ANTIARTHRITIC AND ANTI-INFLAMMATORY ACTIVITY OF GUM GUGGUL (BALSAMODENDRON MUKUL HOOK)*

By

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Gum guggul is an exudate obtained by incision of the bark of the shrub Balsamodendron mukul Hook. (synonym-Commiphora mukul Engl., N. O. Burseraceae; Hindi-guggul, Sanskrit–Koushikaha, Bengali–mukul, Arabic–mokhil). It occurs as vermicular or stalactic pieces golden yellow to brown or dull green in colour, aromatic and bitter in taste. It is also known as Indian bdellium. It is widely distributed throughout the Indian subcontinent particularly in Assam, Bengal, Mysore, Deccan, Khandesh, Kathiawar, Rajputana, Sind and Baluchistan. Some allied species occur in Arabia and Africa.

Gum guggul has been used in the Ayurvedic system of medicine since antiquity. The ancient Hindu texts like Atharva Veda, the Charaka and the Sushruta Samhitas and the various Nighantus as well as the modern texts like those of Watt (1889), Dey (1896), Chopra (1933) and Kirtikar-Basu (1933) describe its use in rheumatic and inflammatory conditions, indolent ulcers and boils, swellings and tumours, urinary and skin disorders, leprosy, bleeding and worm infestations and in nervous diseases and pain. It is highly reputed in the treatment of rheumatism given internally and applied locally. A large number of preparations compounded from this drug, such as 'Yograj guggul' and 'Mahayograj guggul', are in common use in muscular rheumatism and are said to be more effective when administered with the decoction of 'rasana'. According to Watt it was used in the native medicine as demulcent, carminative and alterative. Chopra (1933) described the gum as resembling copaiba and cubebs in pharmacological actions.

During a survey of the antiarthritic and anti-inflammatory effects of the indigenous drugs, Gujral and Saxena (1956) studied a 5% aqueous emulsion of the gum guggul and of Mahayograj guggul in Brownlee's formalin-induced arthritis in rats, but did not get any encouraging results. The later work of Gujral and coworkers (1957–60), under the ICMR Drug

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Research Unit (Arthritis), using the various fractions of gum guggul, revealed that only a particular fraction of the drug possessed this activity.

Karandikar et al. (1960) have observed significant activity in Mahayograj guggul. As gum guggul is only one of the ingredients of this complex multi-component Ayurvedic preparation, the above activity cannot be attributed to any single constituent without further evidence.

The present communication deals with the studies on gum guggul carried out in this laboratory since 1956.

MATERIALS AND METHODS.

Fractionation of gum guggul.— Gum guggul is an oleo-gum-resin obtained as an exudate from the bark of B. mukul (Chopra, 1933; Wehmer, 1935; Dutta et al., 1942). As the preliminary screening of the crude drug in the form of a 5% aqueous emulsion (Gujral and Saxena, 1956) did not show any promising results, it was thought advisable to test its various constituents after their separation.

The crude drug was directly extracted (Soxhlet) with petroleum ether, benzene, ether, chloroform and ethyl alcohol to separate the oleo-resin from the gum. On removal of the solvent under reduced pressure from the respective extracts, fraction D_1 (16–20%), D_2 (45–48%), D_3 (35–40%), D_4 (45–50%) and D_5 (50–55%) were obtained (Figure I). Different organic solvents were used to ascertain the complete extraction of the active material, if any, from the crude drug and to investigate whether any particular solvent could differentially extract the active and the inactive portions. As all the above fractions were found to be equally active, the crude drug was also submitted to successive extractions (Soxhlet) with petroleum ether (40–60°), benzene and alcohol (95%) to find whether this procedure could concentrate the activity in any single fraction. After the removal of the solvent under reduced pressure, fractions S_1 (18.6%), S_2 (30.6%) and S_3 (6.8%) were obtained (Figure I). The residue, left after extraction with alcohol, was dissolved in hot water, filtered to remove the insoluble materials like sand, woody matter and leaves etc., concentrated in a shallow dish on a water bath, cooled and treated with alcohol to precipitate the gum (S_4) which was filtered and dried in vacuum.

