FURTHER STUDIES ON PHARMACOLOGY OF BERBERINE

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Summary: Berberine produced reversible hypotension in the anaesthetized rat. It was not inhibited by aprotinin which rules out contribution of kininogen-kinin mechanism.

Berberine did not reduce histamine-aerosol-induced bronchospasm in guineapigs nor influenced gastric acidity, fluid volume and ulceration in Shay rats or histamine-induced gastric hypersecretion in anaesthetized dogs. It increased the mortality in guineapigs and dogs receiving safe dose of histamine. These findings failed to support the clinical impression that berberine is useful in hyperacidity and peptic ulcer.

Berberine significantly potentiated the apomorphine-induced emesis in dogs. Therefore, its beneficial effect in acute gastroenteritides cannot be due to central antiemetic component.

It reduced the urine volume and concentration of Na^+, Cl^- and creatinine in anaesthetized dogs, and volume and Na^+ concentration (but not of K^+ and Cl^-) in the urine of conscious saline loaded rats.

Berberine lowered the rectal temperature in normal rats and was 3 times more effective than sodium salicylate in reducing Brewer's yeast-induced pyrexia. This finding confirms its traditional use as an antipyretic.

It was 300 times less effective than classic spermicide p-diisobutylphenoxypolyethoxyethanol in reducing the motility and survival time of bull sperm *in vitro*.

Berberine stimulated the spontaneously beating isolated atria of the rabbit, guineapig and rat.

On the isolated guineapig ileum, physostigmine, neostigmine and smaller doses of berberine consistently potentiated the spasmogenic actions of PGE_1 and $F_{1\alpha}$; with E_2 and $F_{2\alpha}$ only 50-75% tissues showed potentiation. Atropine antagonized the stimulant action of PGs as also their potentiation by berberine, physostigmine and neostigmine.

Larger doses of berberine inhibited the PGs-induced contractions.

Effect of berberine on various agonists is discussed with special reference to potentiation of $CaCl_2$-induced contraction of the depolarized guineapig ileum. It is proposed that at the intracellular sites of excitation-contraction coupling, papaverine and polysorbates reduce but berberine increases the availability of Ca ions.

Key words: Berberine histamine-mediated actions renal functions emesis antipyretic sperm motility guineapig ileum prostaglandins potentiation prostaglandins inhibition by atropine depolarized guineapig ileum

INTRODUCTION

Berberine is an alkaloid obtained from *Berberis aristata* (Sanskrit — *daruharidra, darwi;* Hindi — *daruhaldi*) and some other plants (11, 12, 13). In India, berberine-containing preparations have been in continuous medicinal use for over 3000 years (10) for the treatment of many diseases

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including gastroenteritides and skin and eye affections (10, 12, 13, 16, 29, 39, 49). The recent scientific evidences generally concur with the traditional use of these plants. Its utility in diarrhoeas of diverse origin (17, 18, 26, 27, 42) and in giardiasis (14), amoebiasis (19, 45), dermal leishmaniasis (15, 48) and trachoma (37) has been convincingly established. Recently, it has been shown to inhibit the cholera toxin-induced inflammation of the rat neck (1) and of the fluid accumulation in the gastrointestinal tract of rat (38) and rabbit (23). Curiously, berberine also manifests *in vitro* antiheparin action on the dog and human blood (34). Besides these, berberine has been found to exert a large number of interesting pharmacodynamic actions. Some of these actions have been reported earlier (35). Further work is presented here.

MATERIALS AND METHODS

Berberine sulphate, which was used in these experiments, is mentioned hereafter as "berberine". The pH of stock solution (5 *mg/ml* in distilled water) was 4.05.

Blood pressure of the rat :

The carotid artery blood pressure of male rats (200-300 *g*) anaesthetized with urethane (1.25 *g/kg* sc) was recorded with the help of a Condon's manometer on a smoked drum.

Histamine-aerosol-induced bronchospasm in guineapigs (9) :

Guineapigs (275-400 *g*) of either sex were exposed to finely atomized mist of 1.5% W/V histamine dihydrochloride solution. Compressed oxygen at 200 mm Hg was passed through histamine solution in the nebulizer which was connected to the aerosol chamber (Techno, Lucknow). Berberine or mepyramine maleate was administered, ip, 30 min before exposure.

In some experiments, berberine (0.5% W/V) was administered as aerosol, 3 times, each at an interval of 8 hr, each exposure lasting for 15 min; animals were exposed to histamine-aerosol 15 min after the last exposure. Animals were removed from histamine mist after 4 min, or earlier if they convulsed.

Shay rats (25) :

Drugs were injected into the duodenum distal to the pyloric ligatures; control rats received comparable volume of distilled water. After 18 hr, rats were killed, volume and acidity of gastric fluid measured, gastric ulcers graded and ulcer index calculated.

Histamine-induced gastric secretion in dogs (7) :

Adult mongrel dogs (5-15 *kg*) were anaesthetized with sodium pentobarbitone (35 *mg/kg* ip) and, volume and acidity of gastric secretion measured at 15 min intervals.

Apomorphine-induced emesis in dogs :

Dogs (5-21 *kg*) of either sex were fed normal food before the experiment. Berberine (1 and 6 *mg/kg*) or chlorpromazine hydrochloride (3 *mg/kg*) was injected, iv, 1 hr before the im administ-

ration of apomorphine hydrochloride (0.1 *mg/kg*). The dogs were watched continuously for counting the number of vomits. Each group had 6 dogs.

Volume and composition of urine in dogs (6) :

Dogs of either sex (8-12 *kg*) were anaesthetized with sodium pentobarbitone (35 *mg/kg* ip) and loaded with 0.9% sodium chloride solution (25 *ml/kg* iv) over about 30 min. Drugs were injected through the cannulated femoral vein. Polyethylene catheter (diameter 2.5 mm) was passed in the individual ureter near the urinary bladder and urine from both was collected in a graduated cylinder. Volume was measured at 10 min intervals. Na^+ and K^+ in the urine were measured by flame photometer and Cl^- (40) and creatinine (24) by chemical methods.

