SOME PHARMACOLOGICAL INVESTIGATIONS OF EMBELIN AND ITS SEMISYNTHETIC DERIVATIVES

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Summary : Embelin, obtained from *Embolin ribes* was condensed with different primary amines. Depending on the conditions of reaction, disalts or diimines were formed. Ten such disalts and fourteen diimines were developed. Embelin and all its disalts showed analgesic activity whereas all the diimines derivatives were inactive. The disalt, 2:5 disobutly amine embelin showed maximum action. Analgesic effect was noticed only after intraperitoneal administration but not after subcutaneous, intramuscular or oral administration. The compounds cause some local irritation. The possibility of peritoneal irritation rendering the animals unresponsive to experimental pain seems to deserve consideration. However, analgesic effect could be seen in dogs and cats after intravenous injection. Embelin and its disalt, 2:5 isobutyl amine embelin also exhibited antipyretic and antinflammatory activities.

Key words: Embelin semisynthetic disalts analgesia

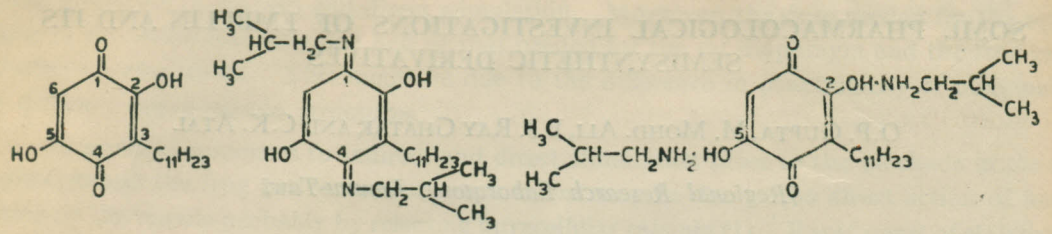
INTRODUCTION

Embelia ribes. Burm (Sans. Vidanga) is a large shrub which grows throughout India. Its berries are reputed in Ayurvedic system of medicine as anthelmintic and alterative, as tonic and for soothing effect on digestive disorders, for strengthening the body and preventing the effect of age and for control of conception (7, 18, 29, 36). The berries as also embelin isolated from these exert anthelmintic activity as reported by Paranjpe, and Gokhale (32) and by others (15,28,33), antibacterial (12) and recently reported for antifertility activity in rats (1,34). The first chemical investigation of the berries appears to be that of Scott (Chemist and druggist, p. 241, 1888), quoted by Heffter and Feuerstein (17) and subsequently investigated by many other workers (2,11,16,21, 30 and 39). The structural formula elucidated by these workers (Fig. 1) revealed that it is a p-quinone. The condensation reaction of embelin with primary amines for preparing diimines has also been reported (31). The present study was taken up because the survey of the literature showed that detailed pharmacological investigation of embelin was not reported. Moreover its derivatives were made with the hope of developing new pharmacologically active compounds. The chemistry part of the work is being communicated separately (38).

MATERIALS AND METHODS

Dry berries of *Embelia ribes* were powdered and extracted with n-hexane. The extract on concentration and cooling deposited crude embelin which on crystallisation from ethanol afforded glistening orange crystals (m.p. 140-141°C). Pure embelin thus obtained was condensed with different primary amines under different conditions of reaction. At low temperature

(optimum 0-5°C) ten disalts and at room temperature fourteen diimines were obtained (Fig. 1). These 24 derivatives were investigated pharmacologically. Embelin and its derivatives were in-



EMBELIN | 1:4-ISOBUTYL DIIMINE EMBELIN | 2:5-DIISOBUTYL AMINO EMBELIN

Fig. 1: Structural formulae of Embelin, its diimine and disalts.

soluble in water. Their gum acacia suspension was used in pharmacological investigations (for 100 *mg* compound, equivalent weight gum acacia and 10 *ml* water). In most of these experiments, morphine was used as positive control and 1% gum acacia in normal saline as negative control.