The direct alcohol extract, D_5 (oleo-resin), was further fractionated by thermal and cold-alkali treatments. The essential oil (D_{5-1}, 1.5–2%) was removed by steam distillation and the residue, after drying, was distilled in a Kugelrohr under high vacuum. It gave a yellow viscous oil (D_{5-2}, volatile fraction I, boiling range 70–150°/0.01 mm), a deep yellow viscous oil (D_{5-3}, volatile fraction II, boiling range 150–220°/0.01 mm) and a non-

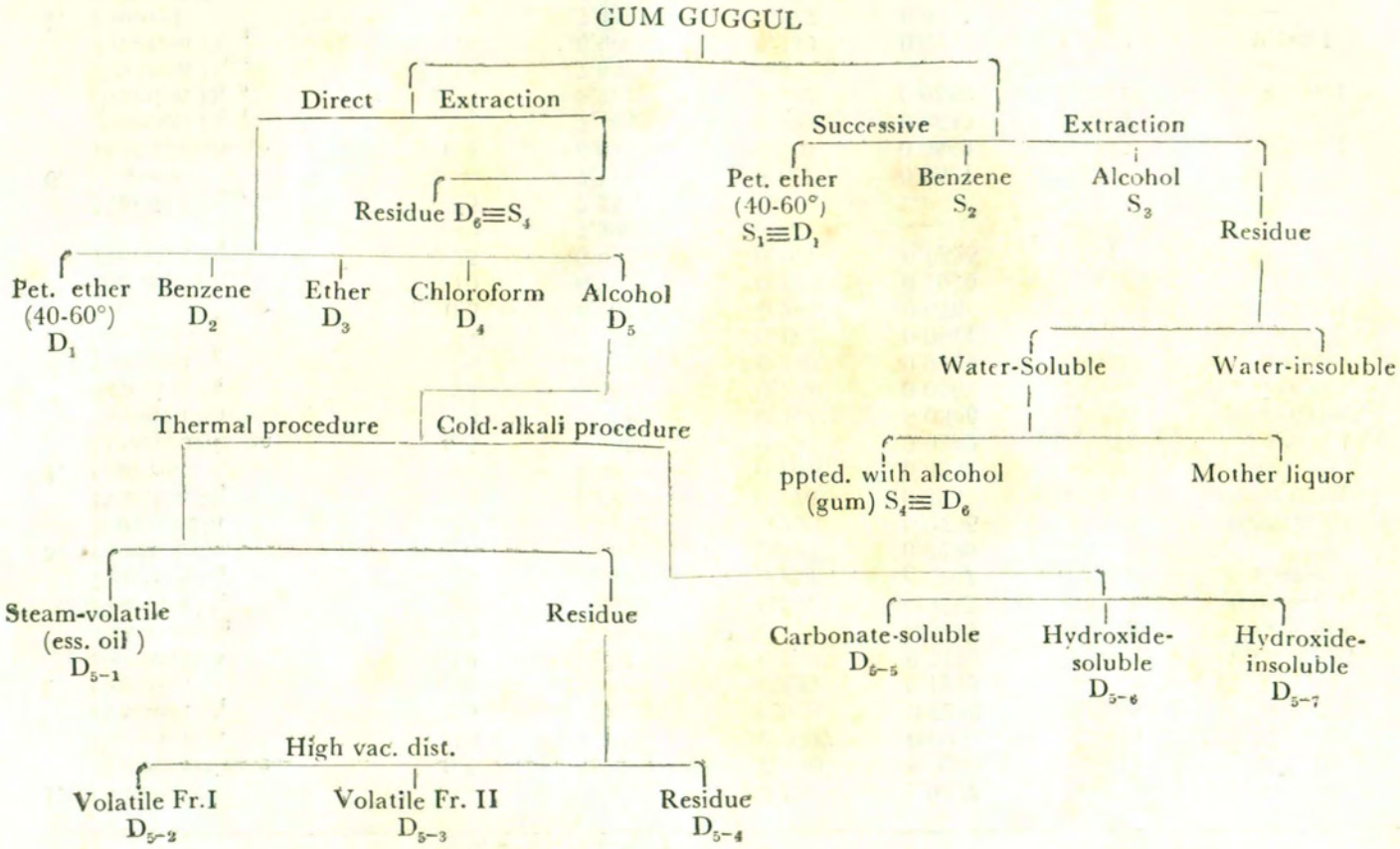
GUM GUGGUL
Direct
Extraction
Residue $D_6 \equiv S_4$
Pet. ether (40-60°) D_1
Benzene D_2
Ether D_3
Chloroform D_4
Alcohol D_5
Thermal procedure
Cold-alkali procedure
Steam-volatile (ess. oil) D_{5-1}
Residue
High vac. dist.
Volatile Fr.I D_{5-2}
Volatile Fr. II D_{5-3}
Residue D_{5-4}
Successive
Extraction
Pet. ether (40-60°) $S_1 \equiv D_1$
Benzene S_2
Alcohol S_3
Residue
Water-Soluble
Water-insoluble
ppted. with alcohol (gum) $S_4 \equiv D_6$
Mother liquor
Carbonate-soluble D_{5-5}
Hydroxide-soluble D_{5-6}
Hydroxide-insoluble D_{5-7}

