PRELIMINARY STUDIES ON NEWER SYNTHETIC ANTI-INFECTIVE COMPOUNDS

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Three compounds 5-formyl-8-hydroxyquinoline, 5-acetyl-8-hydroxyquinoline, and 8-acetoxyquinoline have been studied for their acute toxicity. 5-Formyl-8-hydroxyquinoline possesses a relatively low order of toxicity. Its LD_0 was 1 g/kg intraperitoneally and 1·25 g/kg orally. 5-Formyl-8-hydroxyquinoline produced a mild, precipitous and brief fall of blood pressure. The mode of action seems to be a direct one on the vessels. 5-Formyl-8-hydroxyquinoline possesses good anthelmintic activity.

8-Hydroxyquinoline, one of the seven isomeric monohydroxyquinoline possesses strong antibacterial action (Albert *et al.*, 1941) and significant anti fungal activity (Ringler and Greathouse, 1941). The antibacterial spectrum of 8-hydroxoquinoline is similar to that of penicillin with the exception of its action upon the genus *Haemophilus*. About 65 derivatives of 8-hydroxyquinoline have been prepared and examined for their anti-infective activity by Bahal and Khorana (1961). Some of the compounds were found to possess a wide antibacterial and antifungal spectrum. The authors concluded that 8-acetoxyquinoline and 5-formyl and 5-acetyl derivatives of 8-hydroxyquinoline could be considered worthy of clinical trials.

This paper concerns itself with the preliminary investigation of some derivatives of 8-hydroxyquinoline.

METHODS

Three compounds, 5-acetyl-8-hydroxyquinoline, 5-formyl-8-hydroxyquinoline and 8-acetoxyquinoline were studied for their acute toxicity in mice. Suspensions of the compounds were prepared with 1 per cent starch or 1 per cent Tween 80. Sodium salt of 5-formyl-8-hydroxyquinoline was prepared by adding 1 mole of sodium hydroxide to one mole of the compound. The pH of the solution of the salt used in the study was 7.5.

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Acute toxicity was studied in mice. A uniform strain of albino mice of either sex, weighing 18 to 25 g and obtained from a single source was used throughtout. The animals were fasted for 12 hr prior to drug injection but were allowed food and water after drug administration. The drugs were given either intraperitoneally or orally in a volume of 0.25 ml/10g of body weight. LD_{50} was calculated by the method of Burn *et al.*, (1952).

Albino rats of either sex, weighing 100 to 150 g and bred from a strain originally obtained from Haffkine Institute Bombay were used for studying the chronic toxicity of 5-formyl-8-hydroxyquinoline. Ten rats were divided into two groups of five each. One group received the drug under test and the other served as control. Test animals were given 500 mg/kg of 5-formyl-8-hydroxyquinoline solution daily by the oral route for six months. Body weight and food intake of the animals was regularly charted.

Dogs were anaesthetized with 100 mg/kg of phenobarbitone sodium intravenously after a pre-medication dose of 3 mg/kg morphine hydrochloride and cats with 100 mg/kg of phenobarbitone sodium intraperitoneally. Blood pressure was recorded with a mercury manometer connected to a common carotid artery. Respiration was recorded by cannulating the trachea and connecting the cannula to a tambour. Contractions of the auricle and ventricle were recorded by myocardiograph. The isolated organ studies were made on the rabbit duodenum, guineapig ileum and frog heart using the conventional techniques. Isolated hind leg of rat was perfused with oxygenated Tyrode solution and the number of drops was counted.

Anthelmintic activity was tested on the movement of earthworm (Trendelburg, 1958, Singh *et al.* (1955).

RESULTS

Acute toxicity in mice.—All the three compounds produced the same types of symptoms. The animals slowly became atoxic and depressed. There was no evidence of increased motor activity. Generally speaking, death occured within a periods of 30 min. In non-fatal dose recovery occured within 30 min. All the survivors were observed for a period of one week. Intraperitoneal LD_{50} for the three compounds was 1g/kg for 5-formyl-8-hyhroxyquinoline, 0.5g/kg for 5-acetyl-8-hydroxyquinoline and 0.3g/kg for 8-acetoxyquinoline. The oral LD_{50} of 5-formyl-8-hydroxyquinoline was 1.25 g/kg.

Thus, out of the three compounds tested for the acute toxicity in mice, 5-formyl-8-hydroxyquinoline had relatively a low order of toxicity and,

therefore, pharmacodynamic and chronic toxicity studies were carried out with 5-formyl-8-hydroxyquinoline only.

Blood pressure and respiration.—Intravenous injection of 5-formyl-8-hydroxyquinoline produced a transient fall of blood pressure. The depressor response was related to the dose, increasing doses of 1, 3, 5, and 8mg/kg produced proportionately greater falls of blood pressure (Fig. 1A).