Analgesic activity was tested :

In rats—Hot nichrome wire analgesiometer method (14).
—Measured caudal compression method (13).

In mice—Contact heat method using hot plate at 55-55.5°C (9).
—Caudal immersion in hot water, at 58 ± 0.5°C (3) and
—Acetic acid (23) and phenylquinone (10) induced writhing movement.

In chicks—Squatting posture after injection in wing vein (37).

In dogs, cats and rabbits—Observing the reaction to needle prick on the paws after systemic administration.

Anti-inflammatory activity :

It was tested by using carrageenin induced oedema (41) and cotton pellet granuloma formation (6).

Antipyretic activity :

It was tested against pyrexia induced by brower's yeast in rats (4).

C.N.S. effects :

Gross observations of C.N.S. effects, aggregate amphetamine toxicity (5) and sleeping time potentiation (24) were studied.

Gastrointestinal propulsion :

This was studied by charcoal meal method in 24 hours fasted mice (19). Effect on isolated guinea pig ileum was also studied.

Absorption studies :

Absorption of embelin and its disalt, 2:5 diisobutyl amino embelin (IBAE-1) with various emulsifying, solubilizing and absorption promoting agents (25,40) was studied through oral, subcutaneous and intramuscular routes. It was also studied after their administration into a

loop of inteastine tied at either end, into the stomach after ligating pyloric end and through an indwelling polythene tube passed into the duodenum in unanesthetized dogs.

Local irritant effects :

This was studied in rabbits by dye infiltration method (27).

Acute toxicity :

LD_{50} was determined by the Karber's method (20).

RESULTS

Embelin and its 10 disalts showed clear analgesic activity and the 14 diimines showed none, in the initial testing by hot nichrome wire analgesiometer method. IBAE-1 showed more analgesic activity than the others including embelin. Therefore further detailed work was conducted on IBAE-1, with embelin and morphine as positive controls.

1. **Analgesic activity :**

In rats—(Table I) : Hot nichrome wire analgesiometer method—Embelin and IBAE-1 in 5-15 *mg/kg* doses i.p. raised the pain threshold time. The increase was generally dose depen-

TABLE I : Analgesic activity of embelin and its semisynthetic derivative IBAE—I in rats.

Drug dose mg/kg, & route	*Hot wire analgesiometer*			*Measured caudal compression*	
	Average reaction time in secs. before treatment	*% of animals showing* > 15 *secs reaction time after treatment* (15'–30')	*Average maximum reaction time in secs of animals showing* < 15 *secs. time* (15'–30')	*Average struggle threshold in mm Hg before treatment*	*Average maximum struggle threshold in mm Hg after treatment* (15'-30')
Embelin					
5, i.p.	2.4	Nil	6.5	72	90
10, i.p.	2.6	30	8.4	63	96
15, i.p.	2.3	55	10.0	68	120
20, s.c.	2.6	Nil	2.9	65	67
50, p.c.	2.2	Nil	2.1	65	72
IBAE-I					
5, i.p.	2.3	20	8.2	66	94
10, i.p.	2.4	50	11.5	64	110
15, i.p.	2.3	70	14.5	60	158
20, s.c.	2.5	Nil	3.5	74	76
50, p.o.	2.4	Nil	2.8	68	76
Morphine					
5, s.c.	2.3	Nil	12.6	75	162

At each dose level 15–20 animals were used.

dent. IBAE-1 was found to be more potent than embelin. The peak effect was recorded between 15-30 min and the effect slowly declined in about 3 hours. No analgesia was observed

when IBAE-1 was given by oral and subcutaneous routes. Morphine in 5 *mg/kg* dose by subcutaneous route produced less analgesia than 10 *mg/kg* i.p. dose of IBAE-1.

Measured caudal compression method—15 *mg/kg* (i.p.) of IBAE-1 and 5 *mg/kg* (s.c.) dose of morphine produced comparable analgesia.