Figure I

TABLE I

Effect of the various fractions of gum guggul on the mean ankle cross section (mm.) in formaldehyde-induced arthritis in rats.

	Drug	Dose (mg./100 gm.).	Mean cross section (mm.)	S. D.	S. E.	t	P
1.	Control	—	7.9825	0.15	0.0477	—	—
	Hydrocortisone	0.5	7.4608	0.080	0.0254	9.6611	<0.001
	Butazolidin	10	7.5206	0.0787	0.025	8.4425	<0.001
	Fraction D_1	25	7.5468	0.078	0.0248	8.0985	<0.001
2.	Control	—	8.1948	0.425	0.1353	—	—
	Butazolidin	10	7.8578	0.243	0.0773	2.1602	0.05–0.025
	Fraction D_2	25	7.5931	0.131	0.0417	4.2673	<0.001
	Fraction D_3	25	7.8323	0.255	0.0812	2.3561	0.05–0.025
	Fraction D_4	25	7.8405	0.241	0.0767	2.2711	0.05–0.025
3.	Control	—	7.3641	0.094	0.0299	—	—
	Butazolidin	10	7.0772	0.250	0.0796	3.4154	0.005–0.001
	Fraction D_5	25	7.0968	0.176	0.056	4.2428	<0.001
4.	Control	—	7.4858	0.107	0.034	—	—
	Hydrocortisone	0.5	6.8658	0.095	0.0302	13.5371	<0.001
	Butazolidin	10	6.8962	0.097	0.0308	12.8733	<0.001
	Fraction S_2	25	6.6841	0.176	0 056	12.2396	<0.001
	Fraction S_3	25	6.7832	0.248	0.0789	8.1697	<0.001
5.	Control	—	7.1157	0.192	0.0611	—	—
	Hydrocortisone	0 5	6.6016	0.245	0.078	5.1929	<0.001
	Butazolidin	10	6.7871	0.320	0.1019	2.7613	0.02–0.01
	Fraction S_4	25	6.9939	0.165	0.0525	1.511	0.2–0.1
		40	7.3060	—	—	—	—
	Fraction D_{5-1}	25	7.2870	—	—	—	—
6.	Control	—	7.1112	0.167	0.0531	—	—
	Hydrocortisone	0.5	6.640	0.210	0.0668	5.5435	<0.001
	Fraction D_{5-1}	15	7.1043	0.205	0.0652	0.0821	>0.9
	Fraction D_{5-2}	15	6.7247	0.122	0.0388	5.9461	<0.001
	Fraction D_{5-3}	15	7.637	—	—	—	—
	Fraction D_{5-4}	15	6.9904	0.230	0.0732	1.3422	0.2–0.1
7.	Control	—	7.4767	0.227	0.0722	—	—
	Butazolidin	10	6.914	0.430	0.1369	3.6538	0.005–0.001
	Fraction D_{5-4}	25	7.3175	0.220	0.070	1.592	0.2–0.1

volatile residue (D_{5-4}), which on rubbing with acetone and cooling, yielded a brown amorphous mass with a melting point range 280–320° (decomp.), Figure I. In the cold alkali procedure, fraction D_5 was treated successively with 1% Na_2CO_3 and 0.1% NaOH solutions. The alkali-soluble portions were acidified to precipitate the extracted "acids" which were centrifuged, washed and dried (fractions D_{5-5} and D_{5-6}). The hydroxide-insoluble portion was washed free of alkali and dried (fraction D_{5-7}), Figure I.