Volume and composition of urine in rats :

Adult male albino rats (150-250 *g*), loaded orally with 25 *ml/kg* normal saline were injected berberine (10-30 *mg/kg* ip). 2-3 rats were placed together in a metabolic cage for 24 hr without food or water. Urine was collected in a measuring cylinder containing a few drops of liquid paraffin to prevent evaporation. The urine passed during the first 5 hr and the subsequent 19 hr was measured and expressed as *ml/kg* body weights of the individual groups of animals. Na^+, K^+ and Cl^- in urine were estimated.

Rectal temperature in rats :

Details described for mice (5) were followed for adult male albino rats (150-200 *g*). The rectal temperature was recorded with a clinical thermometer just before, and at $\frac{1}{2}$-1 hr interval for 5 hr after injecting the drugs ip.

Brewer's yeast-induced pyrexia in rats (2) :

Initial rectal temperature of male albino rats (150-200 g) was recorded. Brewer's yeast suspension (15% W/V in 2% gum acacia in water) was injected, sc, in a volume of 10 *ml/kg*. The yeast powder was fairly coarse. It was therefore passed through fine muslin cloth; it was observed that suspension of this fine powder caused pyrexia more consistently than that of the coarse powder. After 18 hr, rats which showed a temperature rise of atleast 1.5°C were given, orally or ip, berberine or sodium salicylate. Comparable volume of distilled water was injected ip to one group of rats which served as common control for all the groups. The temperature of all the rats was recorded at $\frac{1}{2}$-1 hr interval for 5 hr. The room temperature ranged between 30-33°C.

Motility of the bull spermatozoa :

The semen was collected from British Friesian bulls in an artificial vagina using a dummy cow. Aliquots of 0.5 *ml* semen were added to those of 4.5 *ml* of 2.9% sodium citrate containing berberine or p-diisobutylphenoxypolyethoxyethanol (commercially available as Preceptin, a contraceptive jelly manufactured by Ethnor, Bombay). The semen samples were examined (46)

at controlled temperature (37°C) under microscope at frequent intervals till all the sperms were found immotile.

The isolated tissues :

Pieces of isolated seminal vesicle (44), rabbit aortic strip (22, 30), rabbit, guineapig and rat atria (30), guineapig ileum (30) and depolarized guineapig ileum (20) were suspended in 10 *ml* capacity isolated tissue baths containing oxygenated Ringer-Locke (39°C), Krebs-Henseleit (37°C), Ringer-Locke (29-30°C), Tyrode (37°C) and potassium Ringer (37°C) solutions respectively. The contractions were recorded on a smoked drum with a simple or frontal writing lever.

RESULTS

Blood pressure of the rat :

In the anaesthetized rat, the initial blood pressure ranged from 60-70 mm Hg and, berberine (150-300 $\mu g/kg$) reduced it by 20-25 mm Hg (n=12; n=number of experiments). Bradykinin (1 $\mu g/kg$) and kallikrein (Padutin; 0.5-2 $\mu g/kg$) also produced about the same degree of hypotension. Slow iv injection of aprotinin (Trasylol 50,000 to 100,000 U/kg), over 4 min had no effect on the blood pressure; however, it reduced the kallikrein-induced hypotension by about 80% (n=8). Its anti-kallikrein action generally lasted for about 10 min. During this short-lived anti-kallikrein effect of aprotinin, berberine and bradykinin fully manifested their hypotensive actions (n=8).

Histamine-aerosol-induced bronchospasm in guineapigs :

All the 14 animals of control group developed breathing difficulty after an average 83 sec of exposure to histamin-aerosol and 8 of the 14 animals (57%) died in convulsion. Injection of 15 and 30 *mg/kg* of berberine to 12 and 6 guineapigs respectively did not affect the duration (78 and 90 sec respectively) and increased the mortality to 67% (8 out of 12) and 83% (5 out of 6) respectively. Smaller dose (5 *mg/kg*; n=3) of mepyramine offered no protection; however, at 15 *mg/kg*, it manifested its classic protective action against histamine, in that, 5 out of 6 animals did not suffer respiratory distress, convulsion or death with 4 min exposure; only one animal developed convulsions at the end of 4 min and died.

The 4 animals, which received berberine by aerosol to achieve high local concentration in bronchioles, also developed bronchospasm in 66 sec to histamine-aerosol and died in convulsions.

Shay rats :

In the control rats (n=12), the mean volume and total acidity of the gastric contents were 6.2±2.1 and 23.5±11.2 *ml* respectively; the ulcer grade and ulcer index were 2.3±0.3 and 230 respectively. Berberine (10 *mg/kg*; n=12) had no significant effect (volume 5.7±1.2 *ml;* acidity 19.0±5.6 *ml;* ulcer grade 2.7±0.4 and ulcer index 270). Propantheline (Probanthine, 5 *mg/kg*; n=12) was more effective (volume 2.0±0.6 *ml;* acidity 3.4±1.8 *ml;* ulcer grade 2.0±0.3; ulcer index 183) than berberine.

Histamine-induced gastric secretion in dogs :

In this work, the dogs were given histamine and berberine in such doses which they well-tolerate. Interestingly, berberine (6-10 *mg/kg*) produced prompt death of 5 out of 8 dogs which had earlier received histamine (30-60 ug/kg sc). Therefore, the effect of berberine could be studied in only 3 dogs in which 6-20 *mg/kg* berberine did not alter the histamine (30-60 *μg/kg*)-induced gastric hypersecretion. These dogs also died immediately after the administration of a second dose of berberine (10 *mg/kg* iv).

Apomorphine-induced emesis in dogs :

In control dogs, apomorphine induced 6.8±S.E. 1.01 vomits/dog. At 1 *mg/kg* dose berberine had no effect (6.3±S.E. 1.33 vomits/dog). On the other hand, at 6 *mg/kg*, it significantly ($P<0.01$) increased the number of vomits to 11.0±S.E. 1.77. Most of the vomiting episodes occurred within 1 hr of apomorphine administration. Chlorpromazine hydrochloride (3 *mg/kg*) completely inhibited the apomorphine-induced vomiting.