In mice—(Table II) : Contact heat method using hot plate—IBAE-1 (15 *mg/kg* i.p.) and morphine (5 *mg/kg* s.c.) exerted comparable analgesic effects.

Table II : Analgesic activity of embelin and its semisynthetic derivative IBAE-I in mice.

Drug dose mg/kg, & routed	*Hot plate at* 55 + 0.5°C		*Caudal immersion in water* 58 + 0.5°C.		
	Average reaction time in secs. before treatment	*Average maximum reaction time in secs. after treatment* (15'–30')	*Average reaction time in secs. before treatment*	*% of animal showing* > 6 *secs. reaction time after treatment* (15'–30')	*Average maximum reaction time in secs. of animals showing* < 6 *secs. time after treatment* (15'–30')
1	2	3	4	5	6
Embelin					
5, i.p.	5.5	8.7	2.1	–7	3.1
10, i.p.	6.1	10.6	2.4	20	3.8
15, i.p.	5.8	13.8	2.5	35	4.3
20, i.p.	4.9	5.7	2.3	—	2.3
50, i.p.	5.6	6.0	2.5		2.6
IBAE-I					
5, i.p.	5.4	11.3	2.2	—	3.8
10, i.p.	5.6	16.0	2.1	25	4.2
15, i.p.	4.6	21.6	2.6	55	5.1
20, s.c.	6.5	7.0	2.3	—	2.4
50, p.o.	5.8	5.5	2.6	—	2.6
Morphine					
5, s.c.	5.5	20.5	2.3		4.7

At each dose level 15–20 animals were used.

Caudal immersion in hot water—IBAE-1 in 10 *mg/kg* dose by i.p. route showed more analgesic activity than 5 *mg/kg* s.c. dose of morphine.

Acetic acid and phenylquinone induced writhing movements—In 5,10 and 15 *mg/kg* doses through i.p. route, IBAE-1 abolished writhing movements in 25, 45 and 65% animals. Morphine by s.c. route in 3 and 5 *mg/kg* doses abolished the writhing responses in 42 and 72% animals respectively.

In all the experiments described above, however, IBAE-1 and embelin in 20 *mg* and 50 *mg/kg* dose by s.c. and oral routes respectively in rats and mice (Table I and II) did not show

any analgesic activity. Pretreatment with nalorphine or naloxone (5-10 *mg/kg* i.p.) did not detectably reduce the analgesia observed with IBAE-1 by the above tests in mice. Erection of tail (Straub phenomenon) was also not observed with these compounds in mice.

In chicks—(Fig. 2) : Squatting posture—IBAE-1 (5-10 *mg/kg*) injected in the wing vein of 2 months old chicks produced squatting posture. Morphine (20 *mg/kg*) i.v. produced a similar