Testing. The antiarthritic and the anti-inflammatory activity of the above fractions was studied in Brownlee's formaldehyde-induced arthritis in albino rats of either sex weighing 100 ± 10 gm. The test fractions were administered in the doses mentioned (Table I) in the form of an emulsion in 5% gum acacia. The procedure followed was the same as reported earlier (Gujral et al., 1959).

RESULTS AND DISCUSSION.

Table I gives the effect of the various fractions of gum guggul on the mean ankle cross section (mm.) in formaldehyde-induced arthritis in rats with statistical analysis of the data. All the fractions obtained by direct-extraction of the crude drug with the different organic solvents possessed a highly significant activity as compared to hydrocortisone and butazolidin as reference standards. It showed that the active material could be extracted by any of these solvents although the total extractable material was different in all cases, petroleum ether bringing the least and alcohol the maximum amount. There was no differential separation of the active and the inactive material. The activity of the petroleum ether fraction (D_1) showed that the active principle was present either in the lower terpenoids or in the higher terpenoids of the oleo-resin, which were extracted by the solvent action of the lower terpenoids dissolved in the petroleum ether, or in both of them. As fractions S_1, S_2, and S_3, obtained by the successive extraction of the crude drug to decide between the above possibilities, also possessed high activity ($P<0.001$), concentrating the active principle in any single fraction by extraction with organic solvents seemed difficult. The gum portion of the crude drug (Fraction S_4) even upto 40 mg./100 gm. was found to be inactive. The antiarthritic and anti-inflammatory activity of the crude drug was therefore found to reside only in its oleo-resin part. The active principle contained in it seemed to be highly potent as even in the crude state in the doses mentioned, it possessed an activity comparable to that of hydrocortisone and butazolidin.

Further fractionation of the oleo-resin by the thermal procedure yielded fractions D_{5-1} to D_{5-4}. Fractions D_{5-1} (essential oil obtained by steam

distillation of the oleo-resin and consisting mostly of terpenes), D_{5-3} (collected at 150—220°/0.01 mm and consisting mostly of sesquiterpenes) and D_{5-4} the non-volatile residue, mostly di- and triterpenoids) were found to be inactive. The activity of fraction D_{5-2} (collected at 70–150°/0.01 mm and consisting mostly of terpenes and sesquiterpenes) suggested that the active principle or principles may be present, at least partially, in the lower terpenoid portions of the oleo-resin. The inactivity of fractions D_{5-3} and D_{5-4}, however, cannot rule out the possibility of the presence of some active material also in these higher terpenoid portions of the oleo-resin. The highly significant activity of fraction S_3, obtained under relatively mild conditions by the successive removal of the lower terpenoids (S_1 and S_2), suggests the thermo-labile nature of the active material present in these fractions (D_{5-3} and D_{5-4}). Fractions D_{5-5} to D_{5-7}, obtained by the cold-alkali procedure (adopted to avoid the above difficulty), consisting of acidic (resin acids), phenolic (resinotannols) and non-acidic (resenes) portions, gave erratic results in the biological testing. (These results have not therefore been included in Table I). This showed that the active principle, besides being thermolabile, might also be sensitive to alkali or acid treatment. The inactivation may probably be due to thermal–, acid– or base-catalysed isomerisation or decomposition which is so common in the biologically active terpenoids. Further work for the isolation of the active principle from the oleo-resin using still milder techniques (chromatographic or ion-exchange separation, countercurrent distribution etc.) will be reported elsewhere. These experiments have traced the antiarthritic and anti-inflammatory activity of the crude gum guggul to its oleo-resin portion with which further biological work is in progress.

SUMMARY

1. The antiarthritic and anti-inflammatory activity of gum guggul which had eluded detection in the preliminary screening has been shown to reside in the oleo-resin portion of the crude drug. The active principle or principles contained in it appear to be highly potent as compared to hydrocortisone and butazolidin used as reference standards.

2. The concentration of the active material from the oleo-resin by extractive procedures did not meet the requirements; its isolation using thermal- or alkali-treatment gave erratic results in biological testing probably because of its labile and sensitive nature. Work using milder techniques is in progress.

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