Volume and composition of urine in dogs (Table I) :

Berberine (0.1-10 *mg/kg*) consistently reduced the urine volume as also the concentrations of Na^+, Cl^- and creatinine. K^+ concentration remained unchanged or showed rise after berberine; there is no dose-response relation. In anaesthetized dogs mersalyl is known to significantly increase the urine volume and its Na^+ and Cl^- contents (6). In the present study mersalyl (200 mg/animal) could reverse some effects of berberine in that it increased the urine volume and Na^+ and Cl^- levels. However, mersalyl did not affect the reduction of creatinine excretion rate which was lowered by previous berberine injection. Neither berberine nor mersalyl changed the pH of urine which was between 6 and 7.

TABLE I: Effect of berberine on volume and composition of urine in anaesthetized saline loaded dogs.

Drugs/kg	*Urine values*				
	Volume ml/10 min	*Na+ mEq/lit*	*K+ mEq/lit*	*Cl+ mEq/lit*	*Creatinine excretion in mg/10 min*
Nil; Initial control values	4.7	112	48	56	3.5*
Berberine 0.1 *mg*	2.2	77	77	40	2.0*
Berberine 0.3 *mg*	1.8**	81**	98**	34**	—
Berberine 1.0 *mg*	1.7*	76*	103*	27*	1.0*
Berberine 3 *mg*	2.7	71	81	54	1.3*
Berberine 10 *mg*	1.6*	58*	45*	25*	—
Mersalyl 200 *mg* per animal	13.9	216	40	203	1.1*

* Average values from 2-3 animals; **from 1 animal; the remaining values are averages from 4-8 animals.

Volume and composition of urine in rats (Table II) :

Berberine (10 *mg/kg*) reduced the urine volume. This was accompanied by a decline in Na^+ and some rise in K^+ and Cl^- concentrations. These effects were enhanced by increasing berberine dose to 30 *mg/kg*. Berberine did not alter the pH of urine which was between 6 and 7.

TABLE II: Effect of berberine on volume and composition of urine @ in conscious rats orally loaded with normal saline (25 *ml/kg*).

Drugs/kg	*Urine during the first 5 hr*				*Urine over subsequent 19 hr*			
	Volume ml/kg	*mEq/lit*			*Volume ml/kg*	*mEq/lit*		
		Na^+	K^+	Cl^+		Na^+	K^+	Cl^-
Distilled water 6.0 *ml* (control)	10.2	134	166	176	8.8	212	216	285
Berberine 10 *mg*	4.0	112	290	190	8.9	212	261	210
Berberine 30 *mg*	1.3	112	300	279	7.8	161	378	373

@ All values are averages from 8 rats.

Rectal temperature in rats (Fig. 1) :

Distilled water and 10 and 30 *mg/kg* doses of berberine had no significant effect. However, 50 *mg/kg* berberine and 5 *mg/kg* chlorpromazine significantly ($P<0.01$) lowered the rectal temperature; berberine-induced hypothermia persisted over the entire 5 hr observation period.

Brewer's yeast-induced pyrexia in rats :

Eighteen hr after its injection, Brewer's yeast in 2% gum acacia significantly ($P<0.001$) increased the rectal temperature. Pyrexia then persisted for at least 5 hr. Comparable volume (10 *ml/kg*) of 2% gum acacia had no effect. Intraperitoneal (Fig. 2) or oral (Fig. 3) administration of berberine (10 *mg/kg*) significantly lowered the rectal temperature of pyrectic rats in about 2 hr; the effect disappeared by fifth hr. Larger dose (30 *mg/kg*) was more effective in that, berberine lowered the rectal temperature to near normal within 1 hr and the effect persisted over the 5 hr observation period; ip administration was somewhat more effective than oral. 30 *mg/kg*, berberine was about equieffective with 100 *mg/kg* sodium salicylate.

Motility of the bull spermatozoa :

Sperms remained motile for more than 30 hr. 3, 10 and 50 *mg/ml* (at higher concentrations berberine remains finely suspended) of berberine abolished motility in 217, 153 and 20 min respectively; 100 *mg/ml* produced immediate paralysis of all the sperms. In 0.1 and 0.01 *mg/ml* concentrations, p-diisobutylphenoxypolyethoxyethanol produced immobility of the sperms in 40 and 265 min respectively; 0.3 *mg/ml* dose had immediate paralysing effect. Repeated washes with drug-free sodium citrate solution could not revive paralysed sperms.

Isolated guineapig seminal vesicle :

Berberine (0.01-0.5 *mg/ml*) had no direct effect of its own on the seminal vesicle. 10, 30, 60 and 100 $\mu g/ml$ of berberine inhibited adrenaline-induced (2 $\mu g/ml$) contractions by about 10, 30, 50