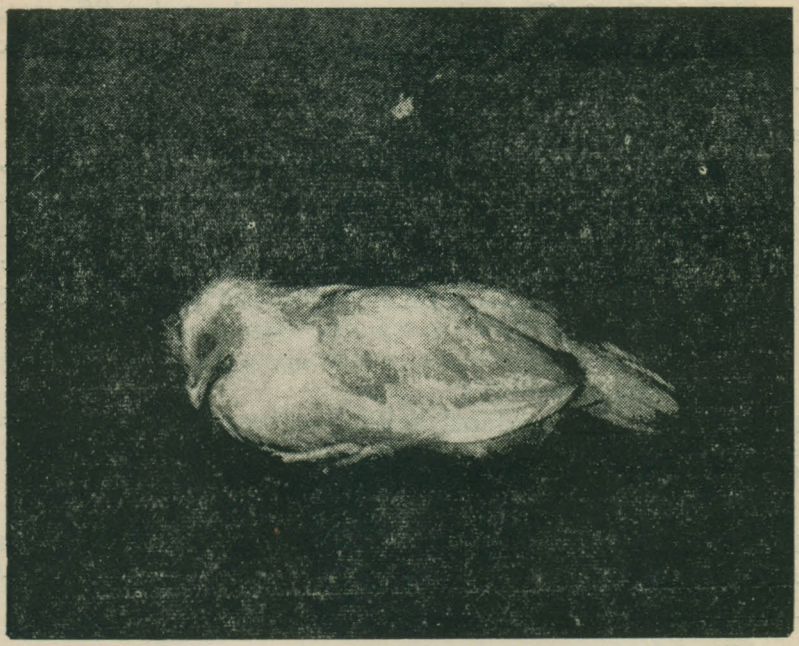


Fig. 2 : Squatting posture in chicks after IBAE-I (5-10 *mg/kg*, i.v.).

posture. In this study, analgesic action was also tested by applying bull dog clamp to the toe. IBAE-1 treated chicks showed analgesia. Morphine (20 *mg/kg*) did not show any evidence of analgesia as also reported earlier (37).

In dogs, cats and rabbits: Reaction to the needle prick—IBAE-1 and embelin in 5-10 *mg/kg* i.p. dose in rabbits, cats and dogs abolished the reaction to needle prick applied to the paws. The onset of effect came in 4-6 min and lasted for 2-3 hrs in dogs and 6-10 hrs in cats. Analgesic effect in equivalent doses after intravenous administration was less marked and lasted for shorter duration. After i.p. administration of IBAE-1 and embelin, signs of restlessness were observed in some of these animals, 2-3 and 5-8 min respectively after their administration.

Some side effects like nausea, vomiting, defecation and anorexia were observed in dogs and cats in 10-15 min of i.p. and i.v. administration. Dilatation of pupil was also observed in cats which lasted for the duration of analgesic effect. There was no morphine rage like syndrome in cats.

2. **Antiinflammatory activity :**

IBAE-1 inhibited carrageenin induced oedema by 35, 52 and 67% and cotton pell induced granuloma formation by 35, 56 and 74% in 5,10 and 15 *mg/kg* i.p. doses respectivel Embelin in equivalent doses showed 28, 39 and 48% inhibition by the former and 25, 42 a 51% by the latter method.

3. **Antipyretic activity :**

An average fall of 1.7 to 3.1°F rectal temperature was observed at different time interva in the course of 3 hours time with 10 *mg/kg* i.p. dose of IBAE-1 and embelin respectively again brewer's yeast induced pyrexia in rats.

4. **Central nervous system effects :**

After i.p. administration of IBAE-1 or embelin in dogs, cats and rabbits, the anima stood or lay down calmly and quietly. The animals did not react on provocation and the furio animals turned docile. Rats and mice also showed similar effects and their spontaneous mot activity was reduced. There was no induction of sleep or any effect on the general awarene of the animals, or aggregate amphetamine toxicity in mice and pentobarbitone (30 *mg/kg* i.p sleeping time in rats.

5. **Effect on gastro-intestinal propulsion :**

IBAE-1 in 10 *mg/kg* i.p. dose reduced the distance traversed by charcoal meal in 20 min tes by 30-40% as compared with control groups.

6. **Effect on isolated guineapig ileum :**

IBAE-1 in 250 to 500 *µg/ml* conc. in bath fluid caused slow developing contracti which was partly inhibited by prior treatment with atropine or mepyramine. Acetylcholi histamine and barium chloride responses were not affected by IBAE-1.

7. **Absorption studies :**

Tested in dogs and rats, IBAE-1 and embelin failed to show any analgesic action throu s.c. and i.m. routes when tried even with different solubilizing and absorption promoti agents like propylene glycol, DMSO, tween 20 and 80, ground nut oil, carboxy methyl cellulo polyethylene glycol 200 and 400 and polvinylpyrrolidine. Orally also, IBAE-1 as such or em sified with above agents and enclosed in the enteric coated capsules or after intraduodenal a ministration in unanesthetized animals through indwelling polythene tube upto 50-100 *mg* failed to show any analgesic action. Intragastric and intestine loop absorption studies a showed lack of absorption. Also, there were no signs of side effects after oral administrati as observed after i.p. or i.v. route.