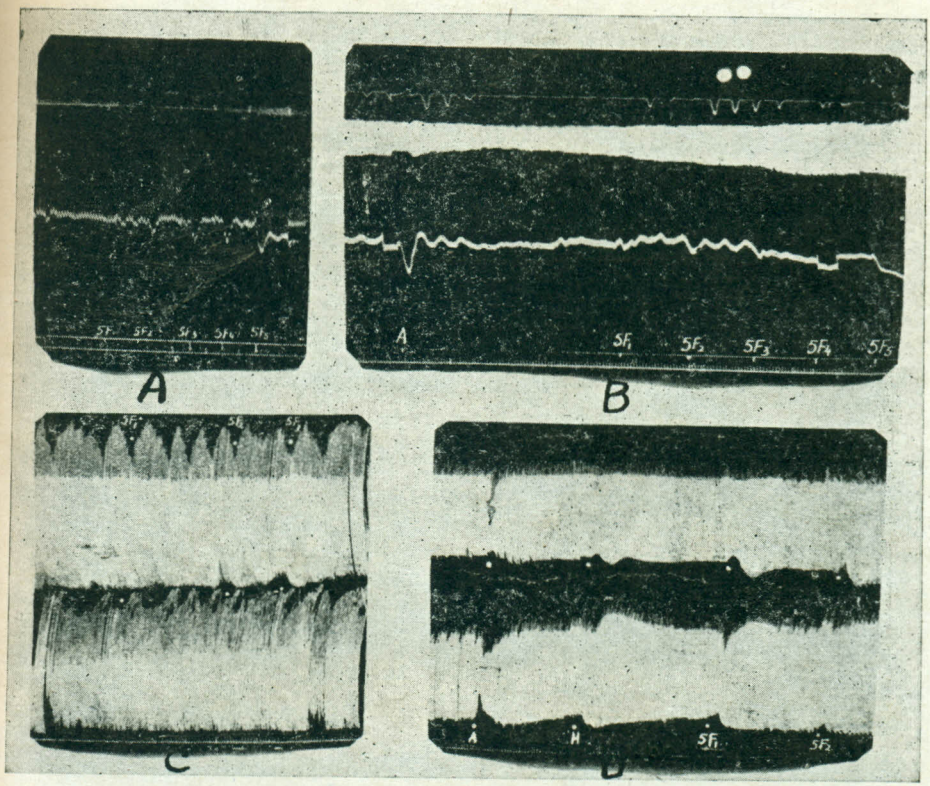

Fig. 1 A & B. Cat, 3 kg, phenobarbitone anaesthesia, intraperitoneal injections, (A) records of respiration and carotid arterial blood pressure (above downwards). (B) records of spleen volume, respiration, and carotid arterial blood pressure (above downwards). At $5F_1$, $5F_2$, $5F_3$, $5F_4$, and $5F_5$ were given 0.5, 1.0, 3.0, 5.0 and 8.0 mg/kg of 5-formyl-8-hydroxyquinoline respectively. Acetylcholine 2 μg/kg was given at A.

C & D: Cat, 2.5 kg, phenobarbitone anaesthesia, intraperitoneal injections. Upper tracing auricular contractions. At $5F_1$, $5F_2$, $5F_3$, were given 3,5 and 8 mg/kg of 5-formyl-8-hydroxyquinoline respectively. Acetylcholine 2 μg/kg was given at A and histamine 2 μg/kg was given at H.

The fall of the blood pressure was not modified in anyway by pretreatment with 2 mg/kg of atropine sulphate or 2 mg/kg of mepyramine maleate (Fig. 2B).

There was no change in the rate and amplitude of respiration upto 6 mg/kg of drug. Only at the dose of 8 mg/kg was there an increase in the rate and amplitude of respiration during the period of fall of blood pressure.

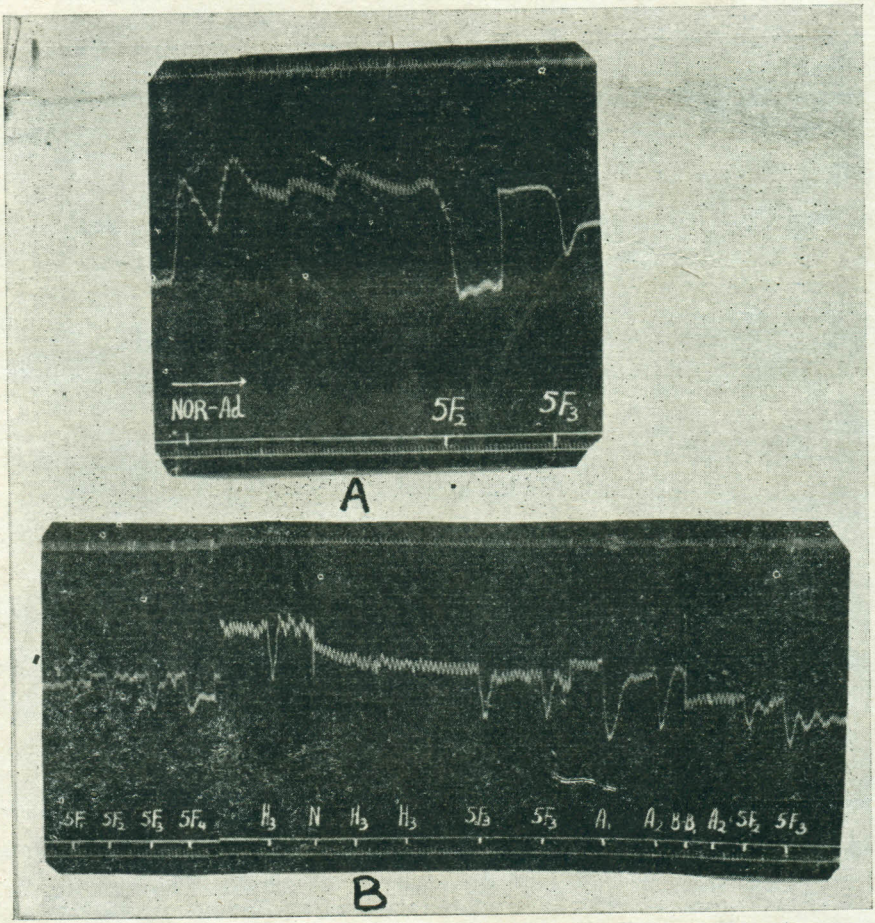

Fig 2. Dog, 12 kg, phenobarbitone anaesthesia, intravenous injections. Records of respiration and carotide arterial blood pressure (above downwards). At Nor-Ad infusion of noradrenaline 1 μg/ml was started and at 5F and $5F_3$ were given 8 and 5 mg/kg of 5-formyl-8-hydroxyquinoline respectively in A. In B 1, 3, 5 and 8 mg/kg of 5-formyl-8-hydroxyquinoline were injected at $5F_1$ $5F_2$, $5F_3$ and $5F_4$ respectively while H_3 represents the injection of histamine 1 μg/kg, N of mepyramine 2 mg/kg, A_1 and A_2 2 and 1 μg/kg respectively acetylcholine and interval B-B, of atropine 2 mg/kg.

Experimental hypertension. —A solution of nor-adrenaline (1 μg/ml) was infused slowly in such a way that the blood pressure was maintained at between 160 to 180 mm Hg. The effect of the drug was not in anyway modified by this procedure (Fig. 2A).

Cardiac contractions.—At a dose of 5 mg/kg the drug elicited a slight initial inhibition followed by stimulation of both auricular and venrticular contractions. Histamine produced a similar effect on the cardiac movements. It may be noted that in lower doses in which the drug produced a definite fall of blood pressure it failed to affect the contractions of auricle and ventricle (Fig. IC & ID).

Spleen volume. —The drug produced a diminition in the spleen volume at doses of 1, 2 and 3 mg/kg (Fig. 1B).

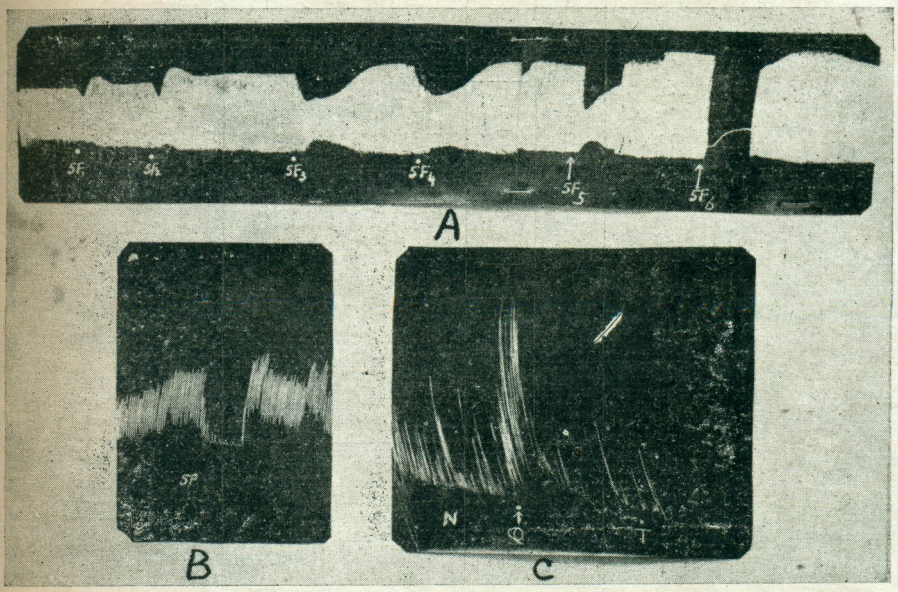

Fig. 3. (A) Isolated frog heart. At $5F_1$, $5F_2$, $5F_3$, $5F_4$, $5F_5$ and $5F_6$ were given 2, 3,4,5, 8 and 10 mg of 5-formyl-8-hydroxyquinoline respectively.

(B) Isolated rabbit duodenum. At 5F, 5-formyl-8 hydroxyquinoline 125 μg/ml was injected into the bath.

(C) Movements of the earthworm suspended in 40 ml bath containing Ringer Locke Solution. N represents control movements, Q represents the beginning of contact with 5-formyl-8-hydroxyquinoline 0.03%. At T drum was stopped for 20 min.

TABLE I

Perfusion of vessels of rat hind-limb

Solutions used	Outflow in no. of drops per min								Total no. of drops in 8 min.
	1	2	3	4	5	6	7	8	
Ringer solution	15	15	15	15	15	15	15	15	125
5-formyl-8-hydroxyquinoline 100 μg	16	22	21	21	21	21	21	21	154
Ringer solution with nor-adrenaline 1 μg/ml	38	33	28	27	27	26	26	24	229
5-formyl-8-hydroxyquinoline 1 mg	27	32	35	35	38	38	36	36	275
Ringer solution with adrenaline 0.1 μg/ml	40	30	20	20	18	10	9	7	154
5-formyl-8-hydroxyquinoline 1 mg	12	12	27	27	28	20	18	18	162

Rat hind limb. —The drug in the doses of 100 and 300 mg increased the rate of outflow of the perfusing Ringer. In some experiments noradrenaline 1 μg/mg or adrenaline 0.1 μg/ml were added to the perfusing Ringer solution. The drug produced an increase in the outflow (Table 1).

Perfused frog heart. —The drug had no marked effect on the rate and amplitude of frog heart at a dose of 1 mg. Partial inhibition was seen at 2 to 5 mg doses and a dose of 10 mg produced complete inhibition (Fig. 3A).

Rabbit duodenum. —5-formyl-8-hydroxyquinoline (100 μg/ml) had little effect on the duodenal strip. Increasing the concentration to 125 μg/ml caused complete relaxation and inhibition of the movements of the strip but the recovery occured quickly (Fig. 3B).

Guineapig ileum. —5 μg/ml, 25 μg/ml and 250 μg/ml of the drug antagonized the spasm induced by barium chloride (250 μg/ml) by 5.8 per cent, 10.7 per cent and 97 per cent respectively.

Chronic toxicity. —No marked difference in the body weight, food intake and behavioural activity was observed between the control and experimental groups.

Anthelmintic activity. —The results of anthelmintic activity are recorded in Table II. When kept in 0.03 per cent solution of the drug, the movements of the worms ceased in 30 min and the worms were dead at 1 hr (Fig. 3C).

TABLE II

Anthelmintic activity of 5-formyl-8-hydroxyquinoline

% Conc.	No. of worms dead out of 6 used after—hours		
	3	6	24
0.0025	1	—	—
0.0050	—	—	2
0.0075	—	3	4
0.01	2	3	6
0.02	3	4	6
0.03	3	6	6
0.04	6	6	6

DISCUSSION

The 5-formyl-8-hydroxyquinoline had the lowest toxicity, further pharmacological screening work was limited to this compound. Increasing doses (1,2,4 & 6 mg/kg) of the sodium salt of the compound produced a precipitous but brief fall of the blood pressure. The compound at 8 mg/kg dose produced a slightly prolonged depressor response.

The depressor response was not modified by the prior administration of atropine or mepyramine an antihistaminic agent. The depressor response continued to be elicited in animals given an infusion of nor-adrenaline. These observations indicate that the hypotensive response is probably due to a direct effect of the drug on the blood vessels. To corroborate this suggestion the drug was tested in the rat hind-leg. In this preparation vasodilator response was elicited. That there is no cardiac component in the depressor response to the drug is clear from the fact that the heart was not depressed. On the contrary the drug produced a mild stimulation of the heart movement as did histamine.

5-Formyl-8 hydroxyquinoline produced inhibition of the isolated frog heart at doses varying from 1 to 5 mg. The drug elicited relaxation of the isolated intestine of rabbit at a dose of 125 μg/ml and antagonized the action of barium chloride on isolated quinea-pig ileum.

The compound produced complete inhibition of the movement of earthworm and the worm ultimately died. On chronic administration, 5-formyl-8-hydroxyquinoline did not affect food intake or body weight.

It may be inferred from these results that out of the three compounds 5-formyl-8-hydroxyquinoline possesses a low order of toxicity besides showing potent anthelmintic activity. Clinical trials of the drug in gastro-intestinal infestations are, therefore, suggested.

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