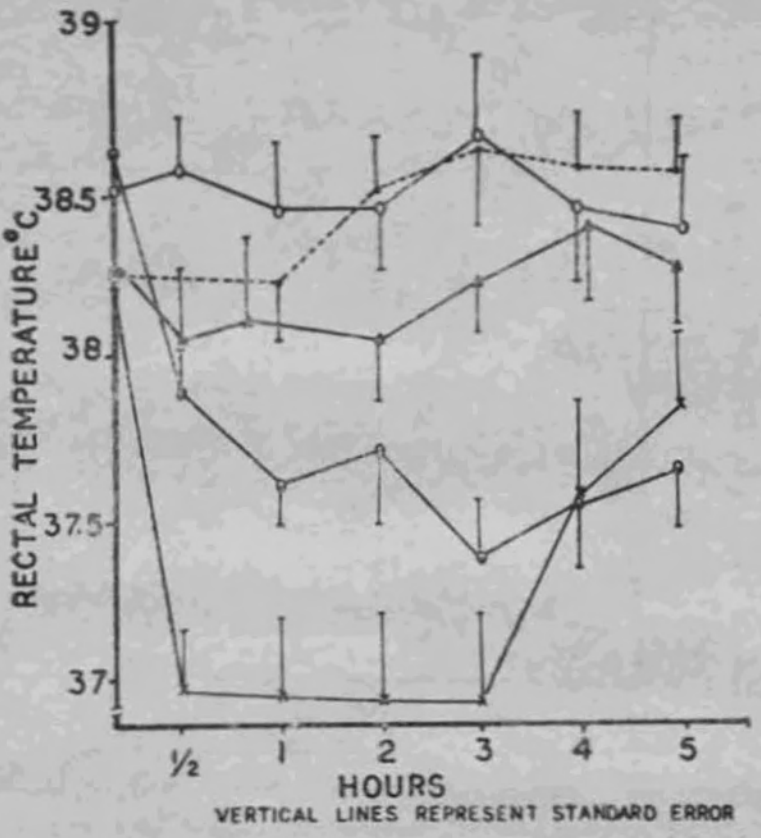

Fig. 1 : Effect of ip berberine or chlorpromazine on the rectal temperature in groups of 8-10 rats.

Distilled water 10 *ml/kg*

Berberine 10 *mg/kg*

Berberine 30 *mg/kg*

Berberine 50 *mg/kg*

Chlorpromazine 5 *mg/kg*

and 70% respectively (n=5). Similarly, the contractions induced by noradrenaline (3 *μg/ml*; n=4) were inhibited by 14, 17, 34, 44 and 80% by 10, 30, 60, 100 and 300 *μg/ml* of berberine respectively. The inhibition was quickly reversible.

Isolated rabbit aortic strip :

Berberine (3-30 *μg/ml*; n=4) had no direct effect of its own on this tissue. 3, 10 and 30 *μg/*

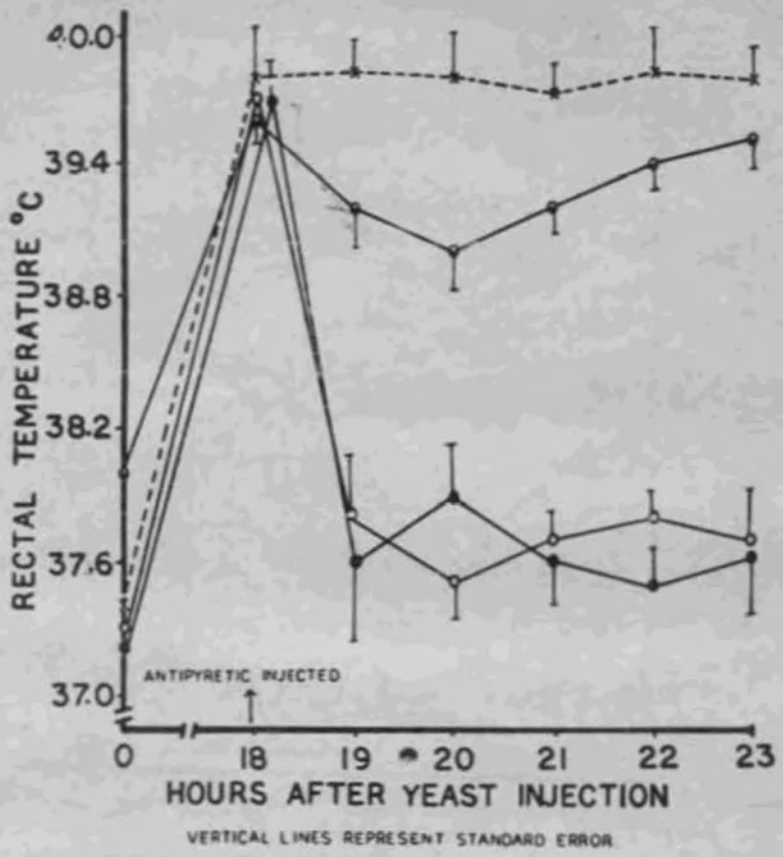

Fig. 2 : Antipyretic effect of ip berberine or sodium salicylate in groups of 10 rats becoming febrile 18 hr after yeast injection.

X- - - - -X Distilled water 10 *ml/kg*

Berberine 10 *mg/kg*

Berberine 30 *mg/kg*

Sodium salicylate 100 *mg/kg*

ml of berberine inhibited the adrenaline-induced contractions by 34, 63 and 71% respectively and those of noradrenaline by 40, 54 and 84% respectively (n=4 each).

Isolated atrial preparations :

Berberine (3-100 *ug/ml*) consistently increased the rate and amplitude of contraction of spontaneously beating isolated atria of the rabbit (n=8), guineapigs (n=3) and rats (n=4). On rabbit atria, 3, 10, 30 and 100 *µg/ml* of berberine increased the amplitude of contraction by 8, 15, 30 and 70% and the rate by 10, 14, 20 and 30% respectively.