8. **Local irritant effects :**

Slight to moderate degree irritation effect by dye infilteration method in rabbit was served with both IBAE-1 and embelin.

9. **Acute toxicity :**

LD_{50} of IBAE-1 and embelin by i.p. route was 62 and 44 *mg/kg* respectively (24 ho mortality).

10. **Study with an analoque and sodium salt of embelin :**

In our attempts to solve the problem of absorption through routes other than i.p., an analogue of embelin, 2:5 dihydroxybenzoquinone which lacked in side chain and sodium salt of embelin which were soluble in water were prepared and tested for analgesic activity. On i.p. administration in dogs both showed marked signs of irritation characterized by vocalization and body movements and there was no analgesia when tested by needle prick both after i.p. and i.v. administration. Embelin and IBAE-1 were found to be without any taste whereas the analogue and sodium salt of embelin were found to be sharply irritant.

DISCUSSION

The foregoing findings indicate that embelin and all its 10 disalts (formed due to reaction of primary amines at OH groups of embelin) showed analgesic, antipyretic and antiinflammatory properties. On the other hand all 14 diimines of embelin (formed due to reaction of primary amines at its keto groups) did not show analgesic action. This suggests that presence of free keto groups at 1:4 position are essential for analgesic activity. In addition, the presence of side chain at position 3 of embelin was also essential as 2:5 dihydroxybenzoquinone which resembled embelin except for absence of side chain, did not show any analgesic action. Compared with embelin, IBAE-1 had the advantage that its analgesic action was quicker to manifest, more marked and lasted longer.

Both IBAE-1 and embelin on the nature of their activities seem to resemble and differ from both narcotic and antipyretic type of analgesics. Observations in favour of former type are (a) high potency of analgesic activity (b) dilation of pupil in cats (c) induction of squatting posture in chicks (d) reduction of gastrointestinal motility and slow developing contraction of the isolated intestine (e) and presence of some side effects like nausea, vomiting and defecation as also encountered with morphine. On the other hand presence of (a) antipyretic and antiinflammatory properties (b) failure of antagonism of analgesia by nalorphine and naloxone and absence of (c) respiratory depression (d) Straub tail phenomenon (e) and morphine rage indicate that embelin and IBAE-1 may be belonging to antipyretic type of analgesics.

The presence of analgesic activity after i.p. administration and its absence after s.c., i.m. and oral administration was either due to lack of absorption through these routes or it could be that these compounds were causing peritoneal irritation which may be rendering these animals non-responsive to external pain stimulus. But the presence of analgesia after i.v. administration though less marked is against such a possibility. The existence of such possibility was also not supported by our findings with 2:5 dihydroxy benzoquinone, which on i.p. administration caused marked signs of irritation unaccompanied by analgesic action and absence of analgesia after i.v. administration. Therefore, it seems that the analgesic effect recorded was genuine but it was not clear why IBAE-1 and embelin while effective through i.p. route, should fail to show analgesia through other routes.

In gross observations, IBAE-1 and embelin seemed to exert some sedative effect on the treated animals but the lack of their efiect in amphetamine aggregate toxicity and pentobarbitone sleeping time did not substantiate it.

The pain employed in experimental studies is usually brief, sharp, accompanied by a sense of security and is relatively unresponsive to analgesics (8,25). On the other hand pathological pain is prolonged, associated with anxiety and respond to analgesic drugs (22). Therefore, it is possible that IBAE-1, like morphine may prove to be effective in clinical pain (35). However the intravenous route of administration would seem to be unacceptable.

The absence of straub tail phenomenon in mice and morphine rage like syndrome in cats, the two tests which are considered predictive of addiction liability of narcotic analgesics and which were not exhibited by IBAE-1, indicated that IBAE-1 may not have significant addiction liability.

