypercholestremia was produced by giving atherogenic diet (2 percent cholesterol and 5 percent cotton seed oil mixed with the low fat and low protein diet—plain mash) for two to three weeks. At this stage, serum cholesterol and phospholipids were again tested and if the increase was two to three fold, different groups were put on different fractions of guggul with one group serving as

control. The fractions 1 gm/kgm (average weight of the group) were mixed with the feed and were given to the chicks. At the start of administration of fractions, all the animals including the control ones, were again put back to only plain mash without any high cholesterol diet. Serum cholesterol, phospholipids and C/P ratio were tested after one week and two weeks of drug treatment.

In the second set of experiments the different fractions of guggul were given just after one week of acclimatization of the animals along with atherogenic diet to see whether the drug prevents any rise in serum cholesterol. The control group received only atherogenic diet. The serum cholesterol and phospholipid levels were estimated after one week and two weeks of giving the fractions.

Different fractions of Gum Guggul are as follows :—



ESTIMATION OF SERUM CHOLESTROL

Serum cholesterol was estimated by the modified method of Bloor et al., (1922) 0.2 cc of serum was added to 10 cc of 1:4 ether and absolute alcohol mixture and kept aside for 30-45 minutes at room temperature. Proteins get precipitated and removed off by centrifuging at 2500 r. p. m. The supernatant was evaporated to dryness by keeping the contents at 70-80°C temperature overnight in oven. The residue was dissolved in 5 cc of chloroform. Then 2 cc of acetic anhydride and 0.1 cc conc. sulphuric acid were added and mixed thoroughly. The reaction mixture was kept for 10 minutes in cool dark place. The developed colour was measured colorimetrically at 640 μ wave length.

Concentration of unknown was found by following formula :—

$$T/S \times 100 = \text{mg} / 100 \text{ cc of serum.}$$

T - represents optical density of unknown.

S - represents optical density of standard.

ESTIMATION OF SERUM PHOSPHOLIPIDS

The estimation was carried out according to the method of Youngberg and Youngberg (1930). The lipids were extracted from 0.05 ml. of serum by ether alcohol mixture similarly as in case of the cholesterol. To the residue, 0.5 ml. of 10 N sulphuric acid was added for the digestion, a few drops of hydrogen peroxide were sometimes required for perfect digestion. The volume was then made up to 10 ml. with distilled water. The addition of 1 ml. ammonium molybdate and ammonium hydroxide was followed by the development of colour which was measured at 630 μ immediately.

$$\text{Phospholipid mg/100ml.} = \frac{\text{O. D. of unknown}}{\text{O. D. of known}} \times 2000 \times \text{conc. of standard}$$

O. D. = Optical Density.

DISCUSSION AND RESULTS

Results are given in Table I and II. There is indirect evidence to suggest that maintenance of plasma lipoprotein concentration at low levels by drug or by diet might well reduce the incidence of coronary heart diseases and other forms of atherosclerosis. The desirability of therapy directed towards lowering plasma lipid and lipoprotein concentration is tentative, as sufficient clinical studies on the effect of lowering plasma lipid concentration have not been undertaken so far. Nevertheless, at present this is the only possible therapy, especially the lowering of cholesterol. This problem must be viewed from two view points, firstly, it should significantly reduce the level of cholesterol in hypercholestraemic people as it is seen that in familial hypercholestraemia the occurrence of myocardial infarction is frequent and secondly, any agent which is to be effective in atherosclerosis should prevent rise in plasma lipoproteins and must have large factor of safety as large number of people are involved in this and the treatment has to be prolonged.

TABLE I

Effect of Gum Guggul fractions on hypercholestraemic chicks (white leg-horn)

(A)

	Cholesterol level mg./100 ml. serum			C/P ratio		Percentage fall in total Cholesterol level
	Initial*	After Atherogenic diet**	After drug; treatment***	After Atherogenic diet	After drug treatment	
Control	103	270	113	0.83	0.41	58
Fraction-A	85	381	110	1.20	0.44	71
Fraction-B	71	337	93	1.60	0.44	71
Fraction -C	100	326	91	1.55	0.50	71
Fraction-D	60	283	112	1.50	0.70	58

(B)	Control	Fraction A	Fraction B	Fraction C	Fraction D
Fall in cholesterol level in atherogenic chicks on plain mash or drug.	270—113	381—110	337—93	326—91	283—112
Mean difference	157	271	244	235	154
Standard Deviation	± 42.82	± 64.50	± 73.05	± 30.53	$\pm 51 \pm 11$
Probability		0.01	0.025	0.05	

Cholesterol level at plain mash feeding.

Atherogenic diet (2% cholesterol 5% cotton seed oil) was given to produce hypercholesteremia. Cholesterol level after 2 weeks feeding of drugs (1 gm./kg. Av. wt.) with plain mash.

TABLE II

(A) *Effect of different fractions of Gum Guggul on cholesterol fed chicks (white leg-horn)*

	Cholesterol level mg/100 ml. serum		C/P ratio		Percentage reduction in cholesterol level with respect to control
	Initial*	Final**	Initial*	Final**	
Control	98	270	0.38	1.10	—
Fraction—A	106	238	0.42	0.93	27
Fraction—B	105	224	0.51	0.98	31
Fraction—C	110	241	0.51	0.90	24
Fraction—D	119	260	0.40	0.87	18

(B)	Control	Fraction A	Fraction B	Fraction C	Fraction D
Increase in cholesterol level while feeding the atherogenic diet with drug	98—270	106—232	105—224	0—241	119—260
Mean difference	172	126	114	138	142
Standard Deviation	± 69.2	± 27.07	± 34.91	± 19.14	± 19.91
Probability	± 0.1

*Cholesterol level and C/P ratio at Plain mash feeding.

**Cholesterol level and C/P ratio after 2 weeks of atherogenic diet and fractions were given to different groups including the control which was put only on atherogenic diet.

The present investigations reveal that fractions A, C and B of gum guggul lower the serum cholesterol significantly and also alter C/P ratio in this order. These fractions though prevent the rise in serum cholesterol when given along with atherogenic diet, the results however, are not statistically significant. The exact mechanism by which these fractions lower the serum cholesterol has not been elucidated as yet but the possibilities are that they might be acting by any one or combination of the following mechanism (i) inhibition of the lipid synthesis (ii) inhibition of lipid mobilization from the adipose tissue (iii) inhibition of fat absorption (iii) removal of lipoproteins from the plasma.

SUMMARY

The effect of different fractions of gum guggul was seen in experimentally produced hypercholestraemia in chicks. Experiments were also performed to see the effect of these fractions in the prevention of hypercholestraemia when given along with atherogenic diet.

Fractions A, C and B significantly lower the serum cholesterol. In this respect fraction A is most active, fraction C less and fraction B least as compared to control animals. As far as the prevention of increase in serum cholesterol is concerned, none of the fractions show any statistically significant effects.

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