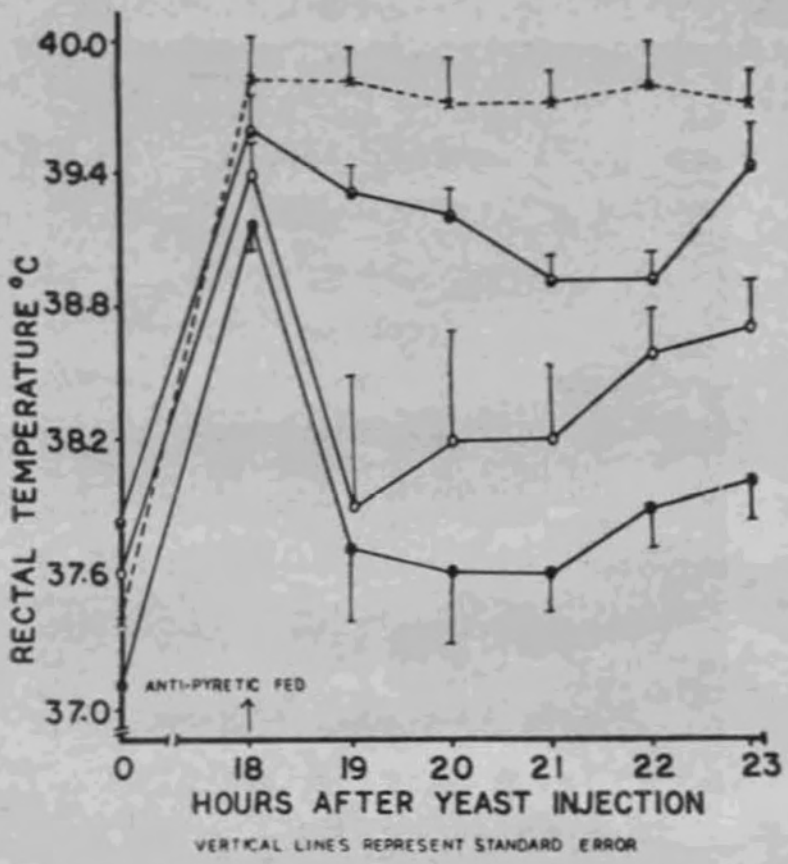

Fig. 3 : Antipyretic effect of orally given berberine or sodium salicylate in groups of 10 rats becoming febrile 18 hr after yeast injection.

X- - - - -X Distilled water 10 *ml/kg*

Berberine 10 *mg/kg*

Sodium salicylate 250 *mg/kg*

Berberine 30 *mg/kg*

Isolated guineapig ileum :

Prostaglandins : Generally berberine augmented the contractions induced by PGs. The potentiation was proportional to their doses, short-lived and could be repeatedly elicited. Larger concentrations (0.1-1 *mg/ml*) of berberine inhibited the PGs-induced contractions.

(a) Prostaglandin E_1 and $F_{1\alpha}$: Berberine (0.1-10 *μg/ml*) potentiated by 18-280%, the contractions induced by PGE_1 (2-100 ng/ml; n=16; Fig. 4). Physostigmine (0.3-3 *ng/ml*; n=4) and neo-

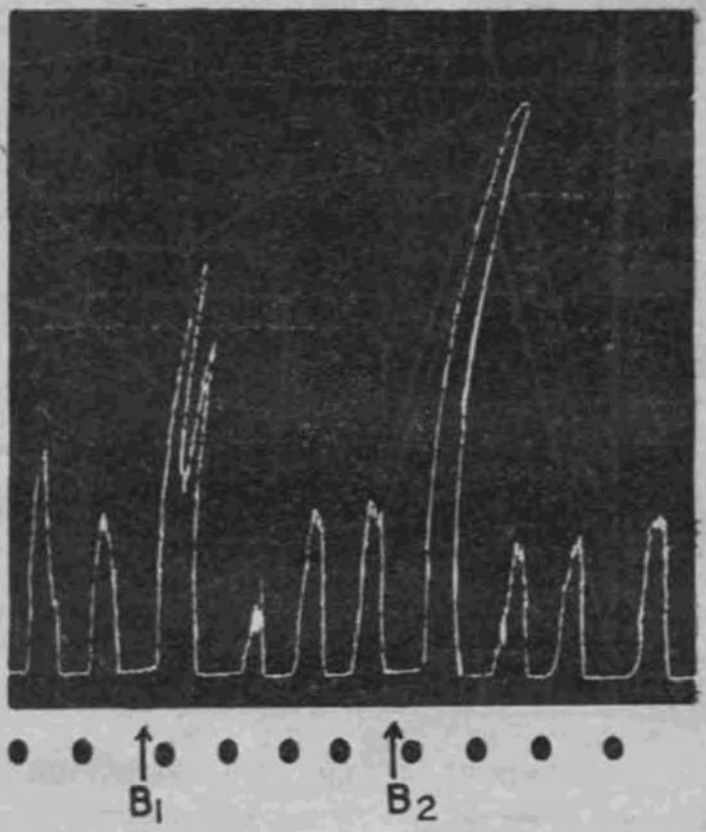

Fig. 4 : Potentiation of prostaglandin E_1 by berberine on the isolated guineapig ileum. Berberine (B_1-3 $\mu g/ml$; B_2-10 $\mu g/ml$) was added at arrows 1 min before prostaglandin E_1 (●=3 ng).

stigmine (10-100 *ng/ml;* n=3) produced potentiation by 80-250%. Atropine (3-10 *ng/ml*; n=3) inhibited the PGE_1 response by 50-70%.

Similarly, berberine potentiated the $PGF_1\alpha$(0.3-3 $\mu g/ml$; n=10)-induced contractions by 20-560%. Physostigmine (0.3 $\mu g/ml$; n=3) and neostigmine (5 *ng/ml*; n=2) also potentiated the response by 450-1200%.

Also, berberine converted the subminimal doses of PGE_1 and $F_1\alpha$ to effective ones.

(b) Prostaglandin E_2 and $F_2\alpha$: The effect of berberine was inconsistent. It (1-6 $\mu g/ml$) inhibited, by 10-64%, the contractions induced by PGE_2 (0.02-0.5 $\mu g/ml$) in 6 out of 9 experiments; in the remaining 3 experiments berberine potentiated the PGE_2 response by 15-165%. Physostigmine (0.3 $\mu g/ml$) potentiated the PGE_2 response by 410% in 5 out of 6 experiments; in the remaining 1 experiment it inhibited the response by 28%. Neostigmine (1 *ng/ml;* n=2) potentiated the PGE_2 response by 125-140%. Atropine (0.3-3 *ng/ml*; n=4) inhibited the contractions by 80%.

Berberine (0.1-30 $\mu g/ml$) inhibited the $PGF_2\alpha$(0.5-1 $\mu g/ml$)-induced contractions by 8-67% in 7 experiments. In the remaining 5 experiments, berberine (1-10 $\mu g/ml$) increased the $PGF_2\alpha$ response by 84-300%; physostigmine (0.3 $\mu g/ml$; n=3) and neostigmine (1 $\mu g/ml$; n=2) potentiated the contractions by 134-261%.