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REFERENCES

1. Arora, R.B., N. Ghatak and S.P. Gupta. Antifertility activity of *Embelia ribes*. *J. Res. Indian Med.*, **6** (2) : 1–5, 1971.
2. Asano, M. and K. Yamaguti. Hydroxyquinones II. Constitution and synthesis of embelin. *J.Pharm. Soc. Jpn.*, **60** : 105–115, 1940.
3. Ben-Bassat, J., E. Peretz and F.G. Sulman. Analgesiometry and ranking of analgesic drugs by the receptacle method. *Arch. Int. Pharmacodyn. Ther*, **122** : 434–447, 1956.
4. Burn, J.H., D.J. Finney and I.G. Goodwin. Biological Standardization, London, New York and Toronto, Oxford University Press, P, 313, 1950.
5. Burn, J.H. and R. Hobbs. A test for tranquilizing drugs. *Arch. Int. Pharmacodyn. Ther.*, **113** : 290–295, 1958.
6. Bush, I.E. and R.W. Alexander. An improved method for assay of antiinflammatory substance in rats. *Acta Endocrinol.* **35** : 268–271, 1960.
7. Chopra, R. N., S. L. Nayar and I. C. Chopra. Glossary of Indian Medicanal Plants., New Delhi, Council of Scientific and Industrial Research, p. 106, 1956.
8. Grossland, J. Lewis's Pharmacology. 4th edn. Edinburgh and London, E. & S. Livingstone, p. 648, 1970.
9. Eddy, N. B. and D. Leimbach. Synthetic analgesics. II, Dithienylbutenyl-and Dithienylbutylamines. *J. Pharmacol. Exp. Ther.*, **107** : 385-393, 1953.
10. Emele, J. F., J. Shanaman and M. R. Warren,. The analgesic activity of phenelzine and other compounds. *J. Pharmcol. Exp. Ther.*, **134** : 206-209, 1961.
11. Fieser, L. F. and E. M. Chamberlin. Synthesis of embelin, repanone and related quinones by peroxide alkylation. *J. Am. Chem. Soc.*, **70** : 71-75, 1948.
12. George, M. and K. M. Pandalai. Investigations on plant antibiotics-Part IV. Further research for antibiotic substances in Indian medicinal plants. *Indian J. Med. Res.*, **37** : 169-181, 1949.
13. Green, A. F. and P. A. Young. A comparison of heat and pressure analgesiometeric methods in rats. *Br. J. Pharmacol.*, **6** : 572-585, 1951.
14. Gujral, M. L. and B. K. Khanna. Comparative evaluation of some of the narcotic analgesics. *J. Sci. Ind. Res. (India)*, **16C** : 11-13, 1957.
15. Guru, L. V. and D. N. Mishra. Anthelmintic activity of Embelia ribes (Burn) (Vayavirang) *in-vitro*. *Antiseptic*, **61** : 545-550, 1964.
16. Hasan, K. H. and E. Stedman, The constitution and synthesis of embelic acid (embelin), the active principle of Embelia ribes. *J. Chem. Soc. (Lond.)*, Trans. Part II, 2112-2123, 1931.
17. Heffter, V. A. and W. Feuerstein. Beitrage zur kenntnis der embelia saure. *Arch. Pharm. (Weinheim)*, **238** : 15-28, 1900.
18. Himes, N. E. Medical History of Contraception. New York, Gamut Press Inc., p.120, 1963.
19. Janssen, P. A. J. and A. H. Jegenear. A new series of potent analgesics : Dextro 2:2- dipnehy 1-3-methy 1-4-morpholinobutyryl pyrrolidine and related amides. Part I-Chemical structure and pharmacological activity. *J. Pharm. Pharmacol.*, **9** : 381-400, 1957.

20. Karber, G. *Aach. Exp. Pathol. Pharmakol,* **162** : 480-484, 1931; as cited by R. A. Turner, 'Screening Methods in Pharmacology' New York, Academic Press, **I,** p. 63, 1965.

