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Preliminary Studies on the Development of a New Non-bitter Chloroquine Formulation Using Tannic Acid

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Chloroquine phosphate is a bitter antimalarial drug belonging to the 4-amino quinoline class of compounds. It was treated with tannic acid and precipitated to form a poorly soluble complex. The complex was analysed and evaluated for taste and *in vitro* release. It was then formulated in a syrup base. When orally administered to healthy human subjects, chloroquine level comparable to marketed preparation was obtained. On the basis of the preliminary results obtained in the present study the method may be further evaluated as a technique for taste masking of bitter amine drugs.

Tannins comprise a large group of complex substances that are widely distributed in plant kingdom possessing astringent action and ability to precipitate proteins and alkaloids.[1] Amine drugs can be treated with tannic acid to form poorly soluble complexes. Such complexes can be obtained by simple acid-base reaction on mixing together solution of individual compounds.[2] Similarly if an alcoholic solution of a basic drug and tannic acid are mixed in 5:1 ratio, tannate complexes containing one amine per digallyl moiety are precipitated. These complexes are split by hydrolysis in gastric and intestinal fluid.[3] Break down of the tannate complex depends on the pH, hydrolysis being faster in acidic medium than basic. Chlorpheniramine, phenylephrine, ephedrine and pyrilamine tannates bear sustained release claims.[4] Long acting narcotic antagonists like naltrexone, nalaxone and cyclozocine form complexes with tannate salt of zinc or aluminium and are used in treatment of narcotic dependence.[5] Cyanocobalamine-tannate complex show sustained release of the drug.[6] Chloroquine phosphate is a bitter amine drug and the taste of the drug presents a problem of having poor patient acceptance. In the present investigation it has been considered as a model drug for taste masking using the principle of complexation with tannic acid.

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MATERIALS AND METHODS

Chloroquine phosphate was obtained from Universal Medicaments, Nagpur and tannic acid (IP grade) was procured from commercial sources. These materials were used as received without purification. All other reagents and chemicals were of either pharmacopoeial or analytical grade.

Twelve healthy human volunteers (age 21–30 y) were used in this study. Written informed consent was obtained after discussing with each subject the inconveniences to be expected. All subjects had normal history with no evidence of cardiac, hepatic, renal or gastrointestinal disease.

Preparation of complex:

Chloroquine phosphate was precipitated using tannic acid by mixing together solutions of individual compounds at various pH conditions. About 4.0 g tannic acid was dissolved in 100 ml distilled water and was kept in magnetically stirred condition. The pH was adjusted to the specific selected value using 1.0 M sodium hydro-

xide. About 16.6 g chloroquine phosphate (equivalent to 9.98 g chloroquine) was dissolved in 150 ml distilled water. The solution was then added gradually to the tannic acid solution, stirring continuously and adjusting the pH after each addition. The precipitate (complex) obtained was filtered and the cake washed with distilled water to remove traces of free chloroquine phosphate and/or tannic acid. The complex was then collected and dried to get a free flowing powder. However, at low or high pH conditions, the precipitate could not be obtained as a free flowing powder.

X-ray diffraction studies:

Formation of a complex between chloroquine and tannic acid was determined by X-ray diffraction studies. X-ray patterns of chloroquine phosphate, its complex with tannic acid and tannic acid were obtained on a Philips Analytical X-Ray diffractometer (PW1710) using Cu radiation, a voltage of 35 kV and a current of 20 mA.

Analysis of complex for chloroquine content :

Weighed quantity of precipitate (complex) was suspended in 5.0 ml water in a separating funnel and the medium was made alkaline with 1 M sodium hydroxide. The drug was extracted in 50 ml chloroform (3–4 times) by equilibrating the two phases. After separation the lower chloroform layer was passed through anhydrous sodium sulphate and re-extracted with 50 ml of 0.1 M hydrochloric acid (3–4 times). The drug concentration in the later medium was determined spectrophotometrically at 343 nm after appropriate dilutions.

Taste evaluation:

The precipitate (complex) obtained at pH 6.0 was subjected to taste evaluation by a trained flavour profile panel, using at least 10 members for each sample.[7] A time-intensity method was used in which a sample equivalent to a normal dose was held in the mouth for 10 s. Bitterness levels were recorded immediately and then after 10 min. Bitterness intensity values were based on a 0–3 scale, with 3 being strongly bitter, 2 moderate, 1 slight and 0 being threshold.[8]

Evaluation of drug release:

In vitro release of chloroquine from the complex into appropriate dissolution medium was conducted using USP dissolution apparatus 2 (rotating paddle method) at 37°. Quantity of complex equivalent to 83 mg of chloroquine (base) was subjected to drug release tests in 1000 ml of stimulated gastric fluid pH 1.2 (without pepsin) and 1000 ml of phosphate buffer pH 7.4 separately at 100 rpm. Aliquots were pipetted out after 1st and 2nd h from pH 1.2 medium and after every hour (up to the 5th h) from pH 7.4 medium. The chloroquine concentration in the dissolution was determined spectrophotometrically at 343 nm.

Formulation of syrup:

The complex prepared at pH 6.0 was dispersed in a syrup base containing 2% carboxyl methyl cellulose sodium. The formulation was so prepared that each 5.0 ml syrup contained 83.0 mg chloroquine (base). The pH of formulation was adjusted to 6.0 using 2% disodium hydrogen phosphate solution.