Depolarized guineapig ileum :

Berberine (1-300 $\mu g/ml$) had no direct effect of its own on this tissue.

It (1-10 $\mu g/ml$) inhibited, by 27-60%, the carbachol (0.5 $\mu g/ml$; n=2)-induced contractions; the inhibition was quickly reversible.

Calcium chloride (0.3-1 mg/ml) produced contractions which were potentiated, by 7-23%, by berberine (1-3 $\mu g/ml$; n=4). Even as high a dose as 300 $\mu g/ml$(n=4) of berberine did not reduce the $CaCl_2$ response. On the other hand, spasmolytic agents like polysorbate 80 (Tween 80 1 $\mu g/ml$; n=3) or papaverine (10 $\mu g/ml$; n=2) reduced the $CaCl_2$-induced contractions by 50-80%. Berberine partly reversed, by 50-60%, the inhibitory effect of polysorbate 80 and papaverine on $CaCl_2$-induced contractions.

DISCUSSION

The present work and previous reports (11, 21, 33, 35) show that intravenously given berberine produces hypotension in all the species tested so far namely, rat, rabbit, cat, dog, fowl and frog. Thus, the hypotensive action might be considered as its universal action.

The mechanism of berberine-induced hypotension has been discussed in details earlier (35). Possibility of hypotension being mediated through the release of kinins from kininogen, by berberine, was worked out in this study. Aprotinin pretreatment is known to inhibit (31) conversion of inactive kininogen into kinins by the enzyme kallikrein. In the present study in rats, aprotinin pretreatment inhibited the hypotensive action of kallikrein but not of berberine or bradykinin. This finding, therefore, suggests that berberine-induced hypotension in the rat (and probably in other species) is not due to the release of kinins from serum kininogen.

Berberine blocks the stimulant action of histamine on the isolated guineapig ileum and also Shultz-Dale anaphylactic contraction of sensitized guineapig ileum (35). However, in *in vivo* experiments, it failed to exert antihistaminic action on the bronchial musculature of guineapig. This once again indicates the limitations of isolated tissue experiments as compared to those on the intact animals.

Animals suffering from experimental cholera have higher levels of histamine, 5-hydroxytryptamine and bradykinin in their blood (4) and, based on isolated tissue findings it has been suggested (3) that the beneficial effect of berberine in cholera patients could, partly, be due to its effect against these autacoids. However, the present results indicate higher motality to histamine in berberine-treated animals. Therefore, our results on intact animals caution against this hypothesis.

In the Ayurvedic (39, 49) and traditional (29) medicine, berberine containing plants are considered to relieve the symptoms of hyperacidity and peptic ulcer. However, the present study on pylorus-ligated rats and on histamine-induced gastric secretion in dogs does not confirm this belief.

In the present work, berberine failed to suppress apomorphine-induced vomiting in dogs. On the other hand, berberine augmented the apomorphine emesis. This could possibly be due to the anticholinesterase action (8) of berberine in the CNS. Thus, berberine lacks the central antiemetic effect although it is known to exert depressant effect on some CNS functions (28, 35, 41, 47). Therefore, whatever beneficial effect berberine might be exerting on the vomiting component of human cholera (and other acute gastroenteritides) cannot be due to any central antiemetic action.

In dogs, creatinine excretion occurs only through the glomerular filtration and it is neither reabsorbed nor excreted by the tubules (43). Therefore, the creatinine excretion rate is proportional to the glomerular filtration rate (GFR). The results (Table I) indicate that berberine reduces the GFR and the reduction is not related to its dose. The reduction in GFR is probably not due to the hypotensive action of berberine because it occurred in doses which do not produce systemic hypotension. Further, the effect on urine composition lasted far too longer than the hypotensive action (35). Possibly, reduction in GFR is due to the direct spasm of the afferent arterioles going to the individual glomeruli. Indeed, the earlier observation that berberine increased the kidney volume (11) supports this possibility.

The marked reduction in the urine volume and urinary Na^+ by berberine could be due to reduced GFR. Reduction in GFR is known to facilitate Na^+ reabsorption (and therefore reduce urinary excretion) much more than of other urinary constituents. The other theoretical possibility of reduced Na^+ in urine is that berberine might potentiate the aldosterone-releasing action of circulating angiotensin. This is because berberine has been found (35) to potentiate two other actions of angiotensin (a) hypertension in the dog and (b) contraction of the isolated guineapig ileum. The excess aldosterone thus released might selectively promote Na^+ reabsorption and the loss of K^+ by the renal tubules; this would also explain some rise of K^+ concentration in the urine by berberine in some experiments (Table I).

The effect of berberine on the renal function of the saline-loaded conscious rats could also be due to reduced GFR or due to excess release of aldosterone.

In rats, berberine produced hypothermia at comparatively higher dose (50 *mg/kg*) and, in this respect, it was about 10 times less potent than chlorpromazine.

Berberine, by ip and oral route, produced, like sodium salicylate, antipyretic effect in rats rendered pyrectic by Brewer's yeast. And, in this respect, berberine was about 3 times more effective than sodium salicylate. If this ratio is extrapolated to man, about 100 *mg* of berberine would be equal to 300 *mg* of sodium salicylate which is its antipyretic dose in man. For approximately 100 *mg* of berberine, about 4 g of "Rasaut" (crude dried extract of *Berberis aristata*) would

be required; indeed, this dose of "Rasaut" is mentioned to be its anti-pyretic dose in Ayurvedic literature (16). Altogether, the results of the present study vindicate its ancient use as an anti-pyretic.

Inhibition of sperm motility may be a manifestation of general inhibition of excitable cells by large doses of berberine. However, it is about 330 times less potent than standard spermicidal agent p-diisobutylphenoxypolyethoxyethanol. Also, in such large quantities berberine is likely to exert local effects on the vaginal and uterine musculature and will produce staining at the site of application. Therefore, berberine has no value as a direct spermicidal agent.