21. Kaul, R., A. C. Ray and S. Dutt. Constitution of the active principle of Embelia ribes-part I. *J. Indian. Chem. Soc.,* **6** : 577-586, 1929.

22. Keats, A. S. and H. K. Beecher. Pain relief with hypnotic doses of barbiturates and a hypothesis. *J. Pharmacol. Exp. Ther.,* **100** : 1-13, 1950.

23. Koster, R., M. Anderson and E. J. De Beer, Acetic Acid for analgesic screening. *Fed. Proc.,* **18** : 412-414, 1959.

24. Kuhn, W. L. and E. F. Van Maanen. Central nervous system effects of Thalidomide *J. Pharmacol. Exp. Ther.,* **134** : 60-68, 1961.

25. Laurence, D. R. and A. L. Bacharach. Evaluation of Drug Activities : Pharmacometrics. London and New York, Academic Press, *I* p. 193, 1964.

26. Levine, R. R. and E. W. Pelikan. Mechanisms of drug absorption and excretion. Passage of drugs out of and into the gastro intestinal tract. *Ann. Rev. Pharmacol.,* **4** : 69-84, 1964.

27. Luduena, F. P. and J. O. Hoppe. Local anaesthetic activity, toxicity and irritancy of 2-alkoxy analogues of procaine and tetracaine. *J. Pharmacol. Exp. Ther.,* **184** : 40-58, 1952.

28. Mukerji. A. K. and N. V. Bhaduri. The treatment of intestinal worms with the indigenous drugs, Butea, Embelia and Kamala. *Indian Med. Gaz.,* **82** : 66-69, 1947.

29. Nadkarni, A. K. Indian Materia Medica. Bombay-7, Popular Book Dept., *I,* p.478, 1954.

30. Nargund, K. S. and B. W. Bhide. Constitution of embelin. *J. Indian. Chem. Soc.,* **8** : 237-240, 1931.

31. Padmanabha Rao, T. V. and V. Venkateswarlu. Chemical examination of Embelin ribes. IV. Condensation reactions of embelin with primary amines. *Tetrahed.,* **20** (4) : 969-71, 1964.

32. Paranjpe, A. S. and G. K. Gohkale. Pharmacological study of embelin, with reference to its use as an anthelmintic. *Arch. Int. Pharmacodyn. Ther.,* **42** : 212-232, 1932.

33. Patel, R. P., C. S. Shah, P. N. Khanna, and T. P. Gandhi. Pharmacognostic and Pharmacological studies of Embelia ribes Burn and Emrebia tsyeriam-Cotton A. DC. (Syn. E. Robusta larke). *Indian. J. Pharm.,* **26** : 168-172, 1964.

34. Radhakrishnan, N. and M. Alam. Antifertility effect of Embelia in albino rats. *Indian J. exp. Biol.,* **13** : 70-71, 1975.

35. Root, W. S. and F. G. Hoffman. Physiological Pharmacology, New York and London, Academic Press, *I* : p. 321, 1963.

36. Sastry, B. N. The wealth of India, New Delhi, Council of Scientific and Industrial Research, III : p.167, 1952.

37. Schneider, C. Effect of morphine-like drugs in chicks. *Nature Lond.,* **191** : 607-608, 1961.

38. Thapa, R. K., R. S. Sawhney and C. K. Atal. Preparation of Pharmacodyname compounds based on embelin. *Indian J. Pharm.,* **38** : 17-18, 1975.

39. Warden, C. J. H. *Pharm. J.,* **18** : 601, 19 : 305, 1888. Quoted by Hasan., K. H. and E. Stedman. of Ref. No. 15.

40. Windsor, E. and G. E. Cronheim. Gastrointestinal absorption of heparin and synthetic heparinoids. *Nature (Lond),* **190** : 263-264, 1961.

41. Winter, C. A., E. A. Risley and G. W. Nuss. Carrageenin-induced oedema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Rxp. Biol. Med. (N.Y.),* **111** : 544-547, 1962.