SEARCH FOR AN ANTIMALARIAL DRUG IN THE INDIGENOUS MATERIA MEDICA

PART I—*Alstonia scholaris*, F. Br.

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Introduction

THE possible shortage of quinine, which has remained the mainstay of the physicians in the treatment of malaria for nearly three centuries, since the discovery of cinchona bark, has naturally stimulated new interest in the search for substitutes which can be used with the same degree of efficiency as quinine. In view of the already established antimalarial value of synthetic derivatives, *e.g.*, Atebrin (Mepacrine Hydrochloride, B. P. Addendum, 1940) and Plasmochin (Pamaquin, B. P. Addendum, 1940), attempts have been made in India to synthesize these products and it is reported that a certain amount of success has already been achieved in this direction. The limitation of synthetic antimalarials, as far as production in India is concerned, is obvious, in that quite a large number of basic and intermediate chemicals necessary for synthesis have to be imported from foreign countries. It seemed worth while, therefore, to explore, by modern scientific methods of investigation, the possibilities of finding a substitute, if possible, from the indigenous materia medica. A number of natural drugs have enjoyed a local reputation as febrifuges and antimalarial remedies for a long time. The present paper deals with one member of this group, *Alstonia scholaris* (N. O. Apocynaceæ), the vernacular (Hindi and Bengali) synonym of which is 'chhatim'.

General description, chemistry, pharmacology, etc.

Alstonia scholaris is a tall evergreen tree widely cultivated throughout India and found in the sub-Himalayan tract from the Jumna eastward ascending to 3,000 feet. The tree is also found in abundance in Bengal. The bark of the tree has been reputed in the Hindu medicine for ages as a febrifuge, alterative, tonic and gastro-intestinal sedative. There is hardly an Ayurvedic prescription for acute and chronic fever or diarrhœa where 'chhatim', in some form or other, is not used. It was also recognized in the B. P. 1914.

An uncrystallizable bitter principle called 'ditain' was isolated and the febrifugal properties of the drug were ascribed to this principle by earlier workers. Later investigations showed that the following constituents were probably present: (1) an alkaloid, ditamine, (2) a substance resembling an alkaloid, (3) a crystallizable acid and (4) a fatty acid and resinous substances. Bacon (1906) found the presence of two alkaloids, 'ditamine' and 'echitamine'. Goodson and Henry (1925) reported that the principal alkaloidal constituent of *A. scholaris* and other allied species such as *A. congensis*, *A. gilletii*, *A. angustiloba* and *A. spathulata* was 'echitamine', ($C_{22}H_{28}O_4N_2$). This alkaloid, however, was found to be absent in other representatives of the Alstonias, *e.g.*, *A. constricta*, *A. macrophylla* or *A. villosa* (Goodson, 1932). Sharp (1934) reported the presence of 4 alkaloids in *A. constricta*, of which 'alstonine' was considered the chief one and was obtained in crystalline form as sulphate.

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resistant. Vitex will possibly inhibit hæmolysis of the more vulnerable cells and will thus prevent relapse of the hæmolytic crisis, diminish the increased disintegration of red cells which is usual in this disease, and thus improve the condition of the blood.

Conclusion

We are now in a position to answer the question quoted above—'It is conceivable that there might be some specific drug that would prevent further hæmolysis and would, therefore, if used prophylactically, prevent the initial attack. Is *Vitex peduncularis* such a drug?' The plant appears to contain some substance or substances with these properties.

It would be unwise from the data given here to draw definite conclusions regarding the value of *Vitex peduncularis* in the prophylaxis or treatment of blackwater fever. So far we have studied only its action on the red cells of healthy animals. Similar work is now being attempted in man in health and disease.

Summary

1. *Vitex peduncularis* increases the osmotic resistance of the red blood cells of animals.

2. It inhibits hæmolysis by saponin, acid, cobra venom and bile salts.

3. It is absorbed when given intramuscularly, the maximum effect being attained within two hours; the injection is apparently without any toxic effect.

4. Absorption through the alimentary tract may take place but the rate is extremely slow and depends on the size of the dose.

5. It is assumed that the action is on the red blood cell surface.

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Bacon (*loc. cit.*) studied the pharmacological action of echitamine on unicellular protoplasm represented by amœbæ. Exposed for 2 hours in a 1 per cent solution, amœbæ were reported to have thrived well and the conclusion was drawn that echitamine would not act as a protoplasmic poison like quinine or emetine. Goodson, Henry and Macfie (1930) tried the alkaloids, 'echitamine', 'ditamine', 'akuammine' and 'harmine', in bird malaria and found them inactive, except 'echitamine' which produced feeble action in doses of 5 mg. per dose. Buttle (mentioned by Sharp, *loc. cit.*) recorded the inactivity of alstonine sulphate in bird malaria.

Experimental

(*a*) *Separation of total alkaloids.*—In view of the conflicting and partly inconclusive data with regard to the efficacy of individual alkaloids of the Alstonias, it was decided to carry out our investigations first with purified total alkaloids of *A. scholaris* obtained from Bengal and, in the event of positive findings, to follow up the work with various related individual alkaloids which could be isolated in a pure state, by following the detailed method employed by Sharp (*loc. cit.*) in the case of *A. constricta*. The method followed for the extraction of total alkaloids is given below :—

About one and a half kilograms of powdered bark were moistened with rectified spirit and extracted in a percolator for several days until exhausted. The solvent was removed and the black viscous residue was extracted with 0.5 per cent H_2SO_4. The aqueous extract was filtered from insoluble resinous and fatty matter, diluted with water, allowed to settle and again filtered. The filtrate was shaken out with ether several times in order to remove the soluble impurities. The aqueous layer was then transferred to a second separator, chloroform was added, the solution made alkaline with Na_2CO_3, and extracted several times with fresh chloroform. It was then made strongly alkaline with 20 per cent NaOH solution and further extracted with chloroform until completely exhausted. The mixed chloroform extract was then evaporated to a small bulk and the total alkaloids were taken up by acidulated water (0.5 per cent H_2SO_4). This process was repeated again for purification. Finally, the chloroform extract was evaporated to dryness in a vacuum and neutralized with alcoholic sulphuric acid. The yield was found to be approximately 0.3 per cent of the bark.

(*b*) *Pharmacological studies.*—A 1–2 per cent solution of the sulphate of the total alkaloids (TAS) was used in these investigations. The important point to find out was whether the toxicity of the total alkaloids would lie within such dose range as to enable it to be used therapeutically without the possibility of any untoward toxic reactions and no attempt was therefore made to work out systematically the minimum lethal dose. It was found that a dose of 20 mg. per kilo. could be well tolerated in the monkey and about 10 mg./kg. in the cat.

Administered intravenously in the anæsthetized cat (chloralose or urethane) in a dose of 4–8 mg. per kilo., a slight fall followed by a rise in blood pressure is produced. The fall in blood pressure is not much affected by atropinization. In spinal cat preparations, a distinct but temporary rise of about 10–15 mm. Hg. is noticeable. This points to a predominance of the central effect to the parasympathetic effect of the drug in bringing about the fall of pressure. The muscular effect of TAS may also be important, as will be evident from the fact that an increased tone and contraction is often observed in isolated rabbit or guinea-pig uterus in as low a dose as 1 in 20,000 to 1 in 10,000. Isolated rabbit intestine also gives indications of stimulation in a concentration of 1 in 20,000. Isolated frog heart is mildly stimulated in 1 in 10,000; in higher concentrations, a depression with measurable diminution of the outflow of the perfusate is observed.

(*c*) *Chemotherapeutic studies.*—These were carried out on rhesus monkeys (*Silenus rhesus*) weighing between 2.5 to 3.0 kilo. infected with *Plasmodium knowlesi*. The inoculum consisted of 1 c.cm. infective blood given intraperitoneally. Injections of TAS in different dosages were given intramuscularly at various stages of the infection. Regular parasite counts were made once a day and sometimes twice a day. Control experiments were run side by side with quinine administered by the intramuscular route. It was found that TAS failed either to retard or to control the progress of infection. If the injections were made when the infection was fairly advanced (say, about 2,000 parasites per 10,000 red cells), the monkeys died invariably, as would have been the case had no treatment been administered at all. Contrary to the findings of Goodson, Henry and Macfie (*loc. cit.*) with echitamine, even a prolongation of the period of survival (when compared with control animals) could not be noticed. Attempts were made to save the animals by intravenous quinine in the late stages but this was ineffective in most cases. In some of the later experiments, quinine was given earlier when the infection was milder (about 1,000 parasites per 10,000 red cells) and in such instances, it was possible to save the animals or establish a chronic infection. The results of a representative series of experiments are given in the following table on the opposite page.

(*d*) *Clinical studies.*—It is a popular belief in Bengal and some other parts of India that genuine 'chhatim' (dita bark), if administered in the form of a decoction ('pachan') according to strict Ayurvedic principles, is almost as effective as quinine. In Manila Hospital, the results of trials in malaria cases were reported to be very satisfactory and it was opined that it would completely replace quinine in malignant tertian fevers (Chopra, 1933). The drug was also tried in India in 14 cases of malaria at the instance of the Indigenous Drugs Committee, Madras, 1921, in all of which it caused the temperature to fall steadily to normal in a short time. Treatment for a few days only was sufficient to cure the patients. In all these early reports, no definite proof was given that the cases treated were truly *malarial* in origin. Presumably purely clinical spot diagnosis was the criterion employed without any laboratory examination of the blood for the presence of parasites. It is therefore difficult to give much credence to such findings.

During the period that the chemotherapeutic studies were proceeding, a tincture (1 in 10), containing approximately 1.3 gr. TAS per ounce, was prepared from the powdered bark of *Alstonia scholaris* and this was administered in doses of one ounce thrice daily in a few patients suffering from malaria. Authentic records of only 6 cases are available, but more than a dozen patients were treated. In 4 cases, malarial infection was definitely proved by the demonstration of parasites (BT parasites in one and MT parasites in three). In 2 cases, the presumption was drawn from symptoms and previous history associated with palpable spleen but parasites were not detected in the peripheral blood, at the time of admission. In none of these cases, according to the opinion of the physicians in charge, did the tincture of alstonia produce any remarkable febrifugal effect or alter in any significant way the course of the disease. The temperature chart of 3 patients, however, showed a distinct drop in fever almost immediately following or about half hour after doses of the tincture were administered. The patients appeared during these periods to be comparatively free from subjective symptoms such as headache, nausea, etc. On critical examination, this mild reduction in temperature has been ascribed by the physicians to simultaneous coincidence rather than to any direct effect of the drug. At any rate, no demonstrable antimalarial action could be proved. It is possible that the slight reduction in temperature may be the result of central action of TAS contained in the tincture, as is

TABLE

Alstonia scholaris alkaloids in monkey malaria (P. knowlesi)

Serial number	Weight of monkey and date of inoculation	Date of commencement of treatment	Daily dosage in mg. and number of daily doses	Number of parasites per 10,000 R.B.C.		Remarks
				Before treatment	During treatment	
I	2.5 kilo, 23-5	27-5	(1) 50 (2) 50 (3) 60 (4) 80 4	840	(1) 3,590 (2) 5,600 (3) 6,820 (4) 6,920	Quinine gr. 2 given on 1–5. Monkey found dead 2-5 morning.
II	2.5 kilo, 12-6	14-6	(1) 50 (2) 50 (3) 50 3	5	(1) 220 (2) 460 (3) 2,430	Quinine gr. 1 given on 17-6. Monkey found dead 18-6 morning.
III	2.6 kilo, 17-6	20-6	(1) 50 (2) 50 2	50	(1) 350 (2) 1,410	Quinine gr. 1 given daily 22-25–6. Parasite counts :—1,840—130 (26-6), 230 (30-6)—70 (9-7) chronic infection established.
IV	3.0 kilo, 20-6	22-6	(1) 60 (2) 60 2	120	(1) 320 (2) 1,220	Quinine gr. 1 given daily 24–28-6. Parasite counts :—1,440–120 (1-7), 60 (2-7)—*nil.*

observed after the administration of centrally-acting antipyretics.

Summary and conclusions

Careful investigations in the laboratory and in the clinic of the total alkaloids isolated from *Alstonia scholaris* (N. O. Apocynaceæ), and also of a tincture (1 in 10) made from the powdered bark show that, contrary to popular belief and the earlier records of clinical trials with the drug, *Alstonia scholaris* has little or no demonstrable action in malaria induced in monkeys or naturally occurring in human patients. It cannot therefore be recommended as a substitute for quinine and other cinchona alkaloids.

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ECONOMY AND SIMPLIFICATION IN THE STAINING OF BLOOD SLIDES

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THE stains commonly used for staining blood films (Giemsa's, Wright's, Leishman's, etc.) have become expensive, and in many cases difficult to obtain. In order to get the best results with these stains, acetone-free alcohol and distilled water of a specified pH are necessary. This again increases costs and complicates technique. Furthermore, films, and particularly thick-drop slides, are usually stained by flooding, which means that the stain is discarded after having been used for a single slide only.

It is the object of this paper to show how a stain devised by Boyé (1940)—during the present campaign in North Africa—for the staining of thick drops can be used in such a way as to make it suitable for routine laboratory blood-film staining in all cases in which the Romanowsky stains are generally used.

The advantages of the methods we are about to describe are that :—

(*a*) The stain itself is 'home-made'.
(*b*) It contains no alcohol.
(*c*) It is suitable for staining by immersion instead of flooding.
(*d*) It does not appear necessary to be fastidious about the pH of the distilled water employed in its manufacture.
(*e*) It improves with use.
(*f*) It reduces the time required for processing drops and films to a matter of seconds.
(*g*) The results are perfectly satisfactory for all routine clinical work.