***In vivo* evaluation:**

Twelve healthy human volunteers (age 21–30 y) were selected and divided into two groups, consisting of six each. One group received the prepared syrup formulation while the other group received marketed chloroquine syrup. Preparation equivalent to 1.0 g chloroquine was administered to the subjects with milk and sufficient food. Blood sample (5.0 ml) was drawn from each volunteer at the end of 4, 8 and 12 h of the sample administration. The volunteers were kept under constant observation during the course of study. Estimation of chloroquine in plasma was done by an HPLC method.[9] Thermal Separation Products' HPLC system equipped with L1 (C_{18}) column and a UV detector (measuring wavelength of 254 nm) was used for the purpose. The flow-rate of the mobile phase [acetonitrile-phosphate buffer (40:60), ionic strength 0.1, pH 3.0 and perchlorate 75 mmol/l] was 0.8–1.0 ml/min. The mobile phase was degassed immediately before use and the experiments were performed at room temperature. A 500 ml sample of plasma was deproteinized by adding it drop wise to a solution (2.0 ml) of trichloroacetic acid 5% w/v in methanol containing the internal standard of chloroquine in concentrations of 100–200 nmol/l, during vigorous vortex mixing for 30 sec. The sample was left standing at room temperature for 10 min and after centrifugation for 20 min, 150 µl of the supernatant were used for chromatographic determination. Microprecipitates were removed by a 0.45 mm filter inserted before the injection loop.

RESULTS AND DISCUSSION

Chloroquine phosphate is precipitated by tannic acid

TABLE 1: ANALYSIS OF THE COMPLEX

pH	Quantity of complex obtained g	Yield of chloroquine in the complex g	% Yield of chloroquine
3.5	—	—	—
4.0	41.54 (± 2.16)	6.45 (± 0.32)	64.63
5.0	48.29 (± 2.76)	9.09 (± 0.12)	91.07
6.0	56.06 (± 3.84)	9.79 (± 0.12)	98.08
7.0	58.48 (± 1.12)	9.18 (± 0.09)	91.90
8.0	27.16 (± 1.37)	5.43 (± 0.50)	54.33

All values are mean of three determinations. Numbers in parentheses indicate standard deviation.

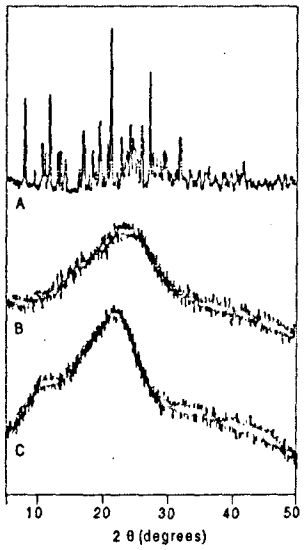

Fig. 1: X-ray diffraction pattern of chloroquine phosphate (A), its complex with tannic acid (B) and tannic acid

to form a poorly soluble complex. The formation of the complex depends upon the pH of the medium. Better yield and quality of the complex is obtained at near neutral pH conditions (Table 1). Confirmation of complexation between chloroquine phosphate and tannic acid was done by X-ray diffraction studies. Fig. 1 depicts the X-ray diffraction patterns of tannic acid, chloroquine phosphate and their complex. A reduction in peak intensity of chloroquine phosphate (fig. 1B) suggested the conversion of crystalline chloroquine phosphate into an amorphous complex with tannic acid. The complexation between tannic acid and chloroquine phosphate is a simple acid-base reaction. The complex obtained at pH 6.0 when considered for taste evaluation seems to be largely acceptable and the taste of the drug is considerably masked (Table 2). *In vitro* release studies on the drug complex performed at pH 1.2 and pH 7.4 indicated that chloroquine is released from the complex in acidic as well as basic medium (Table 3). The human volunteer study indicated comparable blood levels of chloroquine between the prepared formulation and the marketed preparation (Table 4).

Thus, the chloroquine phosphate-tannate complex is precipitated in a less soluble and less bitter form and is suitable for formulation as palatable syrup for oral

TABLE 2: BITTERNESS EVALUATION OF CHLOROQUINE PHOSPHATE AND PREPARED COMPLEX

Drug form	Dosage form	Bitterness level after 10 seconds*	Bitterness level after 10 minutes*
Chloroquine phosphate	Powder	>3	3
Prepared Complex	Powder	1	0

* Bitterness intensity values (on a 0–3 scale) with 3 being strongly bitter, 2 moderate, 1 slight and 0 being threshold.

TABLE 3: EVALUATION OF RELEASE OF CHLOROQUINE FROM THE COMPLEX IN GASTRIC AND INTESTINAL MILIEU

pH	Amount of drug released at different intervals (mg)				
	1 h	2 h	3 h	4 h	5 h
1.2	45.78 (± 0.07)	46.14 (± 0.22)	—	—	—
7.4	32.45 (± 1.42)	33.27 (± 1.32)	35.27 (± 0.73)	36.40 (± 0.35)	36.70 (± 0.25)

All values are mean of six determinations. Numbers in parentheses indicate standard deviation.

TABLE 4: CHLOROQUINE BLOOD LEVELS IN HUMAN VOLUNTEERS

Group	Chloroquine level at different time intervals µg/ml		
	4 h	8 h	12 h
Marketed preparation	3.02 (± 0.06)	7.66 (± 0.47)	11.0 (± 0.82)
Prepared formulation	1.97 (± 0.16)*	6.17 (± 0.41)*	10.66 (± 1.21)*

All values are mean of sample size of six. Numbers in parentheses indicate standard deviation. Asterisk indicates significant difference from marketed preparation at $P < 0.05$.

administration. On the basis of the preliminary results obtained in the present study, more detailed studies should be undertaken for greater acceptance of the method. The complex formed also needs more detailed chemical evaluation.

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