Berberine produced a positive inotropic and chronotropic effect on the isolated atrial preparations of rabbits, rats and guineapigs. Cardiac stimulant action of berberine both in intact animals and isolated heart preparations have been reported earlier (21, 35). The nature of cardiac stimulant action of berberine is not clear. In anaesthetized dogs, propranolol inhibits the cardiac stimulant action of berberine (35) but does not block its direct peripheral hypotensive action. It is possible that berberine specifically stimulates B_1 adrenergic receptors present in the heart. This action needs further study.

Berberine has been reported (3, 35), like adrenaline and isoprenaline, to inhibit diverse type of spasmogenic agents on various isolated tissue preparations. Does berberine act like adrenaline and isoprenaline on the isolated tissues? The following experiments seem to exclude such a possibility — (a) unlike adrenaline, berberine did not contract the isolated seminal vesicles nor the isolated rabbit aortic strip (present work) and (b) its hypotensive action in the dog is not blocked by propranolol (35).

On the isolated guineapig ileum, smaller doses of berberine generally potentiated the spasmogenic action of prostaglandins E_1, E_2, $F_1\alpha$ and $F_2\alpha$ though, the degree and consistency of berberine-induced potentiation varied. It is difficult to explain why in some tissues berberine inhibited PGE_2 and $PGF_1\alpha$. Small doses of physostigmine and neostigmine were also found to potentiate the stimulant actions of PGs. Atropine in small doses was found to inhibit the contractions induced by PGs. Altogether, on the isolated guineapig ileum preparation, the action of berberine, physostigmine and neostigmine on the PGs-induced contraction resembled, in every detail, that of angiotensin (35). Therefore, it is possible that, like angiotensin, PGs stimulate the guineapig ileum by releasing intrinsic acetylcholine and that berberine, physostigmine and neostigmine potentiate their action because they are cholinesterase inhibitors.

The non-specific spasmolytic agents such as papaverine and polysorbates (Tweens) inhibit isolated guineapig ileum contractions induced by diverse agonists including KCl (36) which induces contraction by surface depolarization. In this respect they completely resemble berberine. Therefore, it is reasonable to propose that in a normal isolated guineapig ileum, berberine, papaverine and polysorbates inhibit certain agonists by some mechanism at the cell membrane of the smooth muscles.

On the other hand, in the isolated guineapig ileum depolarized by prolonged exposure to

excess K_2SO_4, the cell membrane is depolarized and non-functional and, drugs like $CaCl_2$ which induce contraction in this preparation do so by directly acting on the contractile elements inside the cell by initiating the excitation-contraction coupling mechanism (32). This $CaCl_2$-induced contraction is blocked by papaverine and polysorbates but, interestingly, is potentiated by berberine. This finding suggests that, at the intracellular sites of excitation-contraction coupling, papaverine and polysorbates reduce but berberine increases the availability of Ca^{++}.

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REFERENCES

1. Akhter, M.H., M. Sabir and N.K. Bhide. Anti-inflammatory effect of berberine in rats injected locally with cholera toxin. *Ind. J. Med. Res.*, **65** : 133-141, 1977.
2. Bhalla, T.N., M.B. Gupta and K.P. Bhargava. Antipyretic and analgesic activity of some natural products. *Ind. J. Pharmac.*, **3** : 194-196, 1971.
3. Bhide, M.B. and N.K. Dutta. The antagonistic action of berberine hydrochloride against certain biogenic substances. *Ind. J. Physiol. Pharmac.*, **12** : 19-20, 1968.
4. Bhide, M.B., V.A. Aroskar and N.K. Dutta. Release of active substances by cholera toxin. *Ind. J. Med. Res.*, **58** : 548-550, 1970.
5. Bhide, N.K. Pharmacological study and fractionation of *Paspalum scrobiculatum* extract. *Br. J. Pharmac. Chemother.*, **18** : 7-18, 1962.
6. Bhide, N.K., D.J. Mehta and R.A. Lewis. Diuretic action of sodium nimbidinate. *Ind. J. Med. Sci.*, **12** : 141-145, 1958.
7. Bhide, N.K., S. Jayasundar, R.C. Setia and M. Sabir. A hypothesis about mechanism of chemotherapeutic action of metronidazole. *Aspects of Allergy and Applied Immunology.*, **4** : 121-125, 1973.
8. Borison, H.L. and S.C. Wang. Physiology and pharmacology of vomiting. *Pharmacological Rev.*, **5** : 193-230, 1953.
9. Broome, J., R.K. Callow, W. Feldberg and B.A. Kovacs. Histamine protection in guineapigs produced by plant tumour extracts. *Br. J. Pharmac.*, **18** : 87-100, 1962.
10. Charak (about 1000 B.C.) Charak Samhita. English translation edited by Shri Gulab Kunverba Ayurvedic Society, Jamnagar, India, 1949.
11. Chopra, R.N., B.B. Dikshit and J.S. Chowhan. Pharmacological actions of berberine. *Ind. J. Med. Res.*, **19** : 1193-1203, 1932.
12. Chopra, R.N., S.L. Nayar and I.C. Chopra. Glossary of Indian medicinal plants, New Delhi, Council of Scientific and Industrial Research, p. 36, 1956.
13. Chopra, R.N., R.L. Badhwar and S. Ghosh. Poisonous plants of India, New Delhi, Indian Council of Agri cultural Research, Vol. I, p. 136, 1965.
14. Choudhry, V.P., M. Sabir and V.N. Bhide. Berberine in giardiasis. *Ind. Pediatrics*, **9** : 143-146, 1972.
15. Das Gupta, B.M. and B.B. Dikshit. Berberine in the treatment of oriental sore. *Ind. Med. Gaz.*, **64**, 67, 1929.
16. Desai, V.G. The Materia Medica and Therapeutics of Indian Medicinal Plants (in Marathi), Bombay, Vaidya Jadhavji Trichumji, Acharya, p. 72-75, 1927.
17. Deshpande, P.R. Trial with berberine tannate for diarrhoea in children. *Bharat Med. J.*, **1** : 28, 1969.
18. Dutta, N.K. and M.V. Panse. Usefulness of berberine (an alkaloid from Berberis aristata) in the treatment of cholera (experimental). *Ind. J. Med. Res.*, **50** : 732, 1962.

19. Dutta, N.K. and S.N. Iyer. Anti amoebic value of berberine and kurchi alkaloids. *Ind. J. Med. Ass.*, **50** : 349-352, 1968.
20. Evans, D.H.L. and H.O. Schild. Mechanism of contraction of smooth muscle by drugs. *Nature*, **180** : 341-342, 1957.
21. Fukuda, H., K. Watanabe and Y. Kudo. Some observations on the cardiovascular effects of 9-substituted berberines. *Chem. Pharm. Bull.*, **18** : 1299-1304, 1970.
22. Furchgott, R.F. and S. Bhadrakom. Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. Exp. Ther.*, **108** : 129-143, 1953.
23. Gaitonde, B.B., P.H. Marker and N.R. Rao. Effect of drugs on cholera toxin-induced fluid in adult rabbit ileal loop. *Prog. Drug. Res.*, **19** : 519-526, 1975.
24. Henry, R.J. Clinical chemistry, Principles and techniques, New York, Hooper and Row Publishers, p. 292, 1964.
25. Jayasundar, S., S.M. Periyasamy and N.K. Bhide. Pharmacological study of antihistaminic principle(s) in the *Rana tigrina* tissues. *Ind. J. Physiol. Pharmac.*, **17** : 213-225, 1973.
26. Kamat, S.A. Clinical trials with berberine hydrochloride for control of diarrhoea in acute gastroenteritis. *J. Ass. Phys. Ind.*, **15**, 525, 1967.
27. Lahiri, S.C. and N.K. Dutta. Berberine and chloramphanicol in the treatment of cholera and severe diarrhoea. *J. Ind. Med. Ass.*, **48** : 1, 1967.
28. Mardikar, B.R. and G.V. Joglekar. Preliminary report on behavioural studies on albino mice with berberine sulphate. *Ind. J. Pharmac.*, **4** : 111-112, 1972.
29. Nadkarni, A.K. Indian Materia Medica. Vol. I, Bombay, Popular Book Depot., p. 188, 1954.
30. Pharmacological experiments on isolated preparations (by the staff of the Department of Pharmacology, University of Edinburgh), Edinburgh, E&S Livingstone, p. 104-107, 1968.
31. Pojda, S.M. and J.R. Vane. Inhibitory effects of aprotinin on kallikrein and kininases in dog's blood. *Br. J. Pharmac.*, **42** : 558-568, 1971.
32. Robertson, P.A. Calcium and contractility in depolarised smooth muscle, *Nature*, **186** : 316-317, 1960.
33. Sabir, M. and N.K. Bhide. Unpublished work, 1970.
34. Sabir, M. and N.K. Bhide. *In Vitro* antiheparin action of berberine on the dog and human blood. *Ind. J. Physiol. Pharmac.*, **15** : 97-100, 1971.
35. Sabir, M., and N.K. Bhide. Study of some pharmacological actions of berberine. *Ind. J. Physiol. Pharmac.*, **15**: 111-132, 1971.
36. Sabir, M., M. Singh and N.K. Bhide. Spasmolytic effect of polysorbates (tweens) 80 and 20 on isolated tissues. *Ind. J. Physiol. Pharmac.*, **16** : 193-199, 1972.
37. Sabir, M., V.M. Mahajan, L.N. Mohapatra and N.K. Bhide. Experimental study of the antitrachoma action of berberine. *Ind. J. Med. Res.*, **64** : 1160-1167, 1976.
38. Sabir, M., M.H. Akhter and N.K. Bhide. Antagonism of cholera toxin by berberine in the gastrointestinal tract of adult rats. *Ind. J. Med. Res.*, **65** : 305-313, 1977.
39. Sathe, A. Gharguti Aushadhe (Home remedies; in Marathi), Bombay, Ayurved Bhavan, 4th ed., p. 154-155, 1947.
40. Schales, O. and S.S. Schales. A simple and accurate method for the determination of chloride in biological fluids *J. Biol. Chem.*, **140** : 879, 1941.
41. Shanbhag, S.M., H.J. Kulkarni and B.B. Gaitonde. Pharmacological actions of berberine on the central nervous system. *Jap. J. Pharmac.*, **20** : 482-487, 1970.
42. Sharda, D.C. Berberine in the treatment of diarrhoea of infancy and childhood. *J. Med. Ass.*, **54** : 22, 1970.
43. Smith, H.W. The kidney, Oxford, Oxford University Press, p. 191, 1951.
44. Stone, C.A. and E.R. Loew. Specificity and potency of aryl haloalkylamine adrenergic blocking drugs as determined on isolated seminal vesicle of guineapigs. *J. Pharmac. Exp. Therp.*, **106** : 226-234, 1952.
45. Subbaiah, T.V. and A.H. Amin. Effect of berberine sulphate on Entamoeba histolytica. *Nature (Lond.)*, **215** : 527-528, 1967.
46. Swanson, E.W. and H.A. Herman. The correlation between some characteristics of dairy bull semen and conception rate. *J. Dairy Sci.*, **27** : 297-301, 1944.
47. Vad, B.G., P.H. Raman and V.K. Deshmukh. Effect of berberine on serum cholesterol and pentobarbitone sleeping time in rats. *Ind. J. Pharmac.*, **33** : 23-24, 1971.
48. Varma, R.L. Berberine sulphate in Oriental sore. *Ind. Med. Gaz.*, **62** : 84, 1927.
49. Wagbhat. Ashtangrhudaya. Original Sanskrit with Marathi translation "Sartha Wagbhat" by G.K. Garde, Poona, Aryabhushan Mudranalaya, 4th ed